Molecular mechanisms of D-cycloserine in a fear extinction posttraumatic stress disorder (PTSD) animal model
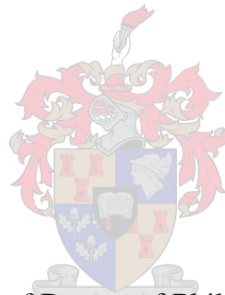
by Stefanie Malan-Müller

Dissertation presented for the Degree of Doctor of Philosophy in the Faculty of Medicine and Health Sciences, at Stellenbosch University

Supervisor: Dr. SMJ Hemmings

Co-supervisor: Prof S Seedat

April 2014

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Signature:

Date:

Abstract

Posttraumatic stress disorder (PTSD) is a severe, chronic and debilitating psychiatric disorder that can present after the experience of a life-threatening traumatic event. D-cycloserine (DCS), a partial *N*-methyl-D-aspartate (NMDA) receptor agonist, has been found to augment cognitive behavioural therapy by facilitating fear extinction; however, the precise mechanisms whereby DCS ameliorates fear triggered by a traumatic context remains to be fully elucidated. This study aimed to (i) identify the molecular mechanisms of intrahippocampally administered DCS in facilitating fear extinction in a rat model of PTSD by investigating gene expression profiles in the left dorsal hippocampus (LDH) of male Sprague Dawley rats and (ii) determine whether microRNA (miRNA) expression and DNA methylation mediated these gene expression changes.

An adapted version of the PTSD animal model described by Siegmund and Wotjak (2007) was utilised. The total number of 120 rats were grouped into four experimental groups (of 30 rats per group) based on fear conditioning and the intrahippocampal administration of either DCS or saline: (1) fear conditioned + intrahippocampal saline administration (FS), (2) fear conditioned + intrahippocampal DCS administration (FD), (3) control + intrahippocampal saline administration (CS) and (4) control + intrahippocampal DCS administration (CD). Behavioural tests (the light/dark [L/D] avoidance test, forced swim test and open field test) were conducted to assess anxiety and PTSD-like behaviours. The L/D avoidance test was the most sensitive behavioural test of anxiety and was subsequently used to differentiate maladapted (animals that displayed anxiety-like behaviour) and well-adapted (animals that did not display anxiety-like behaviour) subgroups. In order to identify genes that were differentially expressed between FS maladapted (FSM) (n = 6) vs. FD well-adapted (FDW) (n = 6) groups, RNA sequencing was performed on the Illumina HiSeq 2000 which generated more than 60 million reads per sample. This was followed by subsequent bioinformatics analyses (using the software programs *TopHat*, *Bowtie*, *Cuffdiff* and Bio-Ontological Relationship Graph (*BORG*) database (that identifies genes that may be biologically relevant) to identify biologically relevant differentially expressed genes between the treatment groups. Epigenetic mechanisms mediating observed differences in gene expression were investigated by conducting DNA methylation and miRNAseq analyses in the FDW and FSM experimental groups. DNA methylation was investigated using real-time quantitative PCR (qPCR) amplification followed by high resolution melt analysis on the Rotor-Gene™ 6000. Differences in miRNA expression levels between the FDW and FSM groups were investigated by sequencing the miRNA fraction on the MiSeq platform.

The bioinformatics pipeline used to analyse the RNAseq data identified 93 genes that were significantly downregulated in the FDW group compared to the FSM group. Forty-two of these genes were predicted to be biologically relevant (based on *BORG* analysis). Integrative network

analyses revealed subsets of differentially expressed genes common across biological functions, pathways and disorders. The co-administration of DCS and behavioural fear extinction downregulated immune system genes and genes that transcribe proinflammatory and oxidative stress molecules. These molecules mediate neuroinflammation and subsequently cause neuronal damage. DCS also regulated genes involved in learning and memory processes. Additionally, a subset of the genes, which have been found to be associated with disorders that commonly co-occur with PTSD (such as cardiovascular disease, metabolic disease, Alzheimer's and Parkinson's disease), was downregulated by the co-administration of DCS and behavioural fear extinction.

In order to determine whether real-time qPCR analysis would be sensitive enough to detect differential expression in those genes found to be differentially expressed in RNAseq analysis, the expression of nine genes was analysed using SYBR Green qPCR technology. In the LDH, six of the nine genes were found to be differentially expressed between FDW and FSM groups and one gene, matrix metallopeptidase 9 (*MMP9*), was observed to be differentially expressed between these two groups in the blood.

Three of the nine genes for which differential expression levels were investigated using SYBR Green real-time qPCR, contained CpG islands and were used for CpG island DNA methylation analysis. Results indicated that CpG island DNA methylation did not mediate differential gene expression of *TRH*, *NPY* or *MT2A*. Bioinformatics analysis of miRNAseq data identified 23 miRNAs that were differentially expressed between the FDW and FSM groups. Several of these miRNAs have previously been found to be involved in brain development and behavioural measures of anxiety. Furthermore, functional luciferase analysis indicated that the upregulation of rno-mi31a-5p could have facilitated the downregulation of interleukin 1 receptor antagonist gene (*IL1RN*) as detected in RNAseq.

RNAseq and miRNAseq analyses in this PTSD animal model identified differentially expressed genes and miRNAs that serve to broaden our understanding of the mechanism whereby DCS facilitates fear extinction. To this end, immune system genes and genes transcribing proinflammatory and oxidative stress molecules were among the genes that were found to be differentially expressed between the FDW and FSM groups. Based on the results obtained, it can be hypothesised that DCS attenuates neuroinflammation and subsequent neuronal damage, and also regulates genes involved in learning and memory processes. Concomitantly, these gene expression alterations mediate optimal neuronal functioning, plasticity, learning and memory (such as fear extinction memory) which contribute to the fear extinction process. Furthermore, biologically relevant differentially expressed genes that were associated with DCS facilitation of fear extinction and with other chronic medical conditions, such as cardiovascular disease and metabolic diseases,

might help to explain the co-occurrence of these disorders with PTSD. In conclusion, Identifying the molecular underpinnings of DCS-mediated fear extinction brings us closer to understanding the process of fear extinction and could, in future work be used to explore novel therapeutic targets to effectively treat PTSD and related disorders.

Opsomming

Posttraumatiese stressindroom is 'n ernstige, kroniese aftakelende psigiatriese toestand wat kan ontwikkel na 'n lewensgevaarlike traumatiese gebeurtenis. Daar is bevind dat die gesamentlike toediening van D-sikloserien (DCS), 'n *N*-metiel-D-aspartaat (NMDA) reseptor agonis, en kognitiewe gedragsterapie effektief is in die bemiddeling van vrees uitwissing; maar die presiese meganisme waar deur DCS die vrees wat deur 'n traumatiese konteks ontlok word verminder, is egter onduidelik. Hierdie studie het beoog om (i) die molekulêre meganismes te identifiseer waardeur intra-hippokampaal toegediende DCS vrees uitwissing fasiliteer, in 'n rot model van posttraumatiese stressindroom, deur geen uitdrukkingsprofiele in the linker dorsale hippokampus (LDH) van manlike Sprague Dawley rotte te ondersoek en (ii) om te bepaal of mikroRNA (miRNA) uitdrukking en DNA metilering die veranderinge in geen uitdrukking bemiddel het.

'n Gewysigde weergawe van die posttraumatiese stressindroom diere model, beskryf deur Siegmund en Wotjak (2007), was gebruik tydens die studie. Rotte was in vier groepe verdeel, vrees kondisionering + soutwater (FS), vrees kondisionering + DCS (FD), kontrole + soutwater (CS) en kontrole + DCS (CD). Gedragstoetse was uitgevoer om angstige, vreesvolle en posttraumatiese stressindroom-tipe gedrag te evalueer. Gedurende die lig/donker (L/D) vermydingstoets het die FS groep aansienlik meer tyd in die donker kompartement deurgebring ('n indikasie van vreesvolle gedrag) in vergelyking met die CS en die FD groepe wat meer tyd in die verligte kompartement deurgebring het ('n indikasie van vreeslose gedrag). Die L/D toets was die mees sensitiewe gedragstoets vir angstige en vreesvolle gedrag en was gevolglik gebruik om die diere te sub-groepeer in wanaangepaste (diere wat angstige en vreesvolle gedrag vertoon het) en goedaangepaste (diere wat nie angstige en vreesvolle gedrag vertoon het nie) subgroepe. Nuwe generasie RNA volgordebepaling (RNAseq) van die LDH RNA en daaropvolgende bioinformatiese analise was uitgevoer om gene te identifiseer wat differensieel uitgedruk is tussen die twee behandelingsgroepe van belang in die betrokke studie, naamlik FS wanaangepaste (FSM) teenoor FD goedaangepaste (FDW) groepe. Epigenetiese analises was uitgevoer om te bepaal of differensieel uitgedrukte miRNAs of CpG-eiland DNA metilasie die differensiële geenuitdrukking bemiddel het.

Bioinformatiese analises van die RNAseq data het 93 gene geïdentifiseer waarvan die geen uitdrukking beduidend onderdruk was in die FDW groep in vergelyking met die FSM groep; 42 van hierdie gene was voorspel om biologies relevant te wees. Geïntegreerde netwerk analise het onthul dat sekere van die differensieel uitgedrukte gene gemeenskaplik was tussen verskeie biologiese funksies, padweë en versteurings. DCS het die uitdrukking van immuun-sisteem gene en pro-inflammatoriese en oksidatiewe stres gene verlaag. Hierdie molekules medieer neuro-inflammasie wat gevolglik tot neurale skade lei. DCS het ook gene gereguleer wat betrokke is by leer en geheue prosesse. DCS het

onder meer ook die geenuitdrukking verlaag van 'n sub-groep van gene wat voorheen geassosier is met komorbiede versteurings van PTSD. SYBR Green real-time qPCR (werklike tyd kwantitatiewe polimerase ketting reaksie) analise was ondersoek om te bepaal of hierdie metode sensitief genoeg sou wees om die verlaagde geen-uitdrukking van verskeie van die biologies relevante differensieel uitgedrukte gene te identifiseer, in dieselfde LDH komplementêre DNA (cDNA) monsters as wat in die RNAseq gebruik is, asook in die bloed cDNA monsters. SYBR Green real-time qPCR was in staat om ses, van die nege, differensieel uitgedrukte gene in die LDH cDNA monsters en een geen, matriks metallopeptidase 9 (*MMP9*), in die bloed cDNA monsters op te tel.

Drie van die gene waarvoor SYBR Green real-time qPCR gebruik is om differensiële geenuitdrukking te toets, het CpG eilande bevat en was gevolglik gebruik in CpG eiland DNA metilering analises. Resultate het getoon dat CpG eiland DNA metilering nie die differensiële geenuitdrukking van *TRH*, *NPY* of *MT2A* gedryf het nie. Bioinformatiese analises van die miRNAseq data het 23 miRNAs geïdentifiseer wat differensieël uitgedruk was tussen die FDW en FSM groepe. Verskeie van hierdie miRNAs is reeds voorheen beskryf om betrokke te wees in brein ontwikkeling en angs gedrags metings. Funksionele luciferase analises het verder aangedui dat die verhoogde uitdrukking van rno-mi31a-5p moontlik die verlaagde geen uitdrukking van *IL1RN*, soos waargeneem in die RNAseq data, kon bewerkstellig het.

RNAseq en miRNAseq analises in hierdie posttraumatiese stressindroom dieremodel het differensieël uitgedrukte gene en miRNAs geïdentifiseer wat dien om die verstaanswyse te verbreed van hoe DCS die vrees uitwissings proses fasiliteer. Die meganismes waardeur DCS vrees uitwissings bewerkstellig het sluit die verlaging van immuun-sisteem geen-uitdrukking in, sowel as verlaagde uitdrukking van gene wat pro-inflammatoriese en oksidatiewe stress gene transkribeer. DCS het daardeur neuro-inflammasie en gevolglike neurale skade voorkom. DCS het daarmee saam ook gene gereguleer wat betrokke is by leer en geheue prosesse. Hierdie gesamentlike veranderings in geen uitdrukking het gelei tot die uiteindelike bewerkstelling van optimale neurale funksionering, plastisiteit, leer en geheue prosesse wat uiteindelik bygedra het tot vrees uitwissing. Biologies relevante differensieël uitgedrukte gene wat ook geassosieer was met ander kondisies, soos middel verwante versteurings en metaboliese versteurings, kan help om die komorbiditeit met posttraumatiese stressindroom te verklaar. Identifisering van die molekulêre grondslae van DCS bemiddelde vrees uitwissing verbreed ons begrip en verstaan van vrees uitwissing en kan moontlik, in toekomstige navorsing gebruik word om nuwe innoverende terapeutiese teikens te verken om sodoende posttraumatiese stressindroom meer effektief te kan behandel.

Acknowledgements

Table of Contents

List of Figures

List of Tables

List of abbreviations

α	alpha
β	beta
γ	gamma
μg	microgram
μl	microliter
μM	micromolar
˚C	degrees Celsius
3'	three prime
3' UTR	three prime untranslated region
5'	five prime
5hmC	5-hydroxymethylcytosine
5-HT$_{2C}$	serotonin receptor gene
5mC	5-methylcytosine
A	adenine
A2M	alpha-2-macroglobulin gene
ABI	Applied Biosystems Incorporated
ABP	arterial blood pressure
AC	adenylyl cyclase
ACTH	adrenocorticotropin hormone
ACC	anterior cingulate cortex
ACTB	β-Actin gene
AD	Alzheimer's disease
ADCYAP1R1	adenylate cyclase activating polypeptide 1 (pituitary) receptor type I gene
ADHD	attention deficit/hyperactivity disorder
AGE	advanced glycation endproducts
AID	activation-induced cytidine deaminase
ALS2	amyotrophic lateral sclerosis 2 gene

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
Amp^R	ampicillin resistance gene
AngII	angiotensin II
ANOVA	one-way analysis of variance
ANXA2	annexin A2 gene
ATPase	adenosine triphosphatase
APA	American Psychological Association
APC5	anaphase promoting complex subunit 5
APOE2	apolipoprotein E2
APR	acute-phase reactants
ASR	acoustic startle response
AVP	arginine vasopressin gene
BBB	blood brain barrier
BDNF	brain-derived neurotrophic factor
BLA	basolateral nucleus
bla	beta- lactamase
BNST	bed nucleus of the stria terminalis
bp	base pair
C	cytosine
C1S	complement component 1, s subcomponent gene
C1QA	complement component 1, q subcomponent, A chain gene
C1QB	complement C1q subcomponent subunit B gene
C1QC	complement C1q subcomponent subunit C gene
C6	complement component C6 gene
Ca^{2+}	calcium
CA1	Cornu Ammonis region 1
CA3	Cornu Ammonis region 3
CAM	cell adhesion molecules
CaSR	Ca^{2+} sensing receptor

CB1	cannabinoid receptor 1
CBT	cognitive behavioural therapy
CD	Control + D-cycloserine
CD44	Cd44 molecule gene
CD4	Cd4 molecule gene
CD74	Cd74 molecule, major histocompatibility complex, class II invariant chain gene
CD8A	Cd8a molecule gene
CDP	chlordiazepoxide
CeA	central nucleus of the amygdala
CGIs	CpG islands
CHRDL1	chordin-like 1 gene
CHRH1	corticotrophin-releasing hormone receptor gene
CHRNA5	cholinergic receptor, nicotinic, alpha 5 (neuronal) gene
CLEC7A	c-type lectin domain family 7, member A gene
CLEC9A	c-type lectin domain family 9 gene
CMS	chronic mild stress
CMV	cytomegalovirus
CNR1	cannabinoid receptor 1 (brain)
CNVs	copy number variants
CNS	central nervous system
COMT	catechol-O-methyltransferase
CP	ceruloplasmin (glycoprotein) gene
CREB	cAMP response element-binding protein
CRF	corticotropin-releasing factor
CRFR2	corticotrophin releasing factor receptor 2
CRH	corticotropin-releasing hormone
CRHR1	corticotropin releasing hormone receptor 1
CS	conditioned stimulus
CS	Control + Saline
CSF	cerebrospinal fluid

CTD	Comparative Toxicogenomics Database
CTSC	cathepsin C gene
Cq	quantification cycle
CXCL13	chemokine (C-X-C motif) ligand 13 gene
CYBB	cytochrome b-245, beta polypeptide gene
CYC A	cyclophilin A
dACC	dorsal anterior cingulate cortex
DAT	dopamine active transporter gene
dB	decibel
DBH	dopamine β-hydroxylase gene
DCS	D-cycloserine
DGCR8	DiGeorge syndrome critical region gene 8
DH	dorsal hippocampus
DLGAP2	disks large homolog-associated protein 2 gene
DMEM	Dulbecco's Modification of Eagle's Medium
DNA	Deoxyribo Nucleic Acid
DNMT	DNA methyltransferase
DNMT1	DNA methyltransferase 1
DNMT3A	DNA methyltransferase 3A
DNMT3B	DNA methyltransferase 3B
DNMT3L	DNA methyltransferase 3L
DRD2	dopamine receptor D2 gene
dNTPs	deoxynucleotide triphosphates
DRD4	dopamine receptor D4 gene
dsDNA	double stranded DNA
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, version 5
DZ	dizygotic
E	epinephrine
E_2	estradiol
EBV	Epstein-Barr virus

ECM	extracellular matrix
ECS	endocannabinoid system
ECT	electroconvulsive shock therapy
EDs	eating disorders
EDG1	endothelial differentiation gene 1
EDTA	ethylene-diamine-tetra-acetic acid
eGFP	enhanced green fluorescent protein
EMBL	European Molecular Biology Laboratory
EMBOSS	European Molecular Biology Open Software Suite
ERα	estrogen receptor α
ESCs	embryonic stem cells
F	forward primer
F10	coagulation factor X gene
FCER1G	Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide gene
FD	fear-conditioned + D-cycloserine
FDM	fear-conditioned + D-cycloserine maladapted
FDW	fear-conditioned + DCS well-adapted
FGF1	fibroblast growth factor 1 gene
Fig.	figure
FKBP5	FK506 binding protein 5 gene
FLU	fluoxetine
FPKM	Fragments Per Kilobase of exon per Million fragments mapped
FS	fear-conditioned + saline
FSM	fear-conditioned + saline maladapted
FST	forced swim test
FSW	fear-conditioned + saline well-adapted
G	guanine
g	gram
GABA	gamma-aminobutyric acid

GABRA2	gamma-aminobutyric acid receptor subunit alpha-2 gene
Gadd45b	growth arrest and DNA-damage-inducible, beta
GAPDH	glyceraldehyde-3-phosphate dehydrogenase gene
GC	glucocorticoid
GCCR	glucocorticoid receptor
G x E	gene-environment
GFOLD	generalized fold change count facility
GH	growth hormone
GILZ	glucocorticoid-Induced Leucine Zipper gene
GLuc	gaussia luciferase
GLYT-1	glycine transporter 1 gene
GnRH	gonadotropin-releasing hormone
GO	gene ontology
GPCR	G-protein coupled receptor
GPNMB	glycoprotein (transmembrane) nmb gene
GR	glucocorticoid receptor gene
GRM5	glutamate receptor 5 gene
GWAS	genome-wide association studies
H^+	hydrogen
H_2O_2	hydrogen peroxide
HA	high swim stress-induced analgesia
HDAC	histone deacetylase
HDACis	histone deacetylase inhibitors
HEK 293	human embryonic kidney 293
HIV	human immunodeficiency virus
HMOX1	heme oxygenase (decycling) 1 gene
HPA	hypothalamic–pituitary–adrenal
HRM	high resolution melt
Hsa	*Homo sapiens*
Hsp90	heat shock protein 90

hPGK	human phosphoglycerate kinase I promoter
HPT	hypothalamic-pituitary-thyroid
HPRT	hypoxanthine phosphoribosyltransferase gene
HTR2A	5-hydroxytryptamine (serotonin) receptor 2A gene
HVA	homovanillic acid
iCRH	immune corticotropin-releasing hormone
ICV	intracerebroventricular
IEG	immediate-early gene
IGF-I	insulin-like growth factor I
IL-6	interleukin 6
IL-16	interleukin 16 gene
IL-18	interleukin 18 gene
IL1RN	interleukin 1 receptor antagonist gene
ILPFC	infralimbic prefrontal cortex
IPA	Ingenuity Pathway Analysis
iPS cells	induced pluripotent stem cells
IPV	intimate partner violence
ITGAL	integrin, alpha L gene
K^+	potassium
kb	kilobase
KEGG	Kyoto encyclopedia of genes and genomes
KET	ketamine
kg	kilogram
kHz	kilohertz
L	long allele
LA	low swim stress-induced analgesia
LBP	lipopolysaccharide binding protein gene
LC	locus coeruleus
LC-NA	locus coeruleus- noradrenergic
LCP1	ceruloplasmin gene

L/D	light/dark
LDH	left dorsal hippocampus
LG-ABN	licking/grooming and arched back nursing
LGALS3BP	lectin, galactoside-binding, soluble, 3 gene
LH	luteinizing hormone
LINE1	long interspersed nucleotide element 1
LS	least square
LSD	least square differences
LTM	long-term memory
LTP	long-term potentiation
LYZ2	lysozyme 2 gene
mA	milliamps
MA	maladapted
MAOIs	monoamine oxidase inhibitors
MBD	methyl CpG-binding domain
MBD1-4	methyl-CpG binding domain 1-4
MDD	major depressive disorder
MeCP2	methyl CpG binding protein 2
mg	milligram
Mg^{2+}	magnesium
mGluR5	metabotropic glutamate receptor type 5
min	minute
miRNA	microRNA
mir	microRNA
miRSVR	microRNA support vector regression
min	minutes
ml	millilitres
mM	millimolar
MMP9	matrix metallopeptidase 9 gene
mmu	*Mus musculus*

MN	motor neurons
mPFC	medial prefrontal cortex
MRI	magnetic resonance imaging
Ms	maternal separation
MS	multiple sclerosis
MSC	mature stem cells
MSR1	Macrophage scavenger receptor 1 gene
MT2A	metallothionein 2A gene
mRNA	messenger ribonucleic acid
MWM	Morris water maze
MZ	monozygotic
n	sample number
Na$^+$	sodium
NaCl	sodium chloride
NADPH	nicotinamide adenine dinucleotide phosphate-oxidase
NCBI	National Center for Bioinformatics
NCF1	neutrophil cytosolic factor 1 gene
NE	norepinephrine
NFI-A	nuclear factor 1 A gene
ng	nanogram
NGFI-A	nerve growth factor-inducible protein A gene
NK1	neurokinin 1
nM	nanomolar
NMDA	*N*-methyl-D-aspartate
NMDAR	*N*-methyl-D-aspartate receptor
NMDAR1	*N*-methyl-D-aspartate receptor subunit 1
NOX	NADPH Oxidase
NOX2	NADPH Oxidase 2
NPY	neuropeptide Y gene
NSC	neuronal stem cells

nt	nucleotide
NTS	nucleus tractus solitarius
NTRK3	neurotrophic tyrosine kinase gene
NR3C1	glucocorticoid receptor gene
O_2^-	superoxide radicals
OCD	obsessive-compulsive disorder
OCT4	octamer-binding transcription factor 4 gene
OFC	orbitofrontal prefrontal cortex
OMIM	Online Mendelian Inheritance in Man
PAG	periaqueductal gray
PBMCs	peripheral blood mononuclear cells
PCL	phospholipase C
PCR	polymerase chain reaction
PET	positron emission tomography
PFC	prefrontal cortex
PGC	primordial germ cell
PGK	phosphoglycerate kinase gene
PKA	protein kinase
PND	postnatal day postnatal day
POMC	pro-opiomelanocortin gene
PP1	protein phosphatase 1 gene
PPARδ	proliferator-activated receptor delta
PPF	paired-pulse facilitation
PPI	prepulse inhibition
PRRs	pattern recognition receptors
pri-miRNA	primary miRNA
PRLR	prolactin receptor gene
PSD	post-synaptic density
PTPRC	protein tyrosine phosphatase receptor type, C gene
PTSD	posttraumatic stress disorder

pUC Ori	pUC plasmid origin of replication
PURO^R	puromycin resistance gene
PVN	paraventricular nucleus
qPCR	quantitative polymerase chain reaction
R	reverse primer
RAC2	Ras-related C3 botulinum toxin substrate 2 gene
RAGE	receptor for advanced glycation end products
RAS	renin-angiotensin system
RD2	dopamine receptor D2 gene
RELN	reelin
RGS2	regulator of G-protein signaling 2 gene
RLU	relative light units
RNA	ribonucleic acid
RNAPII	ribonucleic acid polymerase II
rno	*Rattus norvegicus*
RORA	retinoid-related orphan receptor alpha gene
ROS	reactive oxygen species
RP1	RNA PCR primer
RRM2	ribonucleoside-diphosphate reductase subunit M2 gene
RSA	Republic of South Africa
RT	reverse transcription
S	short allele
S100A3	S100 calcium binding protein A3 gene
S100A4	S100 calcium binding protein A4 gene
S100A9	S100 calcium binding protein A9 gene
S100A10	S100 calcium binding protein A10 gene
SAD	social anxiety disorder
SEAP	secreted alkaline phosphatase
sec	second
SEM	standard error of the mean

SERT	serotonin transporter
SERTPR	serotonin transporter-linked polymorphic region
siRNA	small interfering RNA
SLC6A3	dopamine transporter
SLC6A4	serotonin transporter gene
SLC17A7	solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 7
SNI	spared nerve injury
SNP	single nucleotide polymorphism
SNRIs	serotonin–norepinephrine reuptake inhibitors
snRNA	small nuclear RNA
snRNP	small nuclear ribonucleoprotein
SNS	sympathetic nervous system
SP	substance P
SPP1	secreted phosphoprotein 1gene
SPS	single prolonged stress
ssDNA	single-stranded DNA
SSRI	selective serotonin re-uptake inhibitor
ST14	suppressor of tumorigenicity 14 protein gene
STM	short term memory
SV40	Simian virus 40
SYP	synaptophysin
T	thymine
T	testosterone
Ta	annealing temperature
TCAs	tricyclic / tetracyclic antidepressants
TDG	thymine DNA glycosylase
TENC	trauma-exposed non-PTSD controls
TET	ten-eleven translocation
TF	transcription factors
TG	triglycerides

Tm	melting temperature
TLR8	toll-like receptor 8 gene
TNF	tumour necrosis factor gene
TNF-α	tumor necrosis factor alpha
TPR	translocated promoter region gene
TRH	thyrotropin releasing hormone gene
TrkB	tropomyosin receptor kinase B
tRNA	total ribonucleic acid
TSH	thyroid-stimulating hormone
TSPO	translocator protein gene
TSS	transcription start site
U	units
UK	United Kingdom
US	unconditioned stimulus
USA	United States of America
UTR	untranslated region
UV	ultra-violet
V	volts
VAMP2	vesicle-associated membrane protein 2
VGLUT1	vesicular glutamate transporter 1
VH	ventral hippocampus
VIM	vimentin gene
vmPFC	ventromedial prefrontal cortex
VTA	ventral tegmental area
WA	well-adapted
WFS1	wolframin gene
WT	wild type

1. Introduction

1.1 Background

Posttraumatic stress disorder (PTSD) is a severe, chronic and debilitating psychiatric disorder that can occur after exposure to a potentially traumatic event (DSM-5, APA 2013)[1], significantly impairing normal functioning and quality of life. PTSD is classified as a trauma- and stress-related disorder in the DSM-5 (APA, 2013), and is characterized by the presence of four distinct diagnostic symptom clusters, namely re-experiencing, avoidance and negative cognitions and mood, and arousal (DSM-5, APA 2013). The disorder occurs in about 7% of the general population (Kessler et al., 2005). Stress-related diseases, such as depression and anxiety disorders, place a heavy health and economic burden on society. However, there is a limited range of available pharmacotherapies to treat these disorders and the majority of treatments are suboptimal with regard to efficacy and tolerability (Holmes et al., 2003; Kessler et al., 2005; Kasper et al., 2010).

The development of PTSD is associated with learned fear-conditioned responses, which serve as reminders of traumatic events, and which can persist for several years after the occurrence of the traumatic event (Orr et al., 2000; Blechert et al., 2007). In fact, several forms of psychotherapy, especially cognitive behavioural therapy (CBT), form part of the current recommendations for the treatment of PTSD (Foa et al., 2000), as well as psycho-education and supportive measures (Cohen et al., 2004; Oflaz et al., 2008). Exposure-based CBT is the most commonly used approach for PTSD treatment and relies on extinction-based methods (Norton and Price, 2007). This therapy involves exposing the patient to an anxiety-producing stimulus repeatedly in a controlled setting, thereby reducing the uncontrolled fear associated with the anxiety (Foa and Kozak, 1986).

Pharmacological strategies for the treatment of established PTSD that target the emotional response or other non-cognitive symptoms include selective serotonin re-uptake inhibitors (SSRIs) (Van der Kolk et al., 1994; Connor et al., 1999; Brady et al., 2000; Martenyi et al., 2002), other antidepressants (such as serotonin–norepinephrine reuptake inhibitors (SNRIs), tricyclic and tetracyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs)) Benzodiazepines) (Davidson et al., 1990,2006; Frank et al., 1988; Onder et al., 2006), adrenoceptor agonists and antagonists (Peskind et al., 2003; Raskind et al., 2003, 2007; Taylor et al., 2008) as well as anticonvulsants and antipsychotics (Hageman et al.,2001; Berlin, 2007).

---

[1] American Psychiatric Association. (2013). Diagnostic and   statistical manual of mental disorders (5th ed.). Arlington, VA: American Psychiatric Publishing.

D-cycloserine (DCS) is an antibiotic and partial *N*-methyl-D-aspartate receptor (NMDAR) agonist at the glycine site on the NMDAR1 receptor subunit and has been found to be effective in facilitating extinction learning in rats when administered before or immediately after extinction training (Ledgerwood et al., 2003; 2005; Walker et al., 2002; Yang and Lu, 2005; Philbert et al., 2013). Administration of DCS has been found to result in generalized extinction of fear (Legderwood et al., 2005), a characteristic which could be of clinical benefit to PTSD, as the extinction of a single cue might generalize to other fear-associated cues simultaneously. Additionally, DCS treatment has been found to augment exposure therapy (Smits et al., 2013), especially in patients suffering from more severe PTSD that require longer treatment (de Kleine et al., 2012). DCS has furthermore been shown to reduce the rate of relapse following successful exposure-based CBT (Richardson et al., 2004). DCS has also been shown to be effective in human trials of various anxiety disorders, such as phobias (Ressler et al., 2004), social anxiety disorder (SAD) (Hofmann et al., 2006; Guastella et al., 2008), obsessive-compulsive disorder (OCD) (Kushner et al., 2007; Wilhelm et al., 2008; Storch et al., 2010) and panic disorder (Otto et al., 2010). However, the precise mechanisms by which co-administration of DCS reduces the fear triggered by a traumatic context remain to be fully elucidated. It is therefore imperative to identify the molecular mechanisms that are involved in DCS-induced fear extinction, as this could facilitate a better understanding of PTSD and anxiety disorders.

Animal models provide researchers with the opportunity to perform brain-specific genetic analyses in order to determine the molecular mechanisms involved in disorders or to determine the molecular mechanisms of therapeutic drugs. Gene expression profiling is one of the approaches followed to elucidate the genetic underpinnings of complex disorders or processes, such as fear extinction. Genes that are differentially expressed between trauma-exposed individuals who develop PTSD and those who do not, have extensively been investigated in PTSD and anxiety disorder research and have the potential to unravel the molecular underpinnings of these disorders.

Although quantifying gene expression provides one with an idea of the biological pathways involved in the disorder, it does not provide knowledge of the mechanisms that contribute to observed alterations in gene expression. The term epigenetics literally means 'outside conventional genetics', and is currently used to describe the study of stable alterations in gene expression that are not brought about by changes in DNA sequence (Bjornsson et al., 2004). These epigenetic changes are heritable and potentially reversible, (Jaenisch and Bird 2003) and provide an additional layer of transcriptional control that may mediate the interaction between genetic predisposition, changes in neural functioning and environmental factors (Bjornsson et al., 2004). Epigenetic modifications may thus explain the interindividual variation and the long-lasting effects of trauma exposure (Yehuda and Bierer 2009). Such epigenetic mechanisms include DNA methylation, posttranscriptional modifications of histone proteins (acetylation, methylation, phosphorylation, ubiquitination and sumoylation) and non-coding RNA-mediated alterations (such as micro-RNAs (miRNAs) and small interfering RNAs (siRNAs)) (Yehuda and Bierer 2009).

1.2 Significance of the study

Although studies have been conducted to investigate the mechanism whereby DCS may facilitate fear extinction, the majority of these studies have focused on either intra-amygdalar (Mao et al., 2006; 2008) or systemic (Polese et al., 2002; Yamamoto et al., 2007; Wu et al., 2008; Gabriele and Packard, 2007) administration of the drug. The novelty of the proposed research rests in the fact that DCS was administered intrahippocampally, allowing the direct assessment of the effects of the drug in this brain region. In addition, the study will shed more light on the role that the hippocampus plays in fear extinction. The hippocampus is an important brain region in fear extinction (Barad, 2005; Szapiro et al., 2003) and numerous studies have observed a reduced hippocampal volume in PTSD patients compared to controls (Bremner et al., 1995; 2003; Gurvits et al., 1996; Vythilingam et al., 2005). It is not known whether this reduced volume is a consequence of the disorder, or a pre-existing vulnerability factor (Gilbertson et al., 2006). In addition, recent investigations have indicated enhanced hippocampal activation during associative memory and learning in PTSD patients compared to trauma-exposed (Geuze et al., 2008) and trauma-unexposed controls (Werner et al., 2009). The hippocampus furthermore plays an important role in the processing of emotional behaviour (Kjelstrup et al., 2002; Bannerman et al., 2004; McHugh et al., 2004).

Understanding the molecular mechanisms underlying the fear extinction process mediated by DCS in a PTSD animal model, is crucial to understanding stress-related disorders and the development of effective treatment strategies. Due to the complexity of the fear extinction process and PTSD, it is important to obtain a comprehensive representation of the whole transcriptome and relevant factors that could affect gene transcription. By investigating genes that are differentially regulated in the left dorsal hippocampus (LDH) in a rat animal model of PTSD, we can delineate what is happening on a genomic level during the fear extinction process. Investigating the epigenetic mechanisms involved in the fear extinction process will provide us with insight into how the epigenome mediates gene expression changes, induced by DCS, to facilitate the fear extinction process. The present study represents one of the first to investigate the possible epigenetic effects involved in fear extinction as mediated by intrahippocampal DCS administration. In light of the potentially reversible nature of epigenetic alterations; the epigenetic information gained in this study may provide researchers with exciting and tractable new avenues for pharmacological treatment of PTSD.

1.3 Aims & Objectives

Aim

To investigate the molecular mechanism of action of intrahippocampally administered D-cycloserine in facilitating fear extinction in an animal model of PTSD by performing gene expression and epigenetic analyses.

Objectives

1. To identify genes that are differentially expressed in the LDH of male Sprague Dawley rats following fear conditioning, fear extinction and intrahippocampal DCS administration.
2. To determine whether CpG island DNA methylation mediated the differential gene expression observed in the LDH of fear-saline maladapted (FSM) and fear-DCS well-adapted FDW male Sprague-Dawley rats.
3. To identify differentially expressed microRNAs in the LDH of male Sprague Dawley rats following fear conditioning, fear extinction and intrahippocampal DCS administration, in order to identify miRNAs that are involved in DCS-induced fear extinction (comparing expression profiles of FSM vs. FDW animals).
4. To correlate LDH gene and miRNA expression profiles in order to elucidate which miRNAs possibly mediated expression changes of which genes to facilitate DCS-induced fear extinction

1.4 Brief overview of chapters

The second chapter provides an overview of the PTSD literature, with a brief introduction to the disease pathology, aetiology, prevalence rates, the hypothalamic–pituitary–adrenal (HPA) axis as well as neurobiology of the disorder. An overview of fear conditioning and extinction is also provided followed by neuropeptides and neurotransmitters that play a role in PTSD. The section thereafter focusses on the genetics of PTSD, covering twin and family studies, candidate genes and   gene-environment studies. The larger part of the literature review is dedicated to gene expression studies as well as DNA methylation and miRNA expression studies in PTSD animal models and human studies. The chapter concludes with the treatment strategies of PTSD, with the focus on DCS.

The third chapter outlines the methods utilised in the present study, including the PTSD animal model, animal behavioural tests (note that animal behavioural work was performed by another student for her PhD), statistical analyses of behavioural data and animal selection based on behavioural data. Thereafter, the genetic laboratory methods are described. This methodology includes nucleic acid isolation, gene expression analyses with next generation RNA sequencing, bioinformatics analyses for differential gene

expression analysis and gene enrichment analyses and clustering to facilitate the biological interpretation of the results. Information regarding SYBR Green real-time qPCR techniques is also provided. SYBR Green qPCR technology was assessed for its sensitivity to detect a subset of differentially expressed genes. This is followed by a description of epigenetic methodology used in the present study, including CpG island DNA methylation analysis, as well as miRNA sequencing and bioinformatics analyses to identify differentially expressed miRNAs. SYBR Green real-time qPCR was again investigated to determine its sensitivity to detect differential expression of a particular miRNA of interest. Chapter 3 concludes with a description of the functional luciferase assay that was performed to determine whether a specific miRNA interacted with its predicted mRNA target region. The fourth chapter provides the relevant results generated during the study. This includes the animal behavioural data, gene expression data together with gene enrichment analyses and clustering as well as epigenetic data. Furthermore, gene and miRNA expression data was correlated to determine whether any differentially expressed miRNAs may have mediated the differential expression of certain genes. Lastly, the data of the functional luciferase assay is provided.

The fifth chapter provides a discussion and interpretation of the results of the current study. Note that certain sections in the discussion are italicized to emphasise the main findings of the current study in light of previous literature. This is followed by the sixth and final chapter, which provides a conclusion of the results of the study as well as limitations and proposed future research. Two appendices are provided at the end of the dissertation to supply additional information.

2. Literature review

2.1 PTSD

The global prevalence of anxiety disorders, as reported in 2012, was estimated at 7.3 % (4.8–10.9 %) (Baxter et al., 2012). Prevalence rates range from 5.3 % (3.5–8.1 %) in African cultures to 10.4 % (7.0–15.5 %) in Euro/Anglo cultures (Baxter et al., 2012). South Africa is considered to be among the most violent countries globally and is a county with the unfortunate title of "rape capital of the world" (Human Rights Watch, 1995). Kaminer et al. (2008) found that approximately 75% of South Africans had experienced at least one traumatic event in their lifetime, and that the experience of multiple traumas was the rule rather than the exception. Studies internationally have found that violent trauma, compared with other types of trauma, is more likely to be associated with posttraumatic stress disorder (PTSD) (Breslau et al., 1998; Creamer et al., 2001; Norris et al., 2003; Zlotnick et al., 2006), suggesting that South Africans are particularly at risk for developing PTSD. This underscores the importance of research into the disease aetiology of PTSD.

PTSD is a severe, chronic and debilitating psychiatric disorder that can occur after exposure to a potentially traumatic event (DSM-5, APA 2013). Failure of extinction of fear memories can result in PTSD symptoms that persist for extended periods of time following the traumatic event (Bremner et al., 1996). These symptoms can significantly impair normal functioning and quality of life (Zatzick et al., 1997; Mendlowicz and Stein 2000). PTSD is classified as a trauma- and stress-related disorder in the DSM-5 (APA, 2013), and is characterized by the presence of four distinct diagnostic symptom clusters, namely re-experiencing, avoidance, negative cognitions and mood, and arousal (DSM-5, APA 2013).

Development of the disorder involves a fear conditioning process during which fear and anxiety responses are exaggerated and/or are resistant to extinction (Keane et al., 1985; Cohen et al., 2006; Amstadter et al., 2009). During classical fear conditioning, a neutral (conditioned) stimulus (CS) is temporarily paired with an aversive (unconditioned) stimulus (US). After sufficient pairing of the CS and the US, the CS alone will eventually elicit the same response as the US. This response is referred to as the conditioned response (CR). The US can elicit a natural, physiological fear response, the unconditioned responses (UR). The CS subsequently acquires the ability to elicit a conditioned fear response which can be triggered upon encountering the harmless stimuli associated with the trauma. Analogous to Pavlovian fear-conditioning models, in PTSD, the trauma is considered to be the US, and the conditioned fear response experienced by PTSD patients, even in the presence of seemingly harmless stimuli, is the CR (Foa and Steketee 1989; Grillon et al., 1998; Skelton et al., 2012). This process plays an important evolutionary role by enabling an organism to identify and react to threatening stimuli. Excessive activation of fear responses, however, to non-threatening stimuli forms the basis of PTSD. Furthermore, emotional and physiological responses to stimuli that resemble the original traumatic event are a central characteristic of PTSD.

2.1.1   HPA axis in PTSD

A key feature of PTSD is an inability to initiate a normal stress response that results in part from the dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis. The HPA-axis is a key stress response system that interacts with the immune system to maintain homeostasis (Wong et al., 2002). Corticotropin-releasing hormone (CRH) (also known as corticotropin-releasing factor [CRF]) regulates the stress-induced activation of the HPA axis and mediates autonomic and behavioural changes associated with anxiety disorders (Chrousos 1998). CRH and vasopressin are secreted by the hypothalamus in response to stress. These neuropeptides are secreted into the portal vessels and stimulate the anterior pituitary to synthesise and release adrenocorticotropin hormone (ACTH) into the bloodstream, which in turn leads to the release of glucocorticoids (GCs) (such as cortisol or corticosterone in rodents) by the adrenal cortex. GCs help to control the processes of adaptation to and recovery from stress due to the role they play in the restoration of biological homeostasis (de Kloet et al., 2009; McEwen et al., 2002). The HPA axis is regulated by a negative feedback mechanism; excess cortisol binds to GC receptors in the hypothalamus and pituitary and this subsequently suppresses the release of CRH and ACTH (Fig. 2.1).

The HPA axis plays a vital role in regulating the normal response to stress. Malfunctioning of this system underlies susceptibility to certain anxiety disorders (McEwen et al., 2002). In addition, studies have indicated a link between elevated cortisol and both chronic stress and depression (Cowen et al., 2002). However, more recent evidence suggests that abnormal HPA axis functioning may characterize a subset of anxiety disorders that distinguish them from mood disorders. For example, traditional stress models (which included anxiety disorders and depression) predict HPA axis overactivity, characterized by hypercortisolemia and reduced negative feedback inhibition (as described in mood disorders) (Holsboer, 2003).

To date, there is no consensus regarding the exact nature of HPA alterations in PTSD. Certain studies reported decreased urinary cortisol levels collected over 24 hours (Mason et al., 1986; Yehuda et al., 1990) and in blood plasma collected repeatedly over 24 hours (Yehuda et al., 1994, 1996). Other studies did not find differences in urinary cortisol levels (over 24-hours) between patients and controls (Mason et al., 2002), or a difference between baseline plasma cortisol levels and PTSD symptoms (Goenjian et al., 2003), and even higher cortisol levels have been reported in PTSD urine samples (Lemieux and Coe 1995; Pitman and Orr 1990). A model described by Yehuda proposed that enhanced negative feedback inhibition of cortisol by the pituitary could be involved (Yehuda, 2006). Initial drug sensitivity studies in PTSD (using Dexamethasone, which measures the response of the adrenal glands to ACTH) did not consider the possibility of hypersuppression to DEX, but rather tested non-suppression of cortisol in PTSD patients, similar to patients with major depressive disorder. Halbreich et al. (1989) found lower post-DEX cortisol levels in the PTSD group compared to subjects with depression and controls (Halbreich et al., 1989), leading Yehuda et al. (1993, 1995) to hypothesize that PTSD patients might exhibit enhanced, rather than reduced cortisol suppression to DEX. Indeed, a hyperresponsiveness to low doses of DEX was observed (indicated by

significantly lower post-DEX cortisol levels), was observed in several studies (Stein et al., 1997; Kellner et al., 1997; Yehuda et al., 2002; Yehuda et al., 2004; Newport et al., 2004).

Reports of elevated CRH and subsequent HPA axis alterations in clinically anxious samples have spurred the investigation of CRH-1 receptor antagonists as novel anxiolytics (refer to Section 2.4 Treatment of PTSD, for more detail).
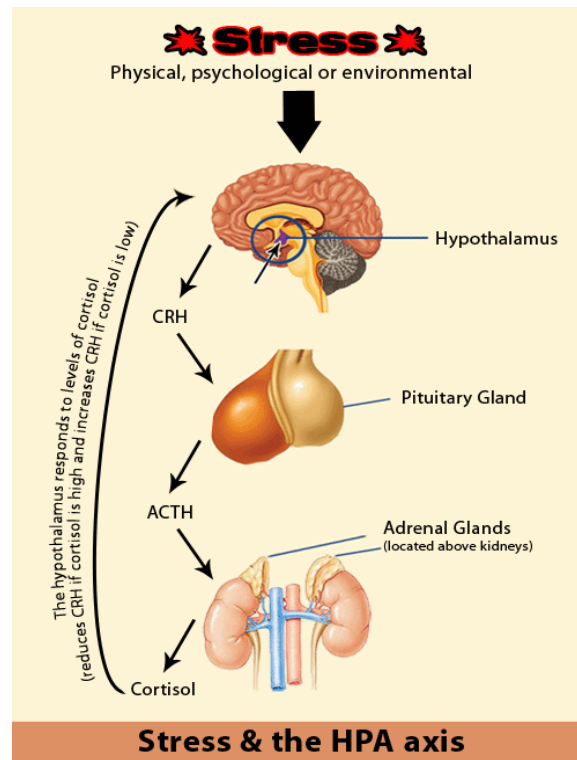
Figure 2.1: Schematic representation of the effect of stress on the HPA axis. CRH is secreted by the hypothalamus in response to stress. CRH is subsequently transported to the pituitary gland, where it stimulates the synthesis and release of ACTH into the bloodstream. ACTH enters the adrenal glands, inducing the release of glucocorticoids (GCs) (such as cortisol or corticosterone in rodents) by the adrenal cortex. This process creates a negative feedback loop whereby the hypothalamus responds to the amount of cortisol it detects and either reduces or increases CRH production (Total Body Psychology website: http://total-body-psychology.com.au/stress-response-hpa-axis/) (copyright granted). ACTH - adrenocorticotropin hormone, CRH - corticotropin-releasing hormone, GCs - glucocorticoid

2.1.2   Neurobiology of PTSD

Processes of fear extinction and retention have been postulated to be deficient in PTSD (Bremner et al., 1996) (refer to Section 2.1.2.3 for more detail regarding fear conditioning and extinction). A network of dysfunctional brain regions, including the hippocampus, amygdala and sub-regions of the medial prefrontal cortex (mPFC) (including ventromedial prefrontal cortex (vmPFC) and dorsal anterior cingulate cortex (dACC)) have been found to contribute to fear extinction and retention abnormalities in PTSD (Fredrikson et al., 1976; Quirk and Mueller 2008). During extinction learning, conditioned fear responses gradually diminish, whilst during extinction recall, the learned extinction memory is retrieved and expressed after a

delay (Quirk et al., 2000). One fear extinction study using a rat model found that extinction recall lasted up to 6 days (Quirk 2002). Behavioural treatment of PTSD (viz. exposure therapy), relies on extinction-based mechanisms (Rothbaum and Foa 2002, Rothbaum and Davis 2003). Thus, a comprehensive understanding of these processes is important to better understand and treat PTSD.

2.1.2.1 Amygdala and hippocampus

Several neurobiological systems are hypothesized to be involved in the aetiology and maintenance of fear conditioning associated with PTSD. These systems include the HPA axis, the locus coeruleus (LC)-noradrenergic (LC-NA) system and connections between the limbic system and frontal cortex. The neural and endocrine structures of the HPA axis coordinate the hormonal response to stress and activate the LC-NA system (Claes, 2004). This system has been implicated in the over-consolidation of fear memories in the aftermath of traumatic exposure (O'Carroll et al., 1999; Southwick et al., 1999; Southwick et al., 2002). The amygdala, located within the temporal lobe, is part of the limbic system. It plays a major role in the detection of threats and in the induction of conditioned and unconditioned fear responses, such as behavioural responses and HPA axis activation. The amygdala consists of several nuclei, including the central nucleus of the amygdala (CeA) and the basolateral nucleus (BLA) which functions in different ways during fear conditioning (Davis 1992).

The fear response is mostly regulated by the CeA. The fear response controls the release of cortisol through the paraventricular nucleus of the hypothalamus, regulates the increase in startle response via the pons in the midbrain, and modulates the autonomic nervous system through the lateral hypothalamus (Davis 1992). Studies in animal models have revealed that lesions in the CeA abolish fear-conditioned responses, such as freezing (LeDoux 1992) and fear-potentiated startle (Davis et al., 1982). The CeA, which receives inputs from BLA projections, is the main locus for associations between the CS and US that ultimately results in fear acquisition (Fanselow and LeDoux 1999). Amygdala activity is regulated in a 'top-down' fashion by the mPFC (including orbitofrontal cortex, subgenual anterior cingulate cortex and hippocampus). The CeA, through an inhibitory or excitatory manner, can either abolish or induce conditioned fear responses (Ehrlich et al., 2009; Milad and Quirk, 2002; Vidal-Gonzalez et al., 2006; Quirk et al., 2006; Peters et al., 2009). This mode of regulation allows appropriate information (such as context, explicit memory representations, and conscious self-regulation) to impact the response to threats. Fear behaviour and inhibition is controlled by complex inhibitory neural circuitry, dysregulation of these circuits are typical in pathological states that are marked by amygdala dysfunction (Jovanovic and Ressler 2010) (Fig. 2.2).

A.

B.
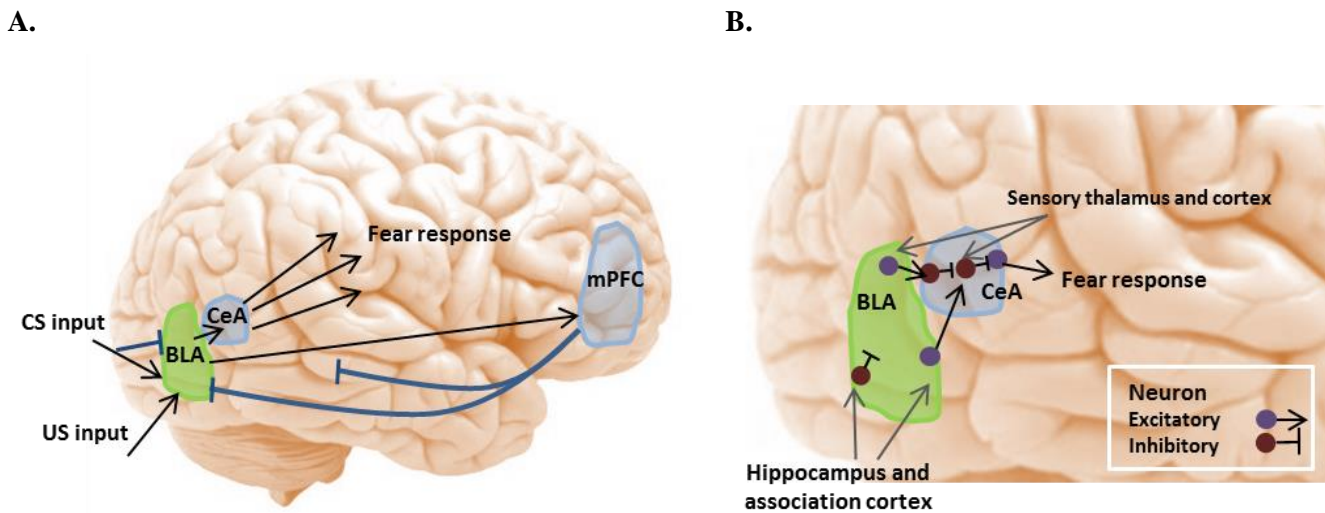
Figure 2.2: Inhibitory control of the amygdala in fear regulation. (A) A schematic diagram of the interaction of the BLA and CeA of the amygdala and the modulatory region, the mPFC. The BLA compares inputs from the CS and US and regulates CeA activation of the fear and stress circuitry. This ultimately leads to inhibition or activation of the fear response. (B) A schematic diagram that illustrates the role of the inhibitory neural circuitry that modulates the fear response at a cellular level. Sensory and associative inputs from the hippocampus and cortex project directly and indirectly to the CeA. Within the CeA "on" and "off" inhibitory circuits are postulated to differentially modulate fear output and fear extinction. In addition, direct projections from the infralimbic region of the mPFC activate inhibitory neurons in the intercalated region between the CeA and BLA, which inhibits the fear output of the CeA in a top-down manner (Adapted from Jovanovic and Ressler 2010).

The hippocampus has been found to be region-specific with regard to its function, the major distinction being between the dorsal hippocampus (DH) (posterior hippocampus in humans) and ventral hippocampus (VH) (anterior hippocampus in humans) (Greicius et al., 2003). For example, the dorsal and ventral regions of the hippocampus have been proposed to play different roles in the processing of emotional behaviour (Bannerman et al., 2004; McHugh et al., 2004; Zhu et al., 2006; Oomen et al., 2010; Korosi et al., 2012). Notably, Bonne et al. (2008) indicated a significant reduction in posterior hippocampal volume in PTSD patients compared to controls, with no significant differences in the size of the anterior hippocampus (Bonne et al. 2008). The DH has exhibited a distinct role in the reconsolidation process of fear memories, indicating that generalization of fear inhibition could be achieved through disruption of memory reconsolidation processes in the DH (Yang et al., 2011).

A related part of the extended amygdala is the bed nucleus of the stria terminalis. This region seems to be associated with nonspecific fear, such as anxiety, which is unrelated to a CS used during fear conditioning. The anxiogenic effects of bright lights and CRH infusions in rodents are eliminated by abrasions to the bed nucleus of the stria terminalis (Davis et al., 1997). This region is postulated to be involved in general, nonspecific anxiety and depression symptoms, whereas the CeA is more involved in cue-specific stress responses, fear and panic. Positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) studies in healthy individuals have shown amygdala activation in response to fearful stimuli (Whalen

et al., 2001; Pine et al., 2001; LaBar et al., 1998; Knight 2005). These findings indicate the extensive role of the amygdala in regulating the fear response in humans as well as in animals.

2.1.2.2 Prefrontal cortex and insula

The prefrontal cortex (PFC) is believed to be important in behavioural inhibition. Animal studies show that lesions of the mPFC, inflicted prior to fear conditioning, delay extinction to a tone (Morgan et al., 1993). Furthermore, studies demonstrate that PFC neurons may elicit inhibitory action on the amygdala (Phelps et al., 2004, Grace and Rosenkranz 2002). Similar to the amygdala, the PFC consists of many subregions, such as the mPFC, anterior cingulate cortex (ACC) and orbitofrontal prefrontal cortex (OFC). The ACC contains ventromedial and dorsolateral components, which may have different functions during expression and inhibition of fear. A study of the rodent neuroanatomical equivalent to the mPFC, the infralimbic prefrontal cortex (ILPFC), showed enhanced activity following learning of an extinguished CS (Milad and Quirk 2002). In addition, fear responses were inhibited when ILPFC activity was enhanced (Milad and Quirk 2002). The authors proposed that during extinction consolidation, a neural circuit running from the BLA to the PFC and back to the amygdala's inhibitory neurons, may be enhanced. This enhancement occurs in such a fashion that during re-experiencing of the extinguished CS, the ILPFC elicits a feed-forward inhibitory projection that competes with the fear pathway elicited by the BLA-to-CeA nucleus projection (Fig. 2.2) (Peters et al., 2009; Vidal-Gonzalez et al., 2006).

Although the majority of research on anxiety disorders has focused on the amygdala and its connections to the PFC, an emerging region of interest is the insula. This region is mainly known for its role in interoception (the sense of the physiological condition of the body) (Critchley et al., 2004). Paulus and Stein (2006) proposed that information from the amygdala, orbitofrontal cortex and nucleus accumbens is integrated by the anterior insula which generates an interoceptive prediction signal (difference between the current body state and a predicted future state). This prediction signal may be increased in anxiety-prone individuals, due to an exaggerated aversive expectation that drives responses such as cognitive and behavioural avoidance. Indeed, brain imaging studies have revealed altered insular function in OCD, PTSD, specific phobia (Rauch et al., 1997; Wright et al., 2003), SAD (Lorberbaum et al., 2004), GAD (Hoehn-Saric et al., 2004), panic disorder (Malizia et al., 1998) patients and individuals with elevated trait anxiety (Paulus and Stein 2006). These studies underscore the importance of detecting and investigating nuances in the neurocircuitry to better understand anxiety disorders.

2.1.2.3 Fear conditioning and extinction

Bremner et al. (2005) were the first to examine fear conditioning and extinction learning in PTSD using positron emission tomography (PET). The study, performed in women with childhood sexual-abuse-related PTSD indicated a reduction in vmPFC activity and an increase in amygdala activity in PTSD cases compared

to controls (Bremner et al., 2005). These results were later confirmed in a study by Milad et al. (2009) whose data suggested that the deficient extinction retention in PTSD might be attributed to dysfunctional responses in brain regions implicated in the recall of fear extinction. To this end, they found reduced hippocampal and bilateral vmPFC activation and increased dACC activation during extinction recall in PTSD cases vs. trauma-exposed non-PTSD controls (TENC). They described a positive correlation between the amount of extinction retention and activation in both the vmPFC and hippocampus and a trend towards a significant negative correlation with activation in dACC (Milad et al., 2009). Their data suggest that dysfunctional activation of particular brain regions in the PTSD group (i.e., increased amygdala activity and decreased vmPFC activity compared with the TENC group) during extinction learning might contribute to inadequate consolidation of extinction memory in PTSD patients. Furthermore, they proposed that a failure to activate vmPFC and hippocampus during recall could contribute to insufficient extinction memory expression in PTSD (Milad et al., 2009).

The molecular mechanism of fear conditioning involves learning, primarily mediated by synaptic plasticity in the amygdala. Associative fear conditioning and extinction of conditioned fear are both learning processes; during extinction of conditioned fear, a CS becomes less associated with the US (refer to Sections 2.1 PTSD and 2.1.2.1 Amygdala and hippocampus). This learning process is dependent on activation of glutamate NMDAR. Extinction of fear memories become blocked by the systemic administration of NMDAR antagonists (Baker and Azorlosa 1996; Cox and Westbrook 1994) or direct infusion of NMDAR antagonists into the BLA (Falls et al., 1992; Lee and Kim 1998). This impairment in extinction training occurs when NMDAR antagonists are administered prior to (Falls et al., 1992; Lee and Kim 1998) or after extinction training, which suggests that NMDARs are involved in the consolidation of extinction memories (Santini et al., 2001). Furthermore, scientists found that 24-hour recall of extinction was impaired following infusion of an NMDAR antagonist into the vmPFC prior to, or immediately after, extinction training (Burgos-Robles et al., 2007). They observed that the NMDAR antagonist selectively reduced burst firing in the neurons of the vmPFC suggesting that extinction memory is stabilized and successful extinction recall is facilitated through NMDAR-dependent bursting in the vmPFC, which subsequently initiates calcium-dependent molecular cascades (Burgos-Robles et al., 2007) (Refer to Section 2.4.1 *N*-methyl-D-aspartate receptors).

Morris and colleagues established a link between NMDAR activity, hippocampal long-term potentiation (LTP) and learning and memory when they developed the Morris water maze (MWM) task for rodents (Morris 1981). The device consists of a large round pool with a small escape platform. The pool is filled with opaque water which obscures the platform. Visual cues (for example coloured shapes) are placed around the pool in plain sight of the animal. During the basic procedure the animal is placed in the pool (at different starting positions) and they have several training trials where they learn to find the platform and escape from the pool (Morris 1981) (more sophisticated variations have also been developed from the basic model) (D'Hooge and De Deyn 2001). During the trials various parameters are recorded, including the time taken to

reach the platform (latency), time spent in each quadrant of the pool and total distance travelled (Morris 1981). With subsequent trials (with the platform in the same position) the animals should be able to locate the platform more rapidly. This performance improvement occurs as a result of learning and memorizing where the hidden platform is located relative to the visual cues. This task evaluates spatial learning and memory in the model organism and is particularly sensitive to the effects of hippocampal lesions in rats. Findings by Morris and colleagues (using rats with hippocampal lesions) suggested that hippocampal NMDAR activity and NMDAR-dependent plasticity are crucial for spatial learning (Morris 1981; Morris et al., 1982). The subregional specificity of NMDAR function was investigated by Lee and Kesner (2002) who found that the Cornu Ammonis region 3 (CA3) hippocampal subfield NMDARs are required in situations requiring reorganization of spatial representation, whereas CA1 and/or the dentate gyrus NMDARs are more involved in memory acquisition that requires retrieval after a delay period (exceeding a short-term range) (Lee and Kesner 2002). The hippocampus is thus an important brain region in PTSD due to high expression levels of NMDARs in this region.

In addition to NMDARs, there are numerous other factors that play a role in the plasticity of learning. Research points to the involvement of voltage-gated calcium channels in the mediation of calcium-dependent synaptic plasticity, a process that is involved in fear extinction (Cain et al., 2002; Cain et al., 2005). Brain-derived neurotrophic factor (BDNF) and its receptor, tropomyosin receptor kinase B (TrkB) (Chhatwal et al., 2006; Rattiner et al., 2004) have also been implicated in plasticity underlying fear and extinction learning. Additionally, regulation of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) was found to be differentially altered during fear acquisition compared to extinction (Harris and Westbrook 1998). Decreased levels of gephyrin (a scaffolding protein that facilitates insertion of GABA into the surface membrane) and decreased surface expression of $GABA_A$ receptors have been found in the BLA after fear acquisition and increased levels of gephyrin and $GABA_A$ receptors have also been observed after extinction learning (Chhatwal et al., 2005; Heldt and Ressler 2007). These results correlate with heightened amygdala excitability during fear learning and amygdala inhibition following extinction. Furthermore, the results indicate that the gephyrin protein is involved in both fear acquisition and extinction and suggest that variation in BLA gephyrin and $GABA_A$ receptor expression contributes to experience-dependent plasticity underlying both types of learning (fear acquisition and extinction). Finally, these results demonstrate that during the consolidation phase of BLA-dependent learning, dynamic alterations of GABAergic synapses occur which may interact with previously described changes in glutamatergic transmission to initiate and stabilize memory formation (Chhatwal et al., 2005).

The expression of genes involved in neural plasticity (such as glutamate receptors and *BDNF*), stress responsiveness (such as glucocorticoid receptors and corticotropin receptor) and neural inhibition (such as GABA receptors), may be associated with extinction learning or impaired fear inhibition. In-depth investigations into these genes are important in PTSD research as the disorder seems to be associated with abnormal fear acquisition or inhibition, either as vulnerability factors prior to trauma exposure or as a result

of trauma-related fear conditioning (Jovanovic and Ressler 2010) (refer to Section 2.2.4 Gene expression analyses in PTSD).

All of the abovementioned neural systems interact during the development and maintenance of fear conditioning, and have been investigated as target regions where candidate genes may be involved in the aetiology of PTSD (Skelton et al., 2012) (refer to Section 2.2.2 Candidate genes in PTSD).

2.1.3   Neuropeptides and neurotransmitters

Neuropeptides are short-chain amino acids which function as neurotransmitters. They are often located in brain regions that mediate emotional behaviours and stress responses, and are subsequently involved in numerous brain functions, including behaviour, learning and memory (Hökfelt et al., 2000). Neuropeptides and peptide hormones are related (both are small protein-like signalling molecules). Occasionally peptides that function as hormones in the periphery also function as neuropeptides with neuronal functions. The difference between peptide hormones and neuropeptides lies in the cell type that release and respond to these molecules. Peptide hormones are secreted by neuroendocrine cells, transported through the blood and elicit a response in distant tissues. Conversely, neuropeptides are secreted by neuronal cells (primarily neurons and sometimes glia) and signal to neighbouring cells (mostly neurons) (Migaud et al., 1995). However, neuropeptides and peptide hormones are synthesized by the same enzymes, including carboxypeptidases and prohormone convertases that cut the peptide precursors at specific sites to generate bioactive peptides (Steiner, 1998). There is a growing list of neuropeptides implicated in stress-related functions. The most extensively studied neuropeptides include tachykinins (substance P (SP) and neurokinin A), vasopressin, CRH and neuropeptide Y (NPY). These molecules and their respective targets have gained popularity as potential novel therapeutic targets to treat stress-related disorders (Varty et al., 2002; Heinrichs et al., 2002; Griebel et al., 2002; Sajdyk et al., 2002), and are discussed in more detail below.

2.1.3.1 Substance P

Substance P (SP) has been one of the most comprehensively studied neuropeptides since its discovery in the 1930s. It elicits its biological actions through G-protein-coupled tachykinin receptors (Neurokinin 1 [NK1]). Levels of SP have been found to be increased in the amygdala of guinea pigs following maternal separation (an early life stressor) (Kramer et al., 1998). In addition, augmented levels of SP were detected at baseline in the cerebrospinal fluid (CSF) of PTSD patients, and increased SP levels accompanied symptom provocation in PTSD patients who viewed a trauma-related video (Geracioti et al., 2006). NK1 receptor antagonists were subsequently investigated as a treatment for PTSD. NK1 receptor antagonists produced anxiolytic responses in various preclinical tests, including elevated plus-maze, transient maternal separation and the social interaction test (Kramer et al., 1998; File, 2000; Varty et al., 2002). A phase II trial of NK1 antagonist, GR205171, has been conducted in chronic PTSD patients (Mathew et al., 2011). This investigational

compound was, however, not significantly superior to placebo for treatment of chronic PTSD (Mathew et al., 2011).

2.1.3.2 Vasopressin

Vasopressin is a nonapeptide synthesized in the paraventricular nucleus (PVN) and supraoptic nucleus and is known for its role in fluid metabolism and regulation of the HPA axis. As in the case of CRH, vasopressin release is induced by stress; it is released from the hypothalamus median eminence into the pituitary portal circulation where it enhances the effects of CRF on ACTH release (Aguilera, et al., 2000). Once released from these neurons, the effects of vasopressin are exerted via a dense localization of vasopressin receptors mainly expressed in limbic areas and the hypothalamus (Lolait et al., 1995). This distribution pattern suggests that vasopressin might have a regulatory effect on limbic function and stress responses (Holmes et al., 2003).

Vasopressin release predicts anxious reactions to stress provocation in healthy individuals (Abelson et al., 2001). Increased vasopressin-mediated control of the HPA axis may therefore contribute to HPA axis dysregulation associated with depression and anxiety disorders (Holsboer and Barden 1996; Dinan et al., 1999). Vasopressin receptor antagonists have consequently been investigated as a treatment option for these disorders. Indeed, the Vasopressin V1b receptor (V1bR) antagonist (SSR149415) has been found to exert noticeable anxiolytic and antidepressant effects in rodent models (Griebel et al., 2002; Serradeil-Le Gal et al., 2002). The antidepressant activity of this compound is comparable to that of reference antidepressant (imipramine); however, the overall anxiety tests profile appears to differ from classical anxiolytics. For example, benzodiazepines are active in a broad range of anxiety models, whereas SSR149415 shows definite effects only in the more stressful test situations (Stemmelin et al., 2005).

2.1.3.3 Corticotropin-releasing factor

Corticotropin-releasing factor (CRF) consists of 41 amino acids and initiates the HPA axis stress response. It has thus been extensively investigated in anxiety disorders and depression. The CRF system in the mammalian brain comprises of CRF-related peptides (urocortin 1, urocortin 2, and urocortin 3) and G-protein-coupled CRF receptor subtypes (CRF1 and CRF2). Neurons containing CRF and CRF receptors are located in brain regions involved in stress responses, including the amygdala, LC, lateral septum and brainstem raphe nuclei as well as the major projections from the PVN to the pituitary corticotropes (Steckler and Holsboer 1999). CRF infusion into rodent brains or constitutive transgenic overexpression of CRF in mice, reproduce certain neuroendocrine and behavioural consequences of stress exposure, such as HPA dysfunction and increased anxiety-like behaviour (Van Gaalen et al., 2002a; Van Gaalen et al., 2002b). PTSD is associated with increased CRF levels in CSF (Bremner et al., 1997; Baker et al., 1999; Sautter et al.,

2003) (as discussed in Section 2.1.1 HPA axis in PTSD), and this has prompted investigation into CRF-1 receptor antagonists as novel anxiolytics. These compounds seem to selectively block CRF- and stress-induced ACTH release without disruption of basal ACTH release (Heinrichs et al., 2002; Gutman et al., 2011; Gully et al., 2002). Preclinical studies suggest that CRF-1 antagonists may reverse stress-related behaviours (Lelas et al., 2004; Timpl et al., 2008; Habib et al., 2000; Heinrichs et al., 2002; Gutman et al., 2011) and similar findings are evident in preliminary data of small clinical trials (Zobel et al., 2001).

2.1.3.4 Neuropeptide Y

Neuropeptide Y (NPY), the most abundant peptide in mammalian brains, constitutes 36-amino-acid polypeptides (Wahlestedt and Reis, 1993) and functions as a sympathetic co-transmitter. NPY is abundantly expressed throughout the brain, including the hypothalamus, LC, hippocampus, amygdala, nucleus accumbens and neocortex. Central NPY is colocalized with GABA, noradrenaline, agouti-related protein and somatostatin (Kask et al., 2002). Heterogeneous G-protein-coupled receptors mediate the actions of NPY with the receptor subtypes Y1, Y2 and Y,5 mediating central nervous system (CNS) effects. NPY is co-released with epinephrine from the cardiac sympathetic nerves and is present in measurable amounts in coronary sinus venous blood (Esler et al., 2004). This is of particular importance in anxiety disorders, where sympathetic hyperactivity occurs during symptoms such as panic attacks (Esler et al., 2004).

NPY may also be involved in the consolidation of fear memories. NPY infusion into the amygdala results in impaired memory retention in a foot-shock avoidance paradigm in rodents (Flood et al., 1989). In combat veterans diagnosed with PTSD, reduced levels of baseline NPY were detected compared to healthy, non-traumatized controls (Rasmusson et al., 2000). In another study, reduced baseline plasma NPY levels were found to be associated with repeated exposure to traumatic stress, rather than the presence of PTSD or PTSD-type symptoms (Morgan et al., 2003). In numerous preclinical models, NPY has been noted to have anxiolytic effects (Heilig et al., 2004). Amygdalar *NPY* mRNA levels were upregulated in response to chronic stress, suggesting involvement in the adaptive responses to stress exposure (Thorsell et al., 1999). To this end, elevated levels of plasma NPY were found in combat-exposed veterans without current PTSD compared to noncombat-exposed veterans (Yehuda et al., 2006). Recovery from previous PTSD symptoms was correlated with NPY elevation (Yehuda et al., 2006).

2.1.3.5 Serotonin

Neurotransmitters are endogenous chemical compounds which transmit signals from neurons to their target cells across synapses, and are thus involved in complex neuronal communication (Sugimori et al., 1994; Lee et al., 2005). Serotonin is one of the neurotransmitters involved in the pathophysiology of PTSD. Graeff et al. (1996) have proposed a "dual serotonin fear hypothesis" based on animal behavioural data. They postulate that in the amygdala, serotonin may augment conditioned fear, whilst in the dorsal periaqueductal gray (PAG), it may inhibit innate fear. The ascending serotonergic pathway, which originates in the dorsal

raphe nucleus and stimulates the amygdala and frontal cortex, facilitates conditioned fear, while instinctive fight-or-flight reactions in response to imminent danger are inhibited by the dorsal raphe-nucleus-periventricular pathway. Finally, the pathway that connects the median raphe nucleus to the dorsal hippocampus facilitates resistance to chronic, unavoidable stress (Graeff et al., 1996). In the region of the LC, serotonin may exert an inhibitory effect on norepinephrine (NE) neurons. Moreover, NE terminals (from the LC) and serotonin terminals (from the dorsal raphe) converge on the amygdala to regulate fear responses. Disruptions in the serotonergic circuits play a role in the aetiology of PTSD (Davis et al., 1997) especially since this system is continuously challenged by life stressors (Stahl, 2005).

2.1.3.6 Dopamine

Dopamine is another neurotransmitter that has been well studied in PTSD. Animal studies have found that dopaminergic innervation of the amygdala, BLA, mPFC and other limbic regions are highly responsive and susceptible to stress (Goldstein et al., 1996; Inglis and Moghaddam, 1999). Administration of dopamine D1 receptor agonists in rats has resulted in an enhanced acoustic startle response, one of the symptoms of PTSD in animal models (Meloni and Davis, 1999). On the other hand, investigation of the D2 receptor revealed that intraperitoneal injection of the dopamine D2 receptor agonist, quinpirole, and the D2 antagonist, sulpiride, resulted in a significant dose-dependent reduction of contextual conditioned freezing. A reduction in the conditioned freezing response was also evident following quinpirole administration into the ventral tegmental area (VTA) and sulpiride injections in the BLA. The data show that the expression of contextual conditioned freezing is influenced by dopamine D2-like receptors. Dopaminergic mechanisms in the mesolimbic circuit should be investigated as potential pharmacological targets for the treatment of fear-related disorders, especially PTSD (de Souza Caetano et al., 2013).

In human studies, a relationship between urinary excreted dopamine and plasma dopamine and severity of PTSD symptoms has also been observed (Yehuda et al., 1992; Hamner and Diamond, 1993). A recent study investigated the levels of the dopamine metabolite, homovanillic acid (HVA), in CSF of individuals with war-related chronic PTSD in response to video provocation (either a trauma-related or neutral video) (Geracioti et al., 2013). A significant increase in anxiety and a drop in mood and blood pressure were evident in the CSF sample taken during the traumatic video screening relative to the neutral video. This was accompanied by significantly reduced concentrations of CSF HVA. The authors concluded that an acute decrease in the concentrations of HVA in the CNS is associated with laboratory-induced symptoms in chronic PTSD patients. Their data suggests that elevation of dopaminergic neurotransmission should be investigated as a potential therapy for PTSD (Geracioti et al., 2013).

Neurotransmitters, neuropeptides and their receptors play a major role in maintaining CNS homeostasis. Significant deviations in the levels of neurotransmitters and neuropeptides in certain brain regions have been

implicated in PTSD aetiology. What then is the underlying cause of these alterations? Assessment of causation leads one to genetic investigations of genes that transcribe these neurotransmitters, neuropeptides and their receptors.

2.2 Genetics of PTSD

Not everybody who experiences a traumatic event will go on to develop PTSD (Monroe et al., 1991; Costello et al., 2002). Thus, traumatic exposure alone cannot account for the development of the disorder. Individuals with an existing genetic vulnerability may have a higher risk of PTSD development, following a traumatic experience and twin and family studies provide evidence for this.

2.2.1   Twin and family studies

Twin and family studies offer the opportunity to delineate the contribution of genetics to the susceptibility of complex disorders, such as PTSD, and although the number of twin and family studies in PTSD is small in relation to other psychiatric disorders, results from studies that have been conducted point towards a genetic aetiology of PTSD. One family study found that as adults, children of Holocaust survivors who suffered from PTSD possessed a higher risk of developing PTSD after experiencing trauma compared to children of Holocaust survivors without PTSD (Yehuda et al., 2001). Furthermore, children of Cambodian refugees were found to be five-fold more likely to develop PTSD when both parents suffered from PTSD compared to refugee children whose parents did not have PTSD (Sack et al., 1995). Similarly, the parents of children who suffered from PTSD following serious physical injury were more susceptible to develop PTSD themselves (Hall et al., 2006). However, since families do not only share genetic material but also similar environments, family studies cannot discriminate whether a disorder that runs in families is due to common genetic or environmental factors. Therefore, twin studies have been conducted to disentangle the role of environmental and genetic influences in PTSD aetiology.

Heritability refers to the amount of variance in a characteristic or disorder that can be explained by genetic factors. Twin studies can be used to calculate the heritability of a disorder. The concordance rate of identical or monozygotic (MZ) twin pairs is compared with that of fraternal or dizygotic (DZ) twin pairs. If MZ twins have a significantly higher concordance rate for a characteristic or disorder compared to DZ twins, then this characteristic is genetically influenced (Koenen et al., 2007).

True et al. (1993) investigated PTSD in a large sample of MZ and DZ US Vietnam War Veteran twins. They reported estimated heritabilities ranging from 0.13 – 0.34 for various PTSD symptoms (including re-experiencing, avoidance and arousal). While the study by True et al. (1993) only included male combat veterans, Stein et al. (2002) broadened the scope by investigating the heritability of trauma exposure and PTSD symptoms in a sample that included female twin pairs (from the general population) and by measuring a wider range of traumatic events (including robbery, sexual assault, motor vehicle accidents, physical

assault, sudden family death and natural disasters). Heritability estimates for the different PTSD symptoms ranged from 0.28 – 0.38 (Stein et al., 2002).

In a study of PTSD in MZ twin pairs who were discordant for combat exposure, similar deficits in verbal memory were evident in the identical co-twins of combat veterans with PTSD, who had no combat exposure or PTSD themselves. To this effect, both the combat veterans with PTSD and their co-twins possessed significantly smaller hippocampal tissue (Gilbertson et al., 2002). These findings suggest that memory impairments and smaller hippocampal size may represent a pre-existing, genetic vulnerability to PTSD (Gilbertson et al., 2006).

A recent study investigated the relative genetic and environmental contributions to trauma exposure, PTSD and major depressive disorder (MDD) in adult twin-pairs who experienced childhood trauma and control twin pairs who did not (Sartor et al., 2012). Additive genetic effects accounted for 47% of the variance observed in low-risk trauma exposure and 60% of the variance in high-risk trauma exposure, while 46% of the variance in PTSD and 27% of the variance in MDD was attributable to heritable influences. High-risk trauma exposure had a high degree of genetic overlap with both PTSD and MDD, suggesting that most of the heritable influences on high-risk trauma exposure, PTSD, and MDD, can be attributed to the same genetic factors.

Results from twin studies underscore the genetic contribution to PTSD. However, twin studies are unable to indicate which genes confer the risk of developing PTSD. Thus in order to identify candidate genes, molecular genetic studies are required (Koenen et al., 2007).

2.2.2   Candidate genes in PTSD

The majority of human genetic variation is attributed to single nucleotide polymorphisms (SNPs), which occur in at least 1% of the population. The human genome harbours about 3 million SNPs; SNPs are one of the most commonly screened for polymorphisms in case-control candidate gene-association studies of PTSD (Risch and Merikangas, 1996; Koenen et al., 2007). These studies rely on the selection of candidate genes based on the current knowledge regarding the neurobiology of the disorder. In PTSD research, such genes typically include those involved in the HPA regulation, noradrenergic system and limbic–frontal brain systems (especially genes that are involved in fear conditioning). Association studies in PTSD have mainly focussed on the following key candidate genes: *BDNF* (Zhang et al., 2006a, Zhang et al., 2006b; Rasmusson et al., 2002; Hemmings et al., 2013), *NPY* (Lappalainen et al., 2002), the serotonin transporter gene (*SLC6A4*) (Stahl, 2005; Davis et al., 1997; Caspi et al., 2003; Mendlewicz et al., 2004; Murphy et al., 2001; Bennett et al., 2002) dopamine (*DRD2*, *DAT*) (Lawford et al., 2003; Young et al., 2002; Gelernter et al., 1999; Comings et al., 1996; Segman et al., 2002; Hemmings et al., 2013), glucocorticoid (*GR*) (Bachmann et al., 2005), GABA (*GABRB*) (Feusner et al., 2001) and the apolipoprotein systems (*APOE2*). Many of these studies have yielded non-replicated or conflicting results, some of which could be attributed to differences in

19

methodological approaches, different sample populations, different index traumas, variations in confounding factors and, in some cases, small sample sizes that result in insufficient statistical power. In addition, the complex aetiology of PTSD complicates the identification of specific genes that contribute significantly to the disorder (Broekman and Boer 2007).

Genome-wide association studies (GWAS) represent an alternative, more robust and hypothesis-neutral approach that can be followed for case-control studies. In GWAS, SNPs (frequencies of SNPs) across the entire genome of cases are compared to controls (Hirschhorn and Daly, 2005). However, to date, very few GWAS studies have been conducted in anxiety disorders and in PTSD. This could be due to difficulties in methodological design, such as requiring very large sample sizes (Koenen et al., 2007), adequate matching of risk factors in cases and controls (especially with regards to trauma exposure and the type of trauma) and small effect sizes (Skelton et al., 2012). Only two GWAS studies were performed thus far in PTSD. In the study by Logue et al., in 2012 the sample population consisted of trauma-exposed Caucasian (non-Hispanic) military veterans and their intimate partners. Several SNPs showed evidence of an association with PTSD. They identified one SNP that reached genome-wide significance (after correcting for multiple testing); rs8042149 is located in the retinoid-related orphan receptor alpha gene (*RORA*) and was significantly associated with a lifetime diagnosis of PTSD (Logue et al., 2012). Recently, Xie et al. (2013) conducted a GWAS study in a study population consisting of European Americans and African Americans in order to find novel common risk alleles for PTSD. They identified a SNP on chromosome 7p12, rs406001, which exceeded genome-wide significance. Furthermore, a SNP that maps to the first intron of the Tolloid-Like 1 gene (*TLL1*) also showed strong evidence of association; however, it did not reach genome-wide significance. However, further analysis of two SNPs in the first intron of *TLL1*, rs6812849 and rs7691872 in 2000 European Americans, replicated the association findings from the GWAS.

Table 2.1 provides a summary of association studies performed in PTSD, also see the review by Cornelis et al., 2010 for more detail.

Table 2.1: Candidate genes that have been investigated in PTSD (adapted from Cornelis et al., 2010)

Gene	Name	Selected feferences
RD2	Dopamine receptor D2	Dragan and Oniszczenko 2009; Gelernter et al., 1999; Voisey al., 2009; Comings et al., 1991; Hemmings et al., 2013; Bailey et al., 2010
DRD4	Dopamine receptor D4	Dragan and Oniszczenko 2009
SLC6A3 (DAT1)	Dopamine transporter	Segman et al., 2002; Drury et al., 2009; Bailey et al., 2010; Chang et al., 2012; Drury et al., 2013; Valente et al., 2011
SLC6A4	Serotonin transporter	Lee et al., 2005; Kolassa et al., 2010; Kilpatrick et al., 2007; Koenen et al., 2009; Thakur et al., 2009; Xie et al., 2009; Grabe et al., 2009; Mellman et al., 2009; Sayin et al., 2010; Valente et al., 2011; Pietrzak et al., 2013

HTR2A	5-hydroxytryptamine (serotonin) receptor 2A	Lee et al.,2007; Mellman et al., 2009
FKBP5	FK506 binding protein 5	Binder et al., 2008; Xie et al., 2010; Sarapas et al., 2011; Boscarino et al., 2011; 2012; 2013; Mehta et al., 2011; van Zuiden et al., 2012
BDNF	Brain-derived neurotrophic factor	Lee et al., 2006; Zhang et al., 2006; Valente et al., 2011; Hemmings te al., 2013; Zhang et al., 2013; Felmingham et al., 2013; Rakofsky et al., 2012
NPY	Neuropeptide Y	Lappalainen et al., 2002
GCCR (NR3C1)	Glucocorticoid receptor	Bachmann et al., 2005; van Zuiden et al., 2012; Hauer et al., 2011
DBH	Dopamine β-hydroxylase	Mustapic et al., 2007
CNR1	Cannabinoid receptor 1 (brain)	Lu et al., 2008
GABRA2	γ-aminobutyric acid receptor subunit alpha-2	Nelson et al., 2009
COMT	Catechol-O-methyltransferase	Kolassa et al., 2010; Boscarino et al., 2011; 2012; 2013
APOE	Apolipoprotein E	Freeman et al., 2005; Kim et al., 2013
RGS2	Regulator of G-protein signaling 2	Amstadter et al., 2009
CHRNA5	Cholinergic receptor, nicotinic, alpha 5 (neuronal)	Boscarino et al., 2011; 2012; 2013
CRHR1	Corticotropin releasing hormone receptor 1	Boscarino et al., 2012; 2013
RORA	Retinoid-related orphan receptor alpha	Logue et al., 2012

2.2.3   Gene-environment interaction studies

As PTSD by definition requires exposure to a traumatic event and only a subset of individuals develop PTSD after trauma, studies of gene-environment (G x E) interactions might be better suited to elucidate the genetic underpinnings of the disorder. These studies have provided evidence that PTSD is influenced by interactive effects from both environmental and genetic factors (Table 2.2). In one of the first G x E studies to be conducted in PTSD, four polymorphisms in *FKBP5* (rs9296158, rs3800373, rs1360780, and rs9470080) were shown to interact with severity of childhood trauma to predict adult PTSD symptom severity in traumatized African-American subjects (Binder et al., 2008). Moreover, these polymorphisms were found to be functional; individuals with PTSD that carried the risk alleles showed enhanced suppression of cortisol in response to dexamethasone (Binder et al., 2008). More recently Xie et al. (2010) partially replicated these findings in an African-American cohort; they found that one of the *FKBP5* polymorphisms (rs9470080) moderated the risk of PTSD associated with childhood adversity. In individuals with a history of childhood

trauma, the *TT* genotype was found to be associated with the highest risk of developing PTSD, whereas it was found to be associated with the lowest risk for PTSD development in individuals with no history of childhood trauma. When Xie et al. (2010) investigated these G x E interactions in a European-American population, they were not significant, however, interactions between alcohol dependence, *FKBP5* polymorphisms (rs3800373, rs9296158, rs1360780 and rs9470080) and childhood adversity were found to increase the risk for PTSD diagnosis.

Furthermore, another polymorphism in *FKBP5* (rs9296158) was found to interact with childhood environment to predict PTSD severity (Mehta and Binder 2012; Mehta et al., 2011). Individuals with adult PTSD, who experienced childhood trauma and were carriers of the risk allele of rs9296158 (*A* allele), displayed excessive GC negative feedback of HPA secretion (Mehta et al., 2011). *FKBP5* regulates GR activity and subsequently influences HPA axis functioning; it also forms part of the mature GR heterocomplex. Furthermore, *FKBP5* is a co-chaperone protein that interacts with heat shock protein 90 (hsp90), another molecular chaperone (Hubler and Scammell, 2004), and controls sensitivity and nuclear translocation of GRs so that reduced *FKBP5* activity increases GR sensitivity (refer to Table 2.2 for more G x E studies conducted in PTSD) (Binder, 2009). These results suggest that various population characteristics, as well as childhood and adult trauma measurements need to be accounted for in the robust identification of G x E effects in PTSD.

Table 2.2: Published G x E studies in PTSD and PTSD phenotypes (adapted from Mehta and Binder 2012)

Gene	Genetic variants	Trauma Type	Environmental variables	Population	Reference
Serotonin transporter (*SLC6A4*)	*5HTTLPR*	Florida Hurricane	Hurricane and social support	Primarily Caucasian	Kilpatrick et al., 2007
	5HTTLPR	Florida Hurricane	Childhood and adult trauma	African American,	Xie et al., 2010
	5HTTLPR, rs25531	Various traumas	Various traumas	Caucasian	Grabe et al., 2009
	5HTTLPR, rs25531	Shooting	Interaction between *SLC6A4* genotype and shooting exposure	Female, primarily Caucasian	Mercer et al., 2013
	5HTTLPR	Rwandan genocide	War and non-war related events	African	Kolassa et al., 2010
Corticotrophin-releasing hormone receptor (*CHRH1*)	rs110402, rs242924, rs7209436	Various traumas	Country crime rate, unemployment	Primarily African Americans	Binder et al., 2008
FK506 binding protein (*FKBP5*)	rs4713916, rs1360780, rs3800373, rs992105, rs9296158, rs737054, rs1334894, rs9470080	Various traumas	Childhood and adult trauma	Primarily African Americans	Binder et al., 2008
	rs3800373, rs9296158, rs1360780, rs9470080	Child abuse	Childhood maltreatment	African American, European American	Xie et al., 2010
Regulator of G-protein	rs4606	Florida Hurricane	Hurricane, social support	Primarily Caucasian	Amstadter et al., 2009

22

signaling (*2RGS2*)					
Gamma-aminobutyric acid (GABA) A receptor, alpha 2 (*GABRA2*)	rs279836, rs279826, rs279858, rs279871	Various traumas	Childhood trauma	Australian	Nelson et al., 2009

Gene	Genetic variants	Trauma Type	Alternate PTSD phenotypes	Population	Reference
FK506 binding protein (*FKBP5*)	rs3800373, rs1360780	Acute medical injury	Peritraumatic dissociation (Peritraumatic Dissociative Experiences Questionnaire and PTSD Reaction Index)	African American, Caucasian	Koenen et al., 2005
	rs4713916, rs1360780, rs3800373, rs992105, rs9296158, rs737054, rs1334894, rs9470080	Various traumas	GR sensitivity (low dose dexamethasone suppression test)	Primarily African Americans	Binder et al., 2008
GR	N363S, BclI, E22E, R23K, F29L, L112F, D233N, K293K	War	GR sensitivity (low dose dexamethasone suppression test and dermal vasoconstrictor assay)	Vietnamese	Bachmann et al., 2005

Serotonergic neurotransmission is influenced by the serotonin transporter (SERT, transcribed by the *SLC6A4* gene). The duration and magnitude of serotonergic neurotransmission is regulated by SERT, which also serves as an initial target site for antidepressants (Graham and Langer, 1992; Schloss and Williams, 1998). A SERT-linked polymorphic region (SERTPR) has been identified in the SERT promoter. This repeat length polymorphism affects the rate of serotonin uptake; the SERTPR consists of repeats of a 20-23-bp-long GC-rich element. The short (*S*) and long (*L*) alleles (14- and 16-repeats, respectively) was reported to originate from an insertion/deletion in the SERTPR (Nakamura et al., 2000). Basal SERT activity in SERTPR long (*L*) allele carriers has been found to be more than twice that of short (*S*) allele carriers (Heils et al., 1996; Lesch et al., 1996). These allelic variants have been shown to be associated with various anxiety responses to stressful events (Table 2.2) (Glatz et al., 2003; Kilpatrick et al., 2007; Koenen et al., 2009; Kolassa et al., 2010; Lee et al., 2005; Xie et al., 2009 Grabe et al., 2009; Sayin et al., 2010; Thakur et al., 2009 Mellman et al., 2009; Valente et al., 2011). Lee et al. (2005) found that the frequency of the *S*/*S* genotype was significantly higher in the PTSD patients compared to controls (Lee et al., 2005). In a study that investigated post-hurricane PTSD and major depression, the *S*-allele was found to increase the risk of developing post-hurricane PTSD and MDD; however, this was observed only under conditions of high hurricane exposure and low social support. High-risk individuals (high hurricane exposure, *S*-allele with low social support) had a 4.5-fold increased risk of developing PTSD and MDD compared to low-risk individuals (low hurricane exposure *L*-allele) (Kilpatrick et al., 2007). Similar findings were published by Xie et al. (2010), who investigated the SERTPR in European American and African American populations. Individuals who experienced childhood adversity and adult traumatic events and who possessed at least one *S*-allele were

23

significantly more likely to develop lifetime PTSD compared to individuals who only experienced one type of adverse event, and were homozygous for the *L*-allele.

The dopamine receptor gene (*DRD2*) and the dopamine transporter gene (*DAT*) directly affect the dopaminergic system (since it transcribes proteins that release dopamine and that regulate transport of dopamine) and are therefore important PTSD candidate genes. Comings et al. (1996) published the first study on the function of *DRD2* in PTSD. They reported a significant association between the *DRD2 A1* allele and PTSD; this was however not confirmed in a later study by Gelernter et al. (1999). Another study later found an association between the *DRD2 A1* allele and PTSD; it was however only seen in individuals who consumed harmful amounts of alcohol on a daily basis (Young et al., 2002). Results on the relationship between *DRD2* and PTSD are thus conflicting. In order to gain more insight into the link between PTSD and *DRD2*, alcohol consumption must be clearly documented in future association studies. Furthermore, research into the role of *DAT* in relation to PTSD should also be explored (Broekman et al., 2007).

An alternative approach to understanding the genetic underpinnings of complex disorders, such as PTSD, includes gene expression profiling studies. Genes that show differential expression profiles between trauma-exposed individuals who develop PTSD and those who don't, also provide good candidate genes for further investigation (Segman et al., 2005). The following sections will review gene expression studies performed in model organisms as well as in human participants.

2.2.4   Gene expression analyses in PTSD

During the process of memory consolidation, short term memory (STM) is converted into stable long-term memory (LTM) over a period of time (Davis and Squire 1984). Fearful experiences and fear conditioning memories are rapidly acquired and therefore easily consolidated into LTM, this is important for survival since these experiences convey vital information regarding danger in the environment (Schafe et al., 2001). LTM is accompanied by long-lasting alterations of synaptic plasticity in particular brain structures (Garcia 2001), such as the hippocampus (Fonta´n-Lozano et al., 2007). In the context of PTSD, cognitive behavioural therapies (CBTs), one of the treatment strategies for PTSD, are based on regulating the processes of fear extinction (McNally, 2007; Otto et al., 2007). Changes in synaptic plasticity also occur during extinction learning to promote long-term memory of extinction. However, the absence of such alterations in synaptic plasticity (during or after the treatment) may lead to the development of PTSD (Herry and Garcia 2002).

The activation of specific signalling cascades contribute to stable, prolonged synaptic changes through the activation of inducible immediate-early gene (IEG) transcription and *de novo* protein synthesis (Davis and Squire, 1984; Bailey et al., 1999; Guzowski, 2002; Ressler et al., 2002). Spontaneous recovery of conditioned fear is a PTSD-like phenotype in animal models, and refers to impaired fear extinction or relapse. Spontaneous recovery of conditioned fear was found to be associated with prolonged long-term

depression of synaptic transmission in the mPFC and the failure to induce the IEGs *c-Fos* and *zif268* in the BLA of the amygdala and the mPFC. These findings suggest that long-term extinction of conditioned fear can be achieved through activity-dependent changes in gene expression in the mPFC and the amygdala (Herry et al., 2004). Given that gene expression alterations mediate fear conditioning and extinction processes, investigating differential gene expression patterns in these brain regions may broaden our understanding of fear extinction as well as the development and maintenance of PTSD.

2.2.4.1 Gene expression analyses in PTSD animal models

Exposing model organisms to acute or chronic stress can alter the activity of the neuroendocrine and neurotransmitter systems that affect behaviour. Stress induces enhanced fear, anxiety and depression in rodents; therefore they have been used extensively in the study of the pathophysiology of PTSD (Bekris et al. 2005; Bergstrom et al. 2008; D'Aquila et al. 1994; Wood et al. 2008; Goswami et al., 2013). Owing to the high degree of similarity between the human genome and that of other mammals, animal studies often identify potential candidates for human genetic studies. Animal models, therefore, provide researchers with the opportunity to perform brain-specific genetic analyses in order to determine the molecular mechanisms involved in disorders such as PTSD (refer to Table 2.3 for an overview of tests performed in PTSD animal models, also refer to reviews by Sousa et al. (2006) and Goswami et al. (2013) for more detail).

Table 2.3: Tests commonly used in animal models of PTSD (adapted from Sousa et al., 2006)

Tests that measure reflexes and sensory function	Measurement	References
Acoustic startle response (ASR)	The magnitude of the reflexive response (muscle contraction) to a loud auditory stimulus is measured.	Koch (1999); Swerdlow et al. (2000); Serova et al. (2013)
Prepulse inhibition (PPI)	Prepulse inhibition includes presentation of a brief subliminal sound stimulus prior to the reflex-eliciting stimulus. Prepulse stimuli inhibit the startle reflex. PPI is a functional measure of sensorimotor gating which could be impaired in PTSD	Yeomans and Frankland (1995) Baisley et al. (2011); Conti 2012
Tests that measure motor function and co-ordination	**Measurement**	**References**
Motor activity	Measures horizontal and/or vertical movements in a test environment (e.g. open field, home cage).	Crusio (2001); Drai and Golani (2001); Holmes et al. (2002); Prut and Belzung (2003); Imanaka et al. (2006); Qiu et al., 2013
Tests that measure exploratory and emotional behaviour	**Measurement**	**References**
Exploration- and Interaction-based anxiety tests (elevated plus-maze test, light/dark test, open field, social interaction)	Measures approach and avoidance behaviour of potentially dangerous environments/stimuli (e.g. open, brightly lit, elevated areas; conspecifics).	Rodgers and Dalvi (1997);File (2001); Hascoet et al. (2001); Prut and Belzung (2003); File and Seth (2003); Kung et al. (2010); Adamec et al. (2010); Krishnamurthy et al. (2013)
Response-based anxiety tests (fear-potentiated startle, unconditioned freezing)	Measures the potentiation of the reflexive startle or freezing response by anxiogenic stimuli or drugs (e.g. bright light).	Davis (1998); Walker et al. (2003); Missig et al. (2010); Norrholm (2013);
Defense-based anxiety tests (rat/ mouse defense test battery)	Measures flight, fight, freezing, defensive threat, defensive attack and risk assessment in	Blanchard et al. (1997, 2003); Borsini et al. (2002); Griebel et al. (1995)

	response to an unconditioned predator stimulus.	
Conflict-based anxiety tests (vogel conflict test)	Measures water consumption in water-deprived animals that receive a mild electric shock to the tongue after a certain number of licks. Measure of anxiolytic-like activity	Vogel et al. (1971); Heilig et al. (1989); Thorsell et al. (2000); Lotarski et al. (2011)
Non-conflict-based conditioned anxiety tests (fear conditioning)	Measures emotional memories by means of potentiation of startle responses, conditioned eyelid closures or conditioned freezing.	LeDoux (2000); Siegmund and Wotjak (2007); Cordero et al. (2003); Takahashi, et al. (2006)
Tests that measure social behaviour	**Measurement**	**References**
Social interaction test	Animal is confronted with an unknown conspecific (animal of the same species) in a novel environment. Measures latencies, frequencies and duration of social behavior (approach, avoidance, exploration, aggression, submission, sexual behavior).	Huhman et al.,(1992); File and Seth (2003); Lijam et al.(1997); Miczek et al. (2001); Strekalova et al. (2004); Huhman (2006) Nanda et al. (2008)
Maternal behaviour	Measures nest building, suckling, active vs. passive nursing.	Levine (1957); Heim et al. (2001); Meaney (2001); Champagne et al. (2003); Siegmund et al. (2009)
Tests that measure cognitive behaviour	**Measurement**	**References**
Passive avoidance (step-down avoidance, step-through avoidance)	Animals are taught to withhold a response following pairing of a cue/test context with the presentation of a mild electric shock. Memory is assessed by the duration of withholding the response following training.	Izquierdo and Medina (1997); Picciotto and Wickman (1998); Szapiro et al. (2003); Padilla et al. (2010)
Spatial learning (Morris water maze, Barnes maze, T-maze)	Animals are taught to navigate in a maze to obtain positive reinforcement (e.g. food) or to avoid negative reinforcement (e.g. escape from water). Measures choice/escape latencies, response accuracy and selectivity of searching.	D'Hooge and De Deyn (2001); Lipp and Wolfer (1998); Graeff et al. (1998); Silva et al. (1998); Whishaw (1998) ; Gerlai (2001); Reisel et al. (2002); Lutz (2009); Diehl et al. (2012)
Recognition memory	Measures frequency and duration of exploration of novel stimuli (e.g. objects, odors, conspecifics) as opposed to exploration of familiar stimuli of the same modality.	Brown and Aggleton (2001); Kogan et al. (2000); Steckler et al. (1998a, 1998b, 1998c); Wang et al. (2012); Goswami et al. (2012)
Classical (Pavlovian) conditioning (fear conditioning)	Measures duration and frequency of conditioned responses (e.g. freezing, fear-potentiated startle, eyelid closures in fear conditioning tasks).	Kamprath and Wotjak (2004); LeDoux (2000); Maren (2001); Medina et al. (2002); Rudy et al. (2004); Norrholm et al. (2013)

In animal models of PTSD, a variety of stressors (physical, psychosocial or psychogenic) are used to examine the long-term effects of severe trauma. Models that use physical stressors include inescapable foot-shocks (Rudy et al., 2004), single prolonged stress (SPS) (Yamamoto et al., 2009), underwater trauma (Moore et al., 2012), restraint stress (Vyas et al., 2002) and variable stress (Molina et al., 1990; McGuire et al., 2010). The models that use psychosocial stressors include housing instability (Rollins et al. 2012; Park et al., 2001; Zoladz et al., 2008, 2012), social defeat (Huhman et al., 1992, 2006) and social isolation (Matsumoto et al., 1999). Models incorporating early life stressors are used to mimic childhood trauma. These models usually use maternal separation (Young et al., 1973; Zhang et al., 2011) either alone or in conjunction with other stressors such as isolation (Diehl et al., 2012). Maternal separation animal models mimic early life stressors by removing rat of mice pups from the dam (for different periods of time depending on the model) (Plotsky and Meaney, 1993; Mourlon et al., 2010; Reus et al., 2011; Leussis et al., 2012). Models that use psychogenic stressors usually involve threat, but not pain. These models include

predator stress or predator threat (usually an odour) (Blanchard and Blanchard, 1988; Dielenberg and McGregor, 2001).

The main aim of the current study was to determine the molecular mechanisms of DCS in facilitating fear extinction in a PTSD animal model; therefore studies that have investigated gene expression profiles following DCS administration will be discussed (refer to Section 2.4.1.1 D-cycloserine, for more information regarding DCS). Several animal studies have investigated the effects of anxiolytic drugs on gene expression profiles in different brain tissues in order to determine the molecular mechanism whereby these drugs facilitate fear extinction. D-cycloserine (DCS) was originally used as an effective antimycobacterial agent used for the treatment of tuberculosis, due to its ability to inhibit mycobacterial cell wall synthesis (Trias and Benz 1994). However, it is rarely prescribed for tuberculosis treatment nowadays and is mostly used in combination with other therapies due to its adverse side-effects at high dosages (Heifets and Iseman 1991; Yew et al., 1993). These side-effects were attributed to the binding of DCS to the NMDAR1 as a partial agonist at the glycine site (Thompson et al., 1992), it also inhibits enzymes that metabolize and synthesize the neurotransmitter γ-aminobutyric acid (GABA) (Wood et al., 1978). It is due to this binding of DCS to the NMDARs that the administration of DCS has been found to facilitate extinction learning in rats when administered before or immediately after extinction training (Ledgerwood et al., 2003; 2005; Walker et al., 2002; Yang and Lu, 2005).

Yamamoto et al. (2008; 2010) investigated the effects of SPS and DCS on hippocampal NMDAR expression in rats. They found a sustained increase in hippocampal NMDAR expression in response to fear conditioning in SPS rats. The increased NMDAR expression was found to be reduced by oral administration of DCS suggesting that DCS may help to reverse hippocampal plasticity, and thus reverse the NMDA compensatory alterations during fear extinction (Yamamoto et al., 2008). In addition, fear conditioning was found to increase the expression of the glycine transporter 1 (*GLYT-1*) gene in the hippocampus and resulted in a reduced extracellular hippocampal glycine concentration (Yamamoto et al., 2010). However, repeated extinction training significantly reduced the enhancement of *GLYT-1* mRNA levels in SPS rats in response to fear conditioning. These findings suggest that impaired fear extinction in SPS rats may be due to reduced activity of the hippocampal glycinergic system (Yamamoto et al., 2010).

Another study also used the SPS model, but combined it with contextual fear and investigated its effects on gene expression in the rat hippocampus (Iwamoto et al. 2007). As with the studies by Yamamoto et al, (2010) the authors reported elevated levels of *GLYT-1* in the SPS treated rats. In addition, they also found a significant increase in levels of vesicle-associated membrane protein 2 (*VAMP2*) mRNA in rats subjected to SPS relative to sham-treated rats. SPS administration alone did not affect the expression of these two genes, suggesting that the upregulation of *GLYT-1* and *VAMP2* in the hippocampus might be involved in enhanced susceptibility to contextual fear in rats subjected to SPS (Iwamoto et al. 2007). Harada et al. (2008) investigated the molecular changes associated with SPS-induced behavioural changes. Their microarray

analysis revealed that the serotonin 5-HT$_{2C}$ receptor gene was overexpressed in the amygdala of SPS rats; these results were confirmed with qPCR analysis. Administration of a selective 5-HT$_{2C}$ receptor antagonist, FR260010, showed a definite anxiolytic effect in the SPS treated rats. Their results were the first to demonstrate that brain-specific 5-HT$_{2C}$ receptor activation is involved in the behavioural abnormalities seen in the SPS exposure model. Selective 5-HT$_{2C}$ receptor antagonists might provide novel therapeutic options for PTSD treatment (Harada et al., 2008).

The majority of earlier PTSD animal models investigated the exposed group of animals as a homogenous PTSD-like population (Pynoos et al., 1996, Koba et al., 2001, Korte et al., 2003). A study by Cohen et al., in 2003 showed that, like humans, animals exhibit heterogeneous reactions to stress, therefore grouping animals accordingly is imperative (Cohen et al., 2003). Kesner et al. (2009) followed this approach in their study where they used exposure to predator odor as an unconditioned stressor. Furthermore, they monitored individual rat behaviour for up to a month, after which animals were grouped into two groups, namely 'exposed PTSD-like' and 'exposed non-PTSD-like'. They then treated the PTSD-like animals with citalopram. They found elevated expression levels of the Wolframin gene (*WFS1*) in the CA1 hippocampal region and basolateral amygdala of PTSD rats compared to control saline-treated rats. Expression levels of *WFS1* in trauma-exposed animals who did not exhibit PTSD-like phenotypes (non-PTSD-like rats) were identical to that of non-trauma exposed controls. Administration of citalopram to PTSD-like animals for 14 days normalized elevated *WFS1* levels in both brain regions (Kesner et al., 2009).

An important neurotrophin involved in plasticity and neuronal survival is BDNF. *BDNF* mRNA expression levels in the CNS are sensitive to factors such as stress (Hyman et al., 1991). Previous studies have reported reduced hippocampal *BDNF* expression levels in the rat pituitary following single or repeated immobilization stress (Givalois et al., 2001; Hyman et al., 1991). Similar results have also been found following psychological and unconditioned physical stress (Rasmusson et al; 2002), where hippocampal *BDNF* expression was found to be reduced. The adrenal hormone, corticosterone, has been hypothesized to be involved in this stress-associated down-regulation of *BDNF*, as exogenous administration of corticosterone reduced *BDNF* expression (Nibuya et al., 1999). Kozlovsky et al. (2007) also observed *BDNF* downregulation and upregulation of the BDNF receptor, TrkB, in the CA1 sub-region of the hippocampus of animals that exhibited fearful behaviour in response to predator stress.

A recent study investigated the effects of chronic mild stress (CMS) on PFC gene expression profiles by using gene expression profiling and bioinformatics analyses on previously studied mice samples (Lisowski et al., 2013; Błaszczyk et al. 2000; Juszczak et al. 2008a; Juszczak et al. 2008b, 2006; Panocka et al. 2001). They used two mice strains that were bred for high (HA) and low (LA) swim stress-induced analgesia (insensibility to or relief from pain). Animals were grouped into two treatment groups: control animals (control HA mice and control LA mice) and stressed animals that received 5 weeks of CMS (CMS HA mice and CMS LA mice). Increased acoustic startle responses (Błaszczyk et al. 2000; Juszczak et al. 2008a),

longer periods of immobility in the tail suspension test (TST) and forced swim tests (FST) were evident in the CMS HA strain (Juszczak et al. 2008b, 2006; Panocka et al. 2001) compared to the CMS LA strain. CMS HA mice thus represent a maladapted group and CMS LA a well-adapted group. CMS induced differential expression in 96 and 92 genes respectively in HA and LA mice compared to the control groups (Lisowski et al., 2013). In the CMS group they found that vesicular glutamate transporter 1(*VGLUT1*) and solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 7 (*SLC17A7*) was significantly upregulated in HA mice and metabotropic glutamate receptor 5 (*GRM5*) was downregulated in LA mice. These results correlated with those from a previous study that also found *VGLUT1* and *VGLUT2* to be significantly upregulated in the hippocampus of LA mice after CMS (Lisowski et al. 2011). Metabotropic glutamate receptor 5 (GRM5), a subtype of group I GRs, exhibits high expression levels in limbic forebrain regions, and possibly modulates anxiety-related processes. The specific antagonist 2-methyl- 6-(phenylethynyl) pyridine, blocks GRM5, thereby reducing extracellular norepinephrine, which may contribute to its anxiolytic effect (Page et al. 2005). The authors suggest that *GRM5* down-regulation and *VGLUT1* up-regulation in LA CMS mice could be involved in a mechanism of adaptation to stress in stress-resistant individuals (Lisowski et al., 2013).

Lisowski et al. (2013) also detected significant up-regulation of the arginine vasopressin (*AVP*) gene in HA mice. Previous research indicates that repeated stress induces increased AVP expression in paraventricular CRH neurons, which acts to enhance the ACTH-releasing capacity of these cells. AVP acts on wide array of neurons throughout the brain. Acute immobilization also induced significant upregulation of *AVP* and *CRF* mRNA expression in the medial parvocellular subdivisions of the paraventricular nucleus (PVN) (Aubry et al. 1999). Makino et al. (1995) found that PVN *AVP* expression levels were more sensitive to GC negative feedback than *CRH* levels. Koob (2008) also suggested that AVP possibly modulates emotional memory and anxiety. Lisowski et al. (2013) concluded that in CMS, increased expression of *AVP* in the PFC is a mechanism that contributes to the maintenance of HPA response following repeated stress. In addition to the amygdala and hypothalamus, the PFC excitatory actions of increased expression of AVP may also contribute to the behavioural stress response (Lisowski et al., 2013).

The endocannabinoid system (ECS) is not only a crucial mediator of emotional learning but also forms part of the intricate circuitry that regulates stress and anxiety (Ganon-Elazar et al., 2012; Marsicano et al., 2002; Abush and Akirav 2012; Akirav 2011; Hill et al., 2009; Viveros et al., 2005), and is a therapeutic target for the treatment of stress and anxiety-related disorders such as PTSD (Marsicano et al., 2002). Indeed, the potential benefits of nabilone, the synthetic cannabinoid, have been demonstrated in PTSD patients (Fraser 2009). Campos et al. (2013) investigated whether the expression of the cannabinoid receptor 1 (CB1) and the synaptic protein, synaptophysin (SYP), were involved in the lasting behavioural effects induced by predator exposure. They investigated this in brain regions implicated in PTSD symptoms, such as the hippocampus, frontal cortex and amygdaloid complex. Male Wistar rats were exposed to predator stress and were evaluated seven days later on the elevated plus-maze test. The authors measured *CB1* receptor and *SYP* expression levels in the hippocampus, frontal cortex and amygdaloid complex. Long-term anxiogenic effects were

29

evident following single predator exposure. Expression levels of *CB1* were downregulated in the frontal cortex and amygdaloid complex seven days after predator threat exposure, while *SYP* was upregulated in the amygdaloid complex. These results suggest that the persistent anxiogenic effects induced by predator exposure are associated with amygdaloid complex hyperactivation and CB1 receptor modulation in brain regions associated with PTSD symptoms (Campos et al., 2013).

Another important aspect to keep in mind when conducting studies in PTSD is comorbidity with other disorders. Comorbidity of PTSD and diabetes has recently become the focus of numerous studies (Goodwin and Davidson 2005; Trief et al., 2006; Boyko et al., 2010). In the brain, insulin influences the structural responses to stressors; insulin deprivation causes a decrease in dentate gyrus neurons and leads to increased remodelling of CA3 neuron dendrites, this effect is further accelerated by exposure to repeated restraint stress. Stress and hyperglycemia both increase oxidative stress in the brain and this is likely to contribute, over time, to impaired neural function in chronic stress and diabetes Lisowski et al. (2013).

Table 2.4 provides a summary of genes that were found to be differentially expressed in PTSD animal models. The majority of these studies have focussed on specific brain regions implicated in the disorder, whereas peripheral blood mononuclear cells (PBMCs) have mainly been used in human expression studies (Zieker et al., 2007; Segman et al., 2005). This raises the question of whether it is possible to obtain consistent expression results from both brain and blood samples. Gene expression analysis in blood provides a much less invasive method of detecting expression changes associated with PTSD. By using animal models to identify stress-related gene expression profiles in the brain that are also reflected in the blood, provide relevant candidate genes to investigate in human studies of the disease (where mostly PBMCs or other peripheral tissues are available for testing). Van Heerden et al. (2009) compared gene expression profiles in PBMCs and brain tissue from a mouse maternal separation model. They found that in the 50 genes selected from the PBMC microarray, the stress-related transcriptome differences in PBMCs were paralleled by stress-related gene expression changes in the CNS target tissues. Similar investigations are required in different animal models and different brain regions to clearly determine the correlation between gene expression profiles in the CNS and in blood.

Gene expression profiles that were found to be associated with anxiety-like behaviours in animal models of PTSD could be further investigated in human samples to unravel the pathophysiology and aetiology of PTSD and direct novel drug targets.

Table 2.4: Summary of differentially expressed genes in animal studies of PTSD

Gene	Association with PTSD	Species	Reference
NPY	Upregulated in amygdala in response to chronic stress	*Rattus norvegicus*	Thorsell et al., 1999
c-Fos	Failure to induce expression of this IEG in basolateral nucleus of amygdala and mPFC lead to spontaneous recovery of conditioned fear	*Mus musculus*	Herry et al., 2004
zif268	Failure to induce expression of this IEG in basolateral nucleus of amygdala and mPFC lead to spontaneous recovery of conditioned fear	*Mus musculus*	Herry et al., 2004
NMDAR	Increased hippocampal expression in response to SPS fear conditioning	*Rattus norvegicus*	Yamamoto et al. (2008)
GLYT-1	Increased hippocampal expression in response to SPS fear conditioning	*Rattus norvegicus*	Yamamoto et al. (2010)
	Elevated levels in hippocampal in response to SPS combined with contextual fear	*Rattus norvegicus*	Iwamoto et al. (2007)
VAMP2	Significant increase in expression in response to SPS combined with contextual fear	*Rattus norvegicus*	Iwamoto et al. (2007)
WFS1	Elevated expression levels in CA1 and BLA in PTSD-like animals vs. stressed animals with no PTSD-like symptoms exposed to repeated stress. 14 day citalopram treatment corrected this effect.	*Rattus norvegicus*	Kesner et al. (2009)
BDNF	Reduced expression in pituitary gland following acute immobilisation stress	*Rattus norvegicus*	Givalois et al., 2001
	Decreased expression following psychological and unconditioned physical stress	*Rattus norvegicus*	Rasmusson et al; 2002
	Downregulated in CA1 of fearful animals following predator stress	*Rattus norvegicus*	Kozlovsky et al., 2007
TRKB	Upregulated in CA1 of fearful animals following predator stress	*Rattus norvegicus*	Kozlovsky et al., 2007
	Reduced expression in pituitary gland following acute immobilisation stress	*Rattus norvegicus*	Givalois et al., 2001
5-HT$_{2C}$	Overexpressed in amygdala following SPS	*Rattus norvegicus*	Harada et al., 2008
P11 (*S100a10*)	Upregulated in the PFC rats exposed to inescapable tail shock	Zhang et al., 2008	
VGLUT1	Upregulated in PFC of fearful animals in response to CMS	*Mus musculus*	Lisowski et al., 2013
	Upregulated in hippocampus of LA mice after CMS	*Mus musculus*	Lisowski et al. 2011
SLC17A7	Upregulated in PFC of HA animals following CMS	*Mus musculus*	Lisowski et al., 2013
GRM5	Downregulated in PFC of LA animals following CMS	*Mus musculus*	Lisowski et al., 2013
VGLUT2	Upregulated in hippocampus of LA mice after CMS	*Mus musculus*	Lisowski et al. 2011
AVP	Upregulated in PFC of HA mi animals following CMS	*Mus musculus*	Lisowski et al., 2013
	Upregulated in PVN following acute immobilization	*Rattus norvegicus*	Aubry et al. 1999
CRH	Upregulated in PVN following acute immobilization	*Rattus norvegicus*	Aubry et al. 1999
CB1	Downregulated in frontal cortex and amygdaloid complex 7 days after predator threat exposure	*Rattus norvegicus*	Campos et al., 2013
SYP	Upregulated in amygdaloid complex 7 days after predator threat exposure	*Rattus norvegicus*	Campos et al., 2013
PRLR	Upregulated in PFC of HA animals following CMS	*Mus musculus*	Lisowski et al., 2013

Please refer to list of abbreviations for full gene names

2.2.4.2 Gene expression analyses in human studies of PTSD

Distinct differences in gene expression patterns have been observed between PTSD-affected and unaffected individuals in genes involved in HPA axis and immune function and genes that transcribe neural and endocrine proteins (Weaver et al., 2002; Segman et al., 2005; Zieker et al., 2007; Uddin et al., 2010; Yehuda et al., 2009, 2010) (Table 2.5). Identification of differentially expressed genes involved in the aetiology of PTSD could help identify pathways that are involved in the disorder. In addition, factors that contribute to altered gene expression patterns may unravel the complex biological underpinnings of PTSD. Table 2.5 provides a summary of the differentially expressed genes that have been described in human PTSD samples.

Table 2.5: Summary of differentially expressed genes in human studies of PTSD

Genes	Association with PTSD	Reference
FKPB5	Baseline differential expression in PBMCs of trauma survivors was predicative of PTSD outcome in later life	Segman et al. 2005
	Low pre-deployment expression levels in PBMCs predictive of PTSD development in response to deployment	Van Zuiden et al., 2013
GILZ	High pre-deployment expression levels in PBMCs predictive of PTSD development in response to deployment	Van Zuiden et al., 2013
*FKBP5, STAT5B, MHC-II	Reduced expression in PTSD patients vs. trauma exposed controls following the 9/11 attacks	Yehuda et al., 2009
*ROS and immune related genes (TXR, SOD, IL-16, IL-18, EDG1)	Downregulated in whole blood of PTSD patients following the Ramstein air show catastrophe, 1989	Zieker et al., 2007
P11 (S100a10)	Upregulated in the post-mortem PFC of individuals who had PTSD	Zhang et al., 2008
	Reduced expression in PBMC of PTSD patients vs. controls. Expression levels can distinguish PTSD from MDD, BP and SCZ.	Su et al., 2009
NFI-A	Reduced expression in PTSD patients vs. trauma exposed controls following the 9/11 attacks	Sarapas et al., 2011

EDG1 - endothelial differentiation gene 1, *FKBP5* - FK506 binding protein 5, *GILZ* - glucocorticoid-induced leucine zipper, interleukin 16 (IL-16) and interleukin 18 (IL-18), *MHC-II* - major histocompatibility complex class II, NFI-A - nuclear factor 1 A, ROS - reactive oxygen species, *SOD* - superoxide dismutase, *STAT5B* - signal transducer and activator of transcription 5B, *TXR* - thromboxane receptor gene (*Refer to reference for full list of differentially expressed genes

2.3 Epigenetics

GWAS and smaller case-control studies have yielded numerous disease-associated variants; however most of these variants have a minor effect on disease and explain only a small amount of the heritability of complex disorders. The search for the missing heritability has shifted attention to rare variants, copy number variants (CNVs), copy neutral variants and epigenetic modifications.

Although quantifying gene expression provides one with an idea of the biological pathways involved in the disorder, it does not provide knowledge of the mechanisms that contribute to observed alterations in gene expression. The question of whether it is transcription of the gene itself that is altered, or whether differences in expression are due to mRNA turnover in the cell, remains unanswered (Akbarian and Huang, 2009).

The term epigenetics literally means 'outside conventional genetics', and is currently used to describe the study of stable alterations in gene expression that are not brought about by changes in DNA sequence (Bjornsson et al., 2004). These epigenetic changes are heritable and potentially reversible, (Jaenisch and Bird 2003) and provide an additional layer of transcriptional control that may mediate the interaction between genetic predisposition, changes in neural functioning and environmental factors (Bjornsson et al., 2004). Epigenetic modifications may thus explain the interindividual variation and the long-lasting effects of trauma exposure (Yehuda and Bierer 2009). Such epigenetic mechanisms include DNA methylation, posttranscriptionional modifications of histone proteins (acetylation, methylation, phosphorylation, ubiquitination and sumoylation) and non-coding RNA-mediated alterations (such as microRNAs (miRNAs) and small interfering RNAs (siRNAs)) (Yehuda and Bierer 2009).

Epigenetic remodelling has been found to be a crucial component of the neuronal changes that underlie memory and learning processes (Bredy et al., 2007; Chwang et al., 2006; Miller and Sweatt, 2007). It has been postulated that epigenetic factors play an important role in the regulation of activity-dependent neuronal gene expression (Martinowich et al., 2003; Chen et al., 2003). In addition, it has been found that contextual fear conditioning can lead to histone H3 acetylation and H3 phosphorylation changes in the hippocampus (Chwang et al., 2006), implicating the role of histone modifications in memory formation. A study by Miller and Sweatt (2007) found that the transcription of DNA methyltransferases (DNMTs), which catalyse DNA methylation, were upregulated in the rat hippocampus during contextual fear conditioning, and that inhibition of DNMT blocked memory formation. Studies have also found various miRNAs in the CNS and they are believed to be crucial in regulating a variety of neurobiological processes that could pertain to the development of PTSD (Parsons et al., 2008; Hunsberger et al., 2009; Uchida et al. 2010; Meerson et al., 2010; Muiños-Gimeno et al., 2011; Malan-Müller et al., 2013; Barbash et al., 2013; Schmidt et al., 2013).

Epigenetic regulation may be particularly important in shaping the effect that the early environment has on the development of dysfunctional fear extinction. It is thought that epigenetic regulation of gene expression underlying neural plasticity is important in the event of early-life adversity. For example, it has been found that early life experience in the form of maternal care results in stable epigenetic markings that contribute towards the anxiety-like phenotype in adult rats (Weaver et al., 2004; 2005; 2006; Szyf et al., 2005; Murgatroyd et al. 2009). These results have recently been extrapolated to human subjects (McGowan et al., 2009; Franklin et al., 2010).

Epigenetic mechanisms represents an exciting frontier to investigate in psychiatric disorders because of the ability to define specific molecular pathways by which environmental risk factors might directly change the

expression of a gene, thus forming a basis for individual differences in gene function and even vulnerability to a specific disorder. This is likely to be the case in PTSD and might explain the origin of GC-related alterations associated with PTSD and PTSD risk (Yehuda and LeDoux, 2007). Indeed, infants of mothers who developed PTSD as a result of direct exposure to the 9/11 World Trade Centre terrorist attacks were found to have lower salivary cortisol levels, reflecting an increase in number of GR, and thus possibly an alteration in *in utero* glucocorticoid programming, thought to be central in susceptibility to PTSD (Yehuda and Bierer, 2009; Yehuda et al., 2005). Exposure to GCs as a result of maternal stress has been found to reduce the birth weight of offspring, which is associated with, amongst others, the development of behavioural and psychiatric problems (Susser et al., 1996). Unpacking the role of the epigenome in PTSD vulnerability is thus of great importance (Kajantie 2006; Seckl 1994; Chertkow-Deutsher et al., 2010; Stankiewicz et al., 2013).

2.3.1   DNA Methylation

The central role of epigenetics, and specifically DNA methylation, in disease susceptibility and progression has become more apparent in recent years. Epigenetic mechanisms facilitate the response to environmental changes and challenges by regulating gene expression (Jaenisch and Bird 2003). This makes the study of DNA methylation in psychiatric disorders such as PTSD highly salient, as the environment plays such a vital role in disease aetiology. The epigenome is dynamic and can be modulated by numerous factors, including learning and memory (Roth et al., 2010), which is important in the context of PTSD. Indeed, numerous studies have shown the effects of early life events, such as maternal separation, as well as traumas during adulthood, on DNA methylation patterns and subsequent gene expression profiles (Mullen et al., 1996; MacMillan et al., 2001; Weaver et al., 2002; Szyf et al., 2005; Han and Kim, 2008; Murgatroyd et al. 2009; Lee et al., 2010; Yang et al., 2012;). Furthermore, aberrations in adaptive DNA methylation contribute to disease susceptibility when an organism is unable to effectively respond to environmental demands (Ozanne and Constância 2007). Epigenetic mechanisms are also involved in higher-order brain functions (Feng et al., 2007). Dysregulation of methylation is associated with neurodevelopmental (Robertson and Wolffe 2000) and neurodegenerative cognitive diseases (Mattson, 2003), affective disorders (McMahon et al., 1995), addictive behaviours (Robison and Nestler 2011) and altered stress resistance. A thorough understanding of how the environment, methylome and transcriptome interact and influence each other in the context of stress and anxiety is integral to our understanding and treatment of anxiety disorders such as PTSD.

2.3.1.1 DNA Methylation (5mC)

In mammals, DNA methylation occurs mainly at the C-5 position of cytosine residues within CpG dinucleotides (Fig. 2.3). However, it was also shown that sequences other than CpG may also be methylated (as seen in the embryonic stem cell) (Salomon et al., 1970; Grafstrom et al., 1985; Ramsahoye et al., 2000). Globally, about 70% – 80 % of all CpG dinucleotides in the human genome are methylated (Ehrlich 1982); however, numerous temporal and spatial variations are evident, especially during early development (Reik et

al. 2001). DNA methylation regulates developmental genes, such as the pluripotency genes *NANOG* (Homeobox Transcription Factor) and the octamer-binding transcription factor 4 gene (*OCT4*), and is vital for genomic imprinting. During specific stages of mammalian development, CpG methylation undergoes dramatic global changes. New methylation patterns are acquired during early development; primordial germ cells (PGCs) are characterised by genome-wide removal of DNA methylation marks, and, following fertilization, the sperm-derived genome is stripped of DNA methylation (Sasaki and Matsui 2008). DNA methylation patterns are maintained after cell division and are consequently passed from parent to daughter cells (Turner, 2001; Taylor and Jones, 1985; Razin, 1998). Dysregulation of methylation can lead to aberrant transcriptional control, and subsequent alterations in gene expression (Yehuda and LeDoux, 2007). Another essential role of DNA methylation is the repression of retrotransposons and other foreign elements (Sasaki and Matsui 2008).

The process of DNA methylation is strongly dependent on DNMTs, namely DNMT1 and *de novo* DNA methyltransferase enzymes DNMT3A and DNMT3B (essential for DNA methylation patterns in early development). DNMT1 acts as a maintenance DNMT which acts on hemimethylated CpG sites (Turek-Plewa and Jagodzinski 2005), whereas DNMT3A and 3B are responsible for *de novo* DNA methylation by acting on hemimethylated and unmethylated CpG sites (Xie et al., 1999). DNMT1 and DNMT3A are abundant in the mature brain (Feng et al., 2010), whereas DNMT3B and DNMT3L are almost undetectable in mature brain. DNMT3L is an accessory protein; it is catalytically inactive and is required to stimulate the DNA methylation activity of DNMT3A and 3B in embryonic stem cells (Turek-Plewa and Jagodzinski 2005). *De novo* methylation in cells that express DNMT3L, requires a tetrameric complex of two DNMT3A2 and DNMT3L molecules as well as the nucleosome. The nucleosome forms the fundamental units of eukaryotic chromatin and consists of DNA wound around eight histone protein cores (McGhee and Felsenfeld 1980).

Active transcription start sites (TSSs) lack nucleosomes and as a result do not contain this substrate for *de novo* methylation (Ooi et al. 2007). A family of methyl CpG-binding domain (MBD) proteins (including methyl CpG binding protein 2 [MeCP2] and methyl-CpG binding domain 1-4 [MBD1-4]) interpret DNA methylation by interacting with histone deactylases and DNA-methyltransferases to induce gene silencing. In addition, the binding of these proteins to methylated DNA seems to be important in maintaining the DNA methylation status since site-specific demethylation is associated with the dissociation of this complex (specifically MeCP2) (Murgatroyd et al., 2009; Chen et al., 2003; Martinowich et al., 2003). The process of active demethylation requires a mechanism that involves cell division or DNA repair and the removal of the base rather than the methyl group directly from the 5mC unit (Bhutani et al., 2010; Popp et al., 2010). Recent studies indicate the involvement of enzymes such as ten-eleven translocation (TET) methylcytosine dioxygenases, thymine DNA glycosylase (TDG) and activation-induced cytidine deaminase (AID) in active and passive demethylation as well as in gene activation (Bhutani et al., 2010; Inoue and Zhang 2011; Iqbal et al., 2011).

It has been hypothesised that DNA methylation and histone deacetylation may function along a common pathway to induce transcriptional repression (Nan et al., 1998; Jones et al., 1998; Cameron et al., 1999). Proteins that contain MBD recognize methylated DNA and recruit a histone deacetylase (HDAC) complex to remodel the chromatin (Nan et al., 1998; Jones et al., 1998; Zhang et al., 1999). Fuks et al. (2000) showed that the association between DNA methylation and histone deacetylation was more direct than originally anticipated. DNMT1 was shown to be directly associated with histone deacetylase activity *in vivo* (Fuks et al., 2000). Results showed that HDAC1 has the ability to bind DNMT1 and to purify methyltransferase activity from nuclear extracts. Furthermore, a transcriptional repression domain in DNMT1 identified which functions partly by recruiting histone deacetylase activity (Fuks et al., 2000). The authors suggested that DNMT1 -mediated DNA methylation may generate, or depend on, a transformed chromatin state through histone deacetylase activity.
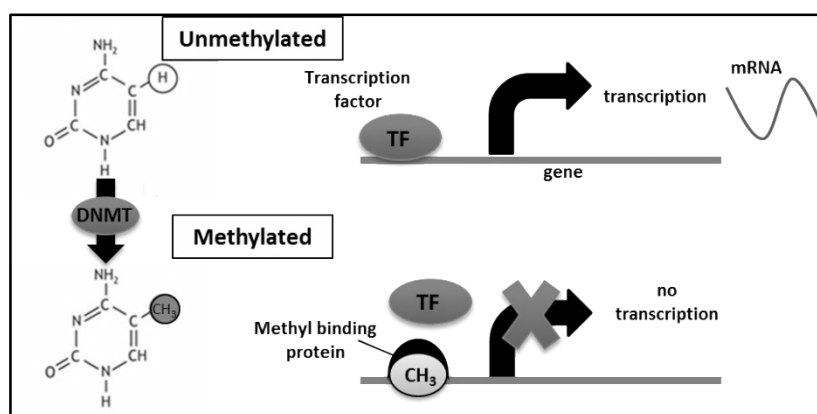
Figure 2.3: Graphical representation of unmethylated and methylated cytosine residues and their respective effects on mRNA transcription. The process of methylation, whereby a methyl group (CH_3) is added to the C-5 position of cytosine residues within CpG dinucleotides, is strongly dependent on the DNA methyltransferases (DNMT) enzymes. The methyl group, together with the methyl binding protein, prevents transcription factors (TF) from binding to transcription start site (TSS) or promoters and hinders transcription of the gene

Methylation in close proximity to the TSS prevents transcription factors and RNA polymerase from accessing the DNA, resulting in silencing of the gene (Fig. 2.3). In addition to gene silencing, these methyl groups also attract other protein complexes which promote histone deacetylation, further inhibiting gene expression (Strathdee and Brown, 2002; Turner, 2001). The bond between the methyl group and the cytosine nucleotide is very strong, resulting in stable, yet potentially reversible, changes in gene expression. It has been well established that transcription cannot be initiated at methylated CpG islands (CGIs) of TSSs after the DNA has been assembled into nucleosomes (Hashimshony et al., 2003; Kass et al., 1997; Venolia and Gartler 1983). However, the question of which comes first, silencing or methylation, has resulted in longstanding and active discussion. In 1987, Lock et al. clearly showed that methylation of the hypoxanthine phosphoribosyltransferase (*HPRT*) gene (on the inactive X chromosome) occurred only after inactivation of

the chromosome. Consequently, it was postulated that methylation serves as a lock that reinforces a previously silenced state of X-linked genes (Lock et al. 1987). However, results from a study by Challen et al. (2011), which investigated the role of DNMT3A in haematopoietic stem cell differentiation have raised questions about the universality of the long-term locking model. Their results indicated that methylase was vital for differentiation of a short-lived cell type. It is thus probable that DNA methylation instructs rather than reinforces gene silencing. It therefore seems likely that there is a general mechanism whereby silencing precedes methylation, although more data is required to confirm this. Nonetheless, it is evident that the process of DNA methylation is a more complex process than initially anticipated, requiring more in-depth research to address a number of unanswered questions.

It is also important to note that the position of methylation affects gene expression. Methylation in the TSS prevents initiation of transcription (as discussed above), whereas methylation in the gene body does not necessarily block transcription, and may even stimulate transcription elongation. It has been suggested that gene body methylation may play a role in splicing (Moarefi and Chedin, 2011). Gene body methylation is a feature of transcribed genes (Wolf et al., 1984), the majority of gene bodies contain a limited amount of CpG dinucleotides, numerous repetitive and transposable elements, and they are extensively methylated. One of the main causes of C→T transition mutations is CpG methylation in gene exons which could result in disease-causing mutations in the germline and cancer-causing mutations in somatic cells (Rideout et al., 1990; Jones et al., 2012). A 'methylation paradox' thus exists, whereby promoter methylation is inversely correlated with gene expression, and gene body methylation is positively correlated with gene expression (Jones et al., 1999). Therefore, initiation of transcription, and not transcription elongation, appears to be sensitive to DNA methylation silencing in mammals. The presence of a 5mC does not, of itself, elicit a transcriptional effect; this effect is elicited by the interpretation of the 5mC in a particular genomic and cellular context (Jones et al., 2012).

Since most genes have at least two TSSs, it has also been suggested that methylation could help to regulate the process of alternative promoter usage (Maunakea et al., 2010). CpG-rich sequences are abundant in the genome and are referred to as CpG islands (CGIs), most often situated in promoter regions. These CGIs are usually protected from methylation (Yehuda and LeDoux, 2007). A fraction of these CGIs, present in certain tissues during ageing (Issa 2000) or in abnormal cells (such as cancer cells) (Baylin and Herman 2000), are susceptible to progressive methylation. In mammals, the GC-content of CGIs is roughly 65% compared to 40% for the entire genome (Suzuki and Bird 2008). CpG island shores and shelves are regions outside CpG islands. Shores are 0 - 2000 bp outside CpG islands, while CpG shelves flank CpG shores and are 2000 - 4000 bp adjacent to CpG islands (Pastor et al., 2011). Methylation mostly occurs a short distance from the CpG islands, at the CpG island shores.

CpG islands can also exist within the gene bodies and within gene deserts (long stretches of the genome devoid of protein-coding genes) (Jones 1999; Venter et al., 2001). In the human brain, up to 34% of all

intragenic CGIs are methylated (Maunakea et al., 2010), however, the exact function of this remains to be fully elucidated. One hypothesis is that these regions may represent 'orphan promoters' that have escaped methylation in the germline, thus maintaining their high CpG density. It is therefore plausible that they play a functional role during development (Illingworth et al., 2010). The function of gene body methylation outside CGIs was initially assumed to be a mechanism for silencing repetitive DNA elements, such as retroviruses, LINE1 and Alu elements (Yoder et al., 1997). However, more recent whole-genome studies, suggest it may play a role in regulating splicing (Laurent et al., 2010).

Assembly of the spliceosome occurs during the process of transcription and is influenced by the elongation rate of RNA polymerase II (RNAPII) (Kornblihtt 2007). Researchers have hypothesized that exon definition could be promoted by chromatin which alters elongation kinetics or act as an adaptor for the recruitment of splicing factors (Alló et al., 2010; Luco et al., 2010). There is a growing body of evidence that link histone modifications to both these levels of regulation (refer to Alló et al. (2010) and Luco et al. (2010) for a thorough review). Exon definition could thus be directly promoted through the interaction of intragenic DNA methylation with auxiliary proteins and/or through kinetic regulation of RNAPII elongation. Deviations to intragenic DNA methylation could therefore result in alternative pre-mRNA splicing. Intragenic DNA methylation can thus be regarded as an emerging critical determinant of pre-mRNA processing (Oberdoerffer 2012).

In addition to the epigenetic mechanisms themselves, the various enzymes that regulate these mechanisms have also been linked to memory formation (Day and Sweatt, 2010; Ma et al., 2010). One such example is the regulation of active DNA demethylation, with focus on the gadd45 (growth arrest and DNA-damage-inducible, beta) family (Sultan et al., 2012; Leach et al., 2012). In particular, gadd45b has been found to be involved in activity-dependent demethylation in the adult CNS (Sultan et al., 2012). The deletion of *gadd45b* (gadd45b−/−) (the gene that encodes the growth arrest and DNA-damage-inducible, beta protein) leads to the abolishment of neuronal activity-induced DNA demethylation in the adult mouse dentate gyrus at specific genomic loci, including the promoters of the brain-derived neurotrophic factor gene (*BDNF*) and fibroblast growth factor 1 (*FGF1*). This reduces activity-induced adult hippocampal neurogenesis (Ma et al., 2009b). In addition, studies have shown that pharmacological inhibition of changes in DNA methylation also affect synaptic plasticity, learning and memory (Day and Sweatt, 2010).

Two research groups investigated the effects that the deletion of the *gadd45b* gene would have on fear conditioning and memory. Both studies found that *gadd45b* transcription is regulated in an experience-dependent manner and suggested its involvement in regulating memory capacity (Sultan et al., 2012; Leach et al., 2012). However, some conflicting results have emerged from these two studies with regards to the involvement of gadd45b in fear conditioning. On the one hand Sultan and colleagues observed enhanced contextual fear conditioning in *gadd45b−/−* (Sultan et al., 2012) whereas Leach et al. (2012) observed a deficit in contextual fear conditioning. Although there is no clear explanation for these conflicting results,

Sultan et al. (2012) hypothesised that a loss of such a potent epigenomic regulator could be sensitive to the background genome, where strain differences could have arisen during backcrossing. Different training facilities or housing environments could have augmented background genome or epigenome differences in the mutant mice (Crews, 2010). Another factor that could have contributed to the discrepant results between these two studies, are the differences in training paradigms; Leach et. al. (2012) utilized a foreground training paradigm whereas Sultan et al. (2012) used background training for contextual memory assessment. Irrespective of these differences, both of these studies emphasized the importance of epigenetic DNA modification mechanisms in the adult nervous system. They showed that the transcription of *gadd45b* is regulated by experience and that *gadd45b* may play an important role in long-term hippocampal-dependent memory.

However, it is not only a methyl group that occur on the C-5 position of cytosine residues, but also 5-hydroxymethylcytosine (5hmC), and although these two groups are very similar, they could have distinct effects on gene expression. The exact biological function of 5hmC is not fully elucidated but due to its identification in mouse embryonic stem (ES) and neuronal cells (Davis and Vaisvila 2011) it has generated interest as a potential biomarker. It has been postulated to play an important role in the process of demethylation (Guo et al., 2011), where 5hmC facilitates passive demethylation and in turn promotes gene transcription.

Most research techniques aimed at investigating methylation, including the current gold standard bisulfite sequencing, are unable to accurately distinguish between 5mC and 5hmC. This is because bisulfite conversion only converts unmethylated cytosines to uracil, thus both 5mC and 5hmC remain unaffected and cannot be distinguished. This could present some difficulties in identifying which methyl group is present and determining the effect it has on gene expression as these two methylation states can have opposite effects on gene expression (Davis and Vaisvila 2011). It is imperative to accurately discriminate between these methylation states, especially if gene expression studies are to be correlated with methylation status. Although a discussion of the methodologies that can be used to distinguish between 5hmC and 5mc is beyond the scope of this review, the reader is directed towards a review by Booth et al., (2012), which discusses methods that can be used to investigate 5hmC, such as thin layer chromatography, liquid chromatography and mass spectrometry, glucosylation, antibody detection and chemical labelling (Booth et al., 2012).

2.3.1.2 Neuronal DNA methylation in PTSD: animal studies

A key clinical feature of PTSD is dysfunctional fear extinction, which results from, amongst other things, dysregulation of the HPA axis. The HPA axis, arguably the key stress response system, interacts with the immune system in order to maintain homeostasis (Wong et al., 2002). Studies have shown that maternal care in rodents influences the development of HPA axis responses to stress in the pups. Adult offspring of

mothers that exhibit increased levels of licking/grooming and arched back nursing (high LG-ABN mothers) display more modest HPA responses to stress (Weaver et al., 2002). Little is known about the molecular mechanisms by which early environmental influences alter anxiety circuits in the brain. However, researchers have found that these alterations are, in part, mediated by changes in hippocampal GR expression, which mediates the negative feedback regulation of CRF expression. The effects on GR expression have been found to be associated with increased expression of the transcription factor, nerve growth factor-inducible protein A gene (*NGFI-A*), and increased activation of GR gene expression via a promoter on exon 1 (exon lZ) of the GR gene. Adult offspring of the high LG-ABN mothers had reduced methylation levels of exon lZ, which was associated with increased NGFI-A (transcription factor) binding to the GR promoter. Therefore, better maternal care increased NGFI-A expression in the offspring, resulting in differential methylation of specific DNA sequences with subsequent stable, long-term alterations in gene expression (Weaver et al., 2002).

Numerous studies have confirmed the abovementioned results, with reduced expression of GRs in the hippocampal tissue of pups raised by dams exhibiting low rates of maternal licking and grooming, compared to the offspring of mothers exhibiting high rates of maternal care (Weaver at al., 2004; Szyf et al., 2005). The reduced expression of GRs was attributed to increased methylation of the GR gene promoter (Weaver at al., 2004; Szyf et al., 2005). Long-term transcriptional alteration is established within the first week of life and may persist long-term and even be passed to the next generation (Champagne 2008). To this end, these alterations are effectively reversed by cross-fostering the rats with dams who exhibit high maternal care, or by infusion of trichostatin A, a histone deacetylase (HDAC) inhibitor (Weaver at al., 2004).

Lee et al. (2010) investigated glucocorticoid (GC) induced epigenetic changes in candidate HPA axis genes. FKBP5 mediates GR translocation; this GR co-chaperone protein is associated with hsp90 and together they form a chaperone complex that regulates GR dynamics (Hubler and Scammell 2004). Lee and colleagues (2010) found reduced DNA methylation levels in the *FKBP5* gene in brain and blood samples following GC administration. These alterations persisted for up to 4 weeks following GC withdrawal. In addition, these DNA methylation changes were associated with behavioural deficits (such as anxiety-like behaviour in the elevated plus-maze task) in an animal model of Cushing's syndrome (Lee et al., 2010). *FKBP5* genotype and methylation profiles have recently been found to be associated with GR sensitivity and exposure to early childhood trauma (Klengel et al., 2013). Here, a functional polymorphism in *FKBP5* altered the chromatin interaction between the TSS and long-range enhancers. This resulted in an increased risk of developing stress-related psychiatric disorders during adulthood through early-life trauma-dependent DNA demethylation in *FKBP5* functional glucocorticoid response elements (Klengel et al., 2013).

Yang et al. (2012) focused their investigation on the mouse hippocampal dentate gyrus, a vital region in the HPA-axis stress response, to determine if epigenetic alterations are enriched in this region compared to the entire hippocampus. They observed an overall greater decrease in DNA methylation in the dentate gyrus

compared to the entire hippocampal region. Moreover, they assessed whether DNMT1, was involved in these epigenetic alterations. They found that dexamethasone treatment resulted in a dose-dependent decrease in DNMT1 expression in a pituitary adenoma cell line and corticosterone-treated mouse hippocampus. Their research identified methylation as a potential epigenetic mediator of the stress response. In addition, they illustrated that GC-induced loss of methylation in pituitary cells can occur. A thorough understanding of the molecular mechanisms of GC-induced changes in gene function is crucial for improved therapeutic strategies for mood and trauma-related disorders.

Another early life stress study in mice has suggested that vasopressin-induced gene hyperactivity could possibly be involved in the aetiology of PTSD (Murgatroyd et al. 2009). In this study of maternally separated mice, a stable increase in GCs, vasopressin and depressive behaviour was observed in the separated pups. This behaviour was reversed by administration of a vasopressin receptor antagonist. Further investigations revealed that this effect was attributable to a reduction in DNA methylation of the transcription factor that increases vasopressin gene activity. Increased release of vasopressin into brain regions involved in anxiety and fear induced increased anxiety-like behaviour. DNA methylation could, therefore, act as an additional putative neurobiological marker for vulnerability to PTSD development in the context of early life stress (Murgatroyd et al. 2009).

It is clear that early life stress has a profound impact on gene expression profiles and subsequent behavioural abnormalities. This is further supported by the fact that some of these effects are heritable. Franklin and colleagues (2010) investigated the transgenerational effects of early stress on behavioural traits and the modes of inheritance in mice. They found that only when maternal separation was unpredictable and combined with unpredictable maternal stress, did it induce long-lasting behavioural effects in the offspring and in subsequent generations. Chronic and unpredictable maternal separation induced depressive-like behaviours as well as altered behavioural responses to aversive environments during adulthood in the separated animals. The male offspring of males subjected to maternal separation also exhibited most of these behavioural alterations, even though they were reared normally. In addition, chronic and unpredictable maternal separation modified the DNA methylation profile (in the germline) of the separated males in promoter regions of several candidate genes (MeCP2, cannabinoid receptor-1 (CB1), corticotrophin releasing factor receptor 2 (CRFR2)). Comparable DNA methylation changes were also evident in the brains of their offspring and were associated with changes in gene expression (Franklin et al., 2010).

A study by Miller and Sweatt (2007) focused on DNMT and its function in DNA methylation and memory. The transcription of DNMTs was found to be upregulated in the rat hippocampus during contextual fear conditioning, while inhibition of DNMT blocked memory formation. Furthermore, fear conditioning was found to be associated with methylation and subsequent transcriptional repression of the protein phosphatase 1 gene (PP1), the memory suppressor gene, and demethylation and transcriptional activation of reelin (RELN), a synaptic plasticity gene. Thus, methyltransferase and demethylase are both involved in the

memory consolidation process. In addition, pharmacological inhibition of DNMT activity blocked normal memory consolidation. This study clearly showed the dynamic regulation of DNA methylation in the adult nervous system and its critical function in memory formation (Miller and Sweatt 2007).

A number of studies have shown that DNA methylation within the brain plays an integral part in PTSD disease aetiology. It is important to note, though, that these DNA methylation patterns differ between different brain regions (Ladd-Acosta et al., 2007; Gibbs et al., 2010). A study investigating the association between *BDNF* DNA methylation and PTSD-like behaviour in an adult rat model, compared methylation levels in the dorsal and ventral hippocampal tissue, mPFC and BLA (Roth et al., 2011). The researchers evaluated DNA methylation patterns in exon IV of *BDNF* and performed subsequent gene expression analysis. They found that psychosocial stress in adulthood resulted in a significant increase in *BDNF* methylation in the dorsal CA1 sub-region. However, in the ventral hippocampus (CA3), stress significantly decreased methylation. Furthermore, decreased expression levels of *BDNF* were evident in both the dorsal and ventral CA1 region. The medial prefrontal cortex and basolateral amygdala exhibited no changes in *BDNF* methylation. These results indicate that traumatic stress can induce DNA methylation in certain parts of the CNS and that hippocampal dysfunction in response to traumatic stress might be induced by *BDNF* methylation. Furthermore, these results also suggest that altered hippocampal *BDNF* methylation is one mechanism underlying the cognitive deficits typical of PTSD pathophysiology (Roth et al., 2011).

Another study that focused specifically on DNA methylation patterns in the hippocampus in a rat PTSD model revealed that maladaptation to traumatic stress is associated with various changes in the methylation pattern of the hippocampus. One of the differentially methylated genes identified using this global screening method was disks large homolog-associated protein 2 (*DLGAP2*). *DLGAP2* had increased methylation levels in a specific site associated with a reduction in *DLGAP2* expression in rats with a PTSD-like (maladapted) phenotype compared to non-PTSD-like (well-adapted) rats. Proteins of the DLGAP family are enriched in the post-synaptic density (PSD) zone, which is regarded as the main region underlying synaptic plasticity. The main PSD scaffolding protein, PSD-95, regulates the development, maintenance and plasticity of synapses and spines (Han and Kim, 2008) and has also been associated with LTP (Migaud et al. 1998). LTP is a model of synaptic plasticity which is proposed to be similar to the plasticity which underlies learning and memory (Hölscher 1999; Bliss and Collingridge 1993), the two cognitive processes that are impaired in PTSD (Friedman 1997; Vermetten and Bremner, 2002). Alterations in methylation patterns could thus be involved in behavioural adaptation to environmental stress and could aid in the identification of possible treatment targets for PTSD (Chertkow-Deutsher et al., 2010).

2.3.1.3 DNA methylation and PTSD: studies in humans

Individuals who suffer from child abuse have a greater risk of developing PTSD and depression in later life (Mullen et al., 1996; MacMillan et al., 2001). These individuals are also prone to exacerbated physiological responses to stress (Weiss et al., 1999; Heim and Nemeroff, 2001) and corresponding alterations in CNS functioning (Liu et al., 1997; Weiss et al., 1999). However, it is not only childhood trauma that alters methylation and gene expression patterns; for example, prenatal exposure to maternal stress and adult exposure in the form of intimate partner violence (IPV) have also been found to induce lasting methylation changes that could affect psychological function in later life. The link between environmental stressors and disease pathogenesis wave been extensively investigated in the context of DNA methylation. Table 2.6 provides a summary of the DNA methylation studies in human subjects that have found associations between trauma and/or PTSD and DNA methylation and gene expression profiles in different tissues (as indicated in the table under the description of the sample group).

Table 2.6: DNA methylation studies in human subjects that describe associations between trauma, DNA methylation profiles, gene expression profiles and PTSD

Association of DNA methylation with trauma, gene expression or PTSD	Sample group	Reference
Increased *SLC6A4* promoter methylation in abused males vs. non-abused males	Iowa adoptee sample (EBV transformed lymphoblast cell lines)	Beach et al. (2010)
Hypermethylation of CpG1 and CpG3 regions of *SLC6A4* promoter in women who experienced child abuse	Iowa adoptee sample (EBV transformed lymphoblast cell lines)	Beach et al. (2010)
Increased methylation of *NR3C1* promoter in suicide victims with childhood abuse history vs. no abuse history and controls	Post-mortem suicide victims (hippocampal tissue)	McGowan et al. (2009)
Hypermethylation of *ALS2* in abused individuals	Individuals that suffered severe childhood abuse (hippocampal tissue)	Labonté et al. (2012)
Hypermethylation of *SLC6A4* at cg22584138 had a protective effect in individuals who experienced a higher number of traumatic events	Detroit Neighbourhood Health Study (whole blood)	Koenen et al. (2011)
Hypermethylation of LINE1 in post- deployment controls vs. pre-deployment controls	US military soldiers deployed to Afghanistan or Iraq (serum samples)	Rusiecki et al. (2012)
Hypomethylation of LINE1 in post-deployment cases vs. controls	US military soldiers deployed to Afghanistan or Iraq (serum samples)	Rusiecki et al. (2012)
Hypermethylation of *Alu* in pre-deployment cases vs. controls	US military soldiers deployed to Afghanistan or Iraq (serum samples)	Rusiecki et al. (2012)
Hypomethylation of genes with immune-related functions in PTSD-affected individuals.	PTSD-affected and -unaffected individuals (blood samples)	Uddin et al. (2010)
Hypomethylation of genes with neurogenesis-related functions in PTSD-unaffected individuals	PTSD-affected and -unaffected individuals (blood samples)	Uddin et al. (2010)
Increased global methylation in subjects with PTSD	Traumatized African American individuals from Atlanta (PBMCs)	Smith et al. (2011)
Differential methylation of *TPR CLEC9A*, *APC5*, *ANXA2* and *TLR8* in PTSD subjects	Traumatized African American individuals from Atlanta (PBMCs)	Smith et al. (2011)
ADCYAP1R1 CpG island methylation directly	Traumatized African American	Ressler et al. (2011

associated with total PTSD symptoms	individuals from Atlanta	
PACAP methylation levels associated with PTSD in females	Traumatized African American individuals from Atlanta (saliva and blood samples)	Ressler et al. (2011)
Total PTSD symptoms associated with methylation at *ADCYAP1R1* CpG island in females	Traumatized African American individuals from Atlanta (saliva and blood samples)	Ressler et al. (2011)
HTR2A minor A-allele (resulting in loss of CpG methylation site at -1,439) associated with disorders including PTSD	Chronic fatigue syndrome patients and non-fatigued controls (PBMCs)	Smith et al. (2008)
COMT Met/Met genotype associated with increased susceptibility to PTSD development and DNA methylation at four CpG sites (two sites found associated with impaired fear inhibition)	Community study in African American individuals from Atlanta (whole blood)	Norrholm et al. (2013)

ALS2 - amyotrophic lateral sclerosis 2 gene, *ADCYAP1R1* - adenylate cyclase activating polypeptide 1 (pituitary) receptor type I gene, *ANXA2* - annexin A2 gene, *APC5* - anaphase promoting complex subunit 5, gene, *BDNF* - brain-derived neurotrophic factor, *CLEC9A* - C-type lectin domain family 9, *COMT* - catechol-O-methyltransferase, *EBV* - Epstein-Barr virus, *HTR2A* - 5-hydroxytryptamine (serotonin) receptor 2A, LINE1 - Long Interspersed Nucleotide Element 1, *NR3C1* - Glucocorticoid receptor gene, PTSD - posttraumatic stress disorder, *SLC6A4* - serotonin transporter gene, *TLR8* - toll-like receptor 8 gene, *TPR* - translocated promoter region gene

2.3.2   MicroRNA (miRNA)

Please note that the following section contains extracts from a review article that I have written under the guidance of my supervisor and co-supervisor and published in 2012 (Malan-Müller S, Hemmings SMJ, Seedat S. Big Effects of Small RNAs: A Review of MicroRNAs in Anxiety. Mol Neurobiol (2012) DOI 10.1007/s12035-012-8374-6). The co-authors, my PhD supervisor and co-supervisor, edited and helped to construct the manuscript and agreed that it can be included in my PhD dissertation. More recent research that has been published after our review, are also included. **Note that the Sections originating from the published manuscript will be indicated by quotation (" ") marks.**

2.3.2.1 MicroRNAs

"MicroRNAs (miRNAs) are a class of small, noncoding RNAs that have recently drawn interest as epigenetic modulators of gene expression in psychiatric disorders (Hunsberger et al., 2009). In 1993, the first miRNA, lin-4, was discovered in *C. elegans* through genetic screening for deficiencies in the temporal control of post-embryonic development (Lee et al., 1993). However, it was only in 2001 that the role of miRNAs as a new layer of gene regulation was finally appreciated (Lagos-Quintana et al., 2001; Lau et al., 2001; Lee and Ambros 2001).

MiRNAs are single-stranded RNA species approximately 22 nucleotides (nt) long that form part of a large class of small, non-coding RNAs. miRBase is the major online repository for all miRNA sequences and annotation, the most recent version of the database, release 19, contains 21 264 hairpin precursor miRNAs entries expressing 25 141 mature miRNA products, in 193 species (Kozomara and Griffiths-Jones 2011). Between 1% and 5% of mammalian genes are comprised of miRNAs (Bartel, 2004), making them one of the most abundant classes of regulators in the genome (Espinosa-Parrilla and Muiños-Gimeno, 2011). Half of all

the miRNAs are expressed from non-protein coding transcripts and the other half from intronic regions of protein-coding genes (Saini et al., 2007). MiRNAs are evolutionarily conserved and are involved in numerous intricate processes including the stress response (He et al., 2007). They are of particular importance in brain functioning and are involved in learning and memory processes (Fiore and Schratt, 2007) as well as synaptic plasticity (Schratt et al., 2006). Certain miRNAs are ubiquitously expressed (e.g. let-7b, miR17-5p and miR21) (Tang et al., 2007) while others have an expression pattern dependent on the specific cell type or developmental stage (Brown and Naldini 2009) (e.g. brain and spinal cord-specific miR34a (Tang et al., 2007), and miR409-3p in brain development in mice (Krichevsky et al., 2003)).

The production of mature miRNAs is a complex process; the primary transcript miRNAs (pri-miRNAs) are cleaved by the ribonuclease III (Drosha) enzymes and the DiGeorge syndrome critical region gene 8 protein (DGCR8) in the nucleus. This cleavage produces a precursor miRNA (pre-miRNA) approximately 70–100 nt in length that is actively transported to the cytoplasm by exportin 5. In the cytoplasm the pre-miR is cleaved by another RNaseIII enzyme, Dicer, and the trans-activation responsive (TAR) RNA binding protein (TRBP) to generate double-stranded miRNAs approximately 22 nt in length. Thereafter, a helicase unwinds the dsRNA of the miRNA and one of the strands is degraded while the other (known as the guide strand) functions as the mature miRNA. The mature miRNA is incorporated into a miRNA-induced silencing complex (miRISC), a complex of proteins that target mRNAs based on sequence complementarity mostly in the 3' untranslated regions (UTRs) (Zhang et al., 2007). In the case of perfect complementarity between the miRNA and target mRNA, the target RNA is degraded. In the absence of perfect complementarity, the target is not cleaved but is deadenylated which leads to decapping and subsequent exonucleolytic digestion or translational repression (through a different mechanism at each translational step, namely initiation, elongation and termination) (Kuss and Chen 2008) (Fig. 2.4) (de Kloet et al., 2009). It is important to note that not all mRNA targets are directly targeted by miRNAs via binding to the 3'UTR of the mRNA. Indirect targets form part of a miRNA-mediated regulatory pathway but do not possess structural affinity for miRNAs. However, the expression of these targets is indirectly affected by another target of the miRNA (Reyes-Herrera et al., 2011). Parker and Wen (2009) have also shown that indirect targets have a delayed response in expression changes over time compared to direct targets (as described for miR-124) (Parker BJ, Wen 2009). It is clear that miRNAs do not simply turn genes on and off, but form part of an interconnected regulatory network that fine-tunes the expression levels of target genes (Sun and Tsao 2008). Variations in target sites could thus result in altered gene expression patterns and ultimately contribute to disease susceptibility (Muinos-Gimeno et al., 2009).
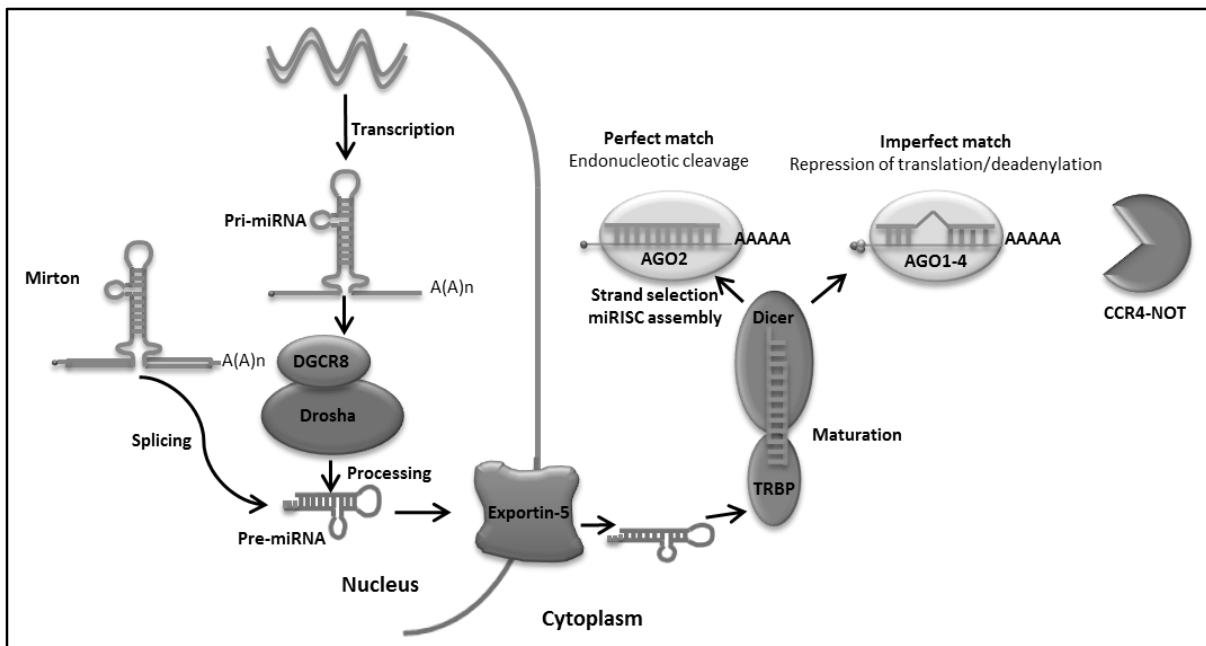
Figure 2.4: Figure depicting the production of mature miRNAs. MicroRNAs (miRNAs) are encoded in the genome, their genes usually transcribed by RNA polymerase II. The transcripts undergo splicing and polyadenylation. The pri-miRNA is processed in the nucleus by the Drosha RNaseIII enzyme and the DGCR8 protein, producing the pre-miRNA. The pre-miRNA is exported to the cytoplasm by exportin-5 where Dicer and the TRBP cleave the pre-miRNA to yield a miRNA duplex (about 22-bp long). One strand is selected to function as a mature miRNA, the other strand is in usually degraded. Mature miRNAs are then incorporated in a miRNA-induced silencing complex (miRISC) that recognises and binds to the 3'UTR of the target mRNA and represses translation (AGO-argonaute) (Kuss and Chen 2008)

Approximately 20% - 30% of all genes are regulated by at least one miRNA (Bartel, 2004; Krek et al., 2005; Lewis et al., 2003). However, computational analysis suggests that a single miRNA can target hundreds of genes and that one gene can be targeted by more than one miRNA (Yang et al., 2011). Although the 3' UTR of mRNAs is a typical target site of miRNAs, target sites in the coding region have also been documented (Duursma et al., 2008; Kloosterman et al., 2005). Nucleotides 2-7 of the miRNA sequence are known as the seed region and are the most critical region for target recognition (Brennecke et al., 2005). MiRNA-mediated regulation of mRNAs is complicated by the fact that miRNAs are prone to tissue-specific RNA editing. RNA editing is a post-transcriptional mechanism whereby some RNA molecules are changed to contain bases not originally encoded in the genome (via nucleotide insertion, deletion or modification). Such events can lead to altered properties of miRNAs and alternative mRNA: miRNA interactions (Erson et al., 2008).

In order to gain insight into miRNAs and their functions, it is essential to identify their mRNA targets. This step has proven to be computationally challenging. Although great advances have been made in the field of miRNA target prediction, with the development of various target-predicting software (Nicolas et al., 2008), their false-positive rates of target prediction range between 24% and 70% (Baek et al., 2008; Easow et al., 2007; Selbach et al., 2008). These high rates emphasize the importance of experimental strategies to validate predicted targets in an endeavour to identify genuine miRNA targets and miRNA function (Thomson et al.,

2011). For more detail on *in vitro* and *in vivo* experimental strategies for miRNA target identification, refer to Thomson et al. (2011), Schratt et al. (2006), Pasquinelli et al. (2012) and Karres et al. (2007).

Recent studies suggest a crucial role for miRNAs in regulating various neurobiological processes, including neurogenesis, neurite outgrowth, synaptogenesis and synaptic and neural plasticity (Zhou et al., 2009). Many putative miRNA targets are involved in neural development; these include mRNAs that encode proteins involved in the maintenance of neuronal function, plasticity of neural networks and specific neurodevelopmental and neurodegenerative diseases (Rogaev, 2005). Studies have also shown that miRNAs are altered by stress, glucocorticoids and mood stabilisers (Hunsberger et al., 2009), suggesting that miRNAs could be vital in the aetiology of anxiety disorders. Hunsberger (2009) suggested that miRNAs could be differentially expressed in patients with various psychiatric disorders, indicating that miRNAs may have the potential to broaden our understanding of the pathophysiology and therapeutics of anxiety disorders (Hunsberger et al., 2009).**"**

2.3.2.2 MiRNAs in anxiety as described in animal Models

"MiRNAs are abundantly expressed throughout the brain where they perform important regulatory functions in the CNS (Kussand Chen 2008; Sun and Tsao 2008). This suggests a role for miRNAs in stress response regulation. Uchida et al. (2008) established and characterized an animal model of vulnerability to repeated stress in F344 rats (Uchida et al., 2008). After investigating neuroendocrine and biochemical responses to repeated restraint stress (RRS) they found lower levels of glucocorticoid receptor (GR) protein expression in the paraventricular nucleus (PVN) in F344 rats compared to control SD rats. Furthermore, they established that miR-18a inhibited translation of GR mRNA (in cultured neuronal cells) and that higher expression levels of miR-18a were present in F344 rats compared with SD rats in the PVN. *In vitro* experiments confirmed the results for miR-18a and also established a similar role for miR-124 (Vreugdenhil et al., 2009). Down-regulation of GR translation via miR-18a may be an important susceptibility mechanism for stress-related disorders (Uchida et al., 2008), and F344 rats could therefore be a useful animal model for studying vulnerability to repeated stress.

Subsequent work by Uchida et al. (2010) focused on the effects of maternal separation and early life adversity on the behavioural response to RRS as well as vulnerability to chronic stress in adult rats (Uchida et al., 2010). Maternally separated rats showed increased expression of repressor element-1 silencing transcription factor 4 (REST4), a neuron-specific splicing variant of the transcriptional repressor REST. REST regulates certain brain-enriched miRNAs postulated to be associated with neuronal functions such as brain development and plasticity (Kosik, 2006; Conaco, Vo et al., 2005; Otto et al., 2007). The maternally separated rats also showed a marked increase in a variety of REST target gene mRNAs and miRNAs in the medial prefrontal cortex (mPFC). The expression of pre-mir132, -124-1, -9-1, -9-3, -212, and -29a as well as the mature miR132, -124, -9, and -29a were found to be significantly up-regulated in maternally separated rats compared to control rats. Interestingly, mir-132, -124-1, -9-1, -9-3, -212 and -29a all possess an

repressor element-1 (RE-1) site within 50 kb of their promoter regions (Otto et al., 2007). The authors hypothesised that the differential expression of mRNAs and miRNAs of genes that contain RE-1 might be due to alterations in RE-1-mediated gene transcription in the mPFC of maternally separated rats secondary to altered REST4 expression. Indeed, results indicated an increased level of expression of genes and miRNAs possibly regulated by REST4, such as glutamate receptor subunit (*GLUR2*), calcium/calmodulin-dependent protein kinase II (*CAMKIIα*) and adenylate cyclase 5 (*ADCY5*) as well as precursors for mir132, -124 and -212. These results suggest a role for an REST4-mediated gene network and specific miRNAs acting in the mPFC. This study provides additional insights into factors that could influence susceptibility to developing mood and anxiety disorders in adulthood following exposure to early life stress (Uchida et al., 2010).

Meerson et al. (2010) predicted that miRNAs mediate stress response regulation through alternative splicing. They studied expression profiles of miRNAs in the hippocampus CA1 region and the central amygdala in both acute and chronically stressed rats. They found that both acute and chronic immobilization stress induced distinct miRNA expression profiles in these two stress-responsive brain regions. MiR-134 and miR-183 were upregulated in the amygdala following acute stress. MiR-134 was downregulated in the amygdala and hippocampus under chronic stress conditions in both the amygdala and CA1. These two miRNAs were further investigated as they shared numerous common predicted mRNA targets that were known mediators of neuronal stress reactions, including the Serine/arginine-rich splicing factor 2 (SC35). SC35 is upregulated in response to stress, promoting the alternative splicing of acetylcholinesterase (AChE) from its synapse-associated isoform (AChE-S) to the rare soluble form of the protein (AChE-R). MiR-183-mediated suppression of SC35 was confirmed in cultured cells. This alternative splicing of AChE affects the local and temporal regulation of cholinergic neurotransmission. The authors were able to demonstrate that stress altered the expression levels of miR-183 and miR-134. Through regulating splicing factors and their targets, these miRNAs were able to modify both alternative splicing and cholinergic neurotransmission under stress conditions in the brain, providing a link between the molecular and physiological responses of different brain regions to psychological stress (Meerson et al., 2010).

The functional role of miRNAs in regulating stress responses were investigated by Haramati et al. (2011); by inactivating the Dicer gene (a key enzyme in miRNA synthesis pathway) they were able to inactivate miRNA processing in the central amygdala (Haramati et al., 2011). A sharp increase in anxiety-like behaviour was evident in mice lacking Dicer (and thus also mature miRNAs) in their amygdala. In addition, acute stress in wild type mice induced differential expression of numerous miRNAs in the amygdala. MiR-34c, one of the prominent stress-induced miRNAs, was further investigated and found to be strongly upregulated by exposure to stress, resulting in reduced symptoms of anxiety in normal mice. Interestingly, corticotrophin releasing factor receptor type 1 (CRFR1) mRNA is one of the targets of miR-34c. The authors showed that miR-34c elicits its effect on the amygdala by targeting an evolutionarily conserved region in the 3'UTR of *CRFR1* mRNA. The authors postulated that miR-34c downregulates stress-related proteins like

CRFR1 and assists in the stress recovery process of these mice. In effect, such miRNAs and their targets may unveil new targets for the treatment of stress-related disorders (Haramati et al., 2011).

By 2008 it was established that miRNAs play an important regulatory role in neuronal development, however the mechanism of regulation of miRNA expression had not been elucidated. Parsons et al. (2008) investigated differential miRNA expression in the hippocampus of four common inbred mouse strains (A/J, BALB/cJ, C57BL/ 6J, and DBA/2J) prone to anxiety-like behaviour They identified 11 differentially expressed miRNAs. The expression of miR-34a, miR-323, miR-378, and miR-451 correlated with behavioural measurements of exploration on the elevated plus maze task (indicative of anxiety levels), with less anxious animals displaying more explorative behaviour. MiR-34c and miR-323 expressions correlated with anxiety (less explorative behaviour) on the elevated plus maze task and expression of miR-34c, miR-323, miR-378, and miR-451 correlated with tests of learning and memory (Parsons et al., 2008). While a role for miRNAs in synaptic development had previously been proposed (Fiore and Schratt 2007), this study was one of the first to demonstrate involvement of miRNAs in anxiety, learning and memory.

Acute and repeated stress affects neural activity in different brain regions (Anisman and Zacharko 1992); short term changes in neural transmission and gene regulation (Alfonso et al., 2006; Gao et al., 2006; Xu et al., 2004) and longer term changes in structural modification (Cook and Wellman 2004; Donohue et al., 2006; Magarinos and McEwen 1995) have, in particular, been documented. It is thus plausible that miRNAs may be involved in these processes. In a recent study investigating the effects of single or repeated exposures to restraint stress on miRNAs in the frontal cortex of CD1 mice, a marked increase in the expression levels of various miRNAs after acute stress was found, while only minor changes were observed after repeated restraint. The authors hypothesized that acute stress rapidly modulates miRNAs, but that these effects are only transient. Northern blot analysis confirmed that after acute restraint an increase in let-7a, miR-9 and miR 26-a/b was observed. These changes were found to be region specific, present in the frontal cortex but not in the hippocampus, providing evidence that miRNAs in the frontal cortex are involved in the process of translating stressful events to alterations in protein expression (Rinaldi et al., 2010).**"**

A recent study suggested that region-specific miRNA-mediated reprogramming are involved in gene expression and epigenomic responses to chronic stress in the brain (Babenko et al., 2012). In this study, rats were grouped into the following groups; those exposed to two weeks of daily restraint stress and those that received two weeks of daily restraint stress + two weeks of recovery from stress and naïve controls. MiR-186 and miR-381were up-regulated, whereas miR-709 was downregulated in the cerebellum of the stressed animals compared to the controls. Remarkably, mir-709 downregulation persisted after two weeks of recovery from stress, suggesting that downregulation of this miRNA in the cerebellum may be involved in long-term adaptation to chronic stress. Interestingly, miR-709 showed different expression patterns in the cerebellum, hippocampus and PFC. Levels of mir-709 in hippocampus and PFC were unaffected after two

weeks of chronic stress and were upregulated in the PFC after two weeks of recovery. These finding emphasized the fact the regulation of mir-709 by stress was brain region-specific (Babenko et al., 2012).

Molitoris et al. (2011) described a regulatory feedback loop that involve the miR-17~92 cluster (which includes miR-18) and the GR. The GR is a main regulator of neurogenesis and structural plasticity in the hippocampus; furthermore Fitzsimons et al. (2012) have previously demonstrated that high levels of miRNA-18 are expressed in the hippocampus during early postnatal development and that miRNA-18 regulates GR protein expression (Vreugdenhil et al., 2009). These results suggest that early postnatal hippocampal neurogenesis may be regulated by mir-18 through its repression of GR expression. Interestingly, a regulatory feedback loop exists between miRNA-18 and the estrogen-receptor α (ERα) (Castellano et al., 2009), suggesting that the sex differences observed in the levels of adult neurogenesis after early-life stress (Oomen et al., 2010, 2011) could involve long-lasting changes in miR-18 expression (Schouten et al., 2013).

The first study to investigate the connection between miRNAs and PTSD was published in 2013. MiRNA profiles from PFC samples from fluoxetine-treated C57BL/6N mice were compared to that of control C57BL/6N mice 74 days after they were subjected to either a single electric footshock or mock-treatment. Fluoxetine is an antidepressant that has previously been shown to be effective in treating PTSD patients and mice suffering from PTSD-like symptoms. The researchers established that the therapeutic action of fluoxetine in shocked mice was associated with significant downregualtion of mmu-miR-1971. The authors hypothesised that fluoxetine interacts with traumatic stress to modify mmu-miR-1971 expression levels (Schmidt et al., 2013). Refer to Table 2.7 for a summary of miRNAs that have been described in animal models of anxiety.

2.3.2.3 MiRNAs in Anxiety as Described in Human Studies

"The role that miRNAs play in synaptic plasticity and neuronal differentiation suggests that miRNAs may be involved in the aetiology of numerous psychiatric disorders. Various miRNA expression studies have been conducted in schizophrenia patients (post-mortem brain samples) (Beveridge et al., 2008; Beveridge et al., 2010; Perkins et al., 2007), autism spectrum disorders (bu-Elneel et al., 2008; Talebizadeh et al., 2008), Rett syndrome (bu-Elneel et al., 2008) and substance abuse disorders (Chandrasekar and Dreyer 2009). To date, there have been few studies of miRNAs in anxiety disorders.

Muiños-Gimeno et al. (2011) selected a panel of SNPs (712 SNPs that covered 325 miRNA regions) to use in association studies of panic disorder (Muinos-Gimeno et al., 2011). Their analysis revealed that the SNP coverage in miRNA regions is much lower than the rest of the genome. None of these SNPs were located within a mature miRNA sequence, which is in line with the reported negative selection at miRNAs and miRNA target sites at 3'UTRs (Chen and Rajewsky 2006). This lower SNP density was confirmed by a

study that re-sequenced 117 miRNAs in four different human reference populations (Quach et al., 2009). It is thus evident that mutations in miRNA binding sites are likely to be deleterious and could have severe phenotypic implications. Re-sequencing of 3'UTRs and miRNAs in patients and controls might cast more light on the role of miRNA-mediated regulation in the susceptibility to anxiety disorders (Quach et al., 2009).

In 2011 Muiños-Gimeno et al. investigated the functional role of miRNAs in panic disorder (PD) in a Spanish cohort of patients with PD. They examined 712 SNPs that tagged 325 human miRNA regions. Two SNPs found to be significantly associated with PD, rs6502892 and rs11763020, were also found to tag miRNAs miR-22 and miR-339, respectively. MiRNA-22 was shown to regulate four candidate genes, namely brain-derived neurotrophic factor *BDNF*, serotonin 5-HT2C receptor (*HTR2C*), monoamine oxidase A (*MAO-A*), and the regulator of G-protein signalling 2 gene (*RGS2*). Target predicting software proposed adenosine receptor A2a (*ADORA2A*), *BDNF*, corticotropin releasing hormone receptor 2 (*CRHR2*) and sodium-dependent noradrenaline transporter (*SLC6A2*) as possible targets of miR-339. In addition, they found SNPs associated with PD sub-phenotypes (PD with and without agoraphobia) that tagged miR-138-2, miR-148a, miR-488 and miR-491. Functional studies indicated that miR-138-2, miR-148a, and miR-488 repressed the expression of certain candidate genes for PD in the region of 30% to 60%, including gamma-aminobutyric acid A receptor, alpha 6 (*GABRA6*), cholecystokinin B receptor (*CCKBR*) and proopiomelanocortin preproprotein (*POMC*), respectively (Muiños-Gimeno et al., 2011). Following transfection with miR-22 and miR-488, neuroblastoma cells showed altered expression of a subset of potential target genes for these miRNAs and genes that might affect physiological pathways related to anxiety. An association between rs73531, which tagged the intergenic miR-148a, and age at onset (AAO) (p = 0.0007) was observed. The average AAO was 23 years for the GG homozygotes and 30 years for the AG heterozygotes and AA homozygotes (Muiños-Gimeno et al., 2011).

Neurotrophin-3 growth factor receptor (*NTRK3*) was also investigated as a candidate susceptibility factor in PD and obsessive-compulsive disorder (OCD). After re-sequencing the 3'UTRs in two different isoforms of *NTRK3* in PD and OCD patients, they found that in the truncated isoform of *NTRK3* (located in a functional target site for miR-485-3p) the C allele of rs28521337 was significantly associated with the hoarding phenotype of OCD. Additionally they identified two new rare variants, ss102661458 (located in a functional target site for miR-765) and ss102661460 (located in a functional target sites for miR-509 and miR-128), in the 3'UTR of *NTRK3*, present in one chromosome of a PD patient (Muinos-Gimeno et al., 2009). MiR-128 is a brain-enriched miRNA that is involved in synaptic processing and neuronal differentiation and miR-509 shares the target site of miR-128, its expression is restricted to the testis (Betel et al., 2008), suggesting tissue-dependent regulation of *NTRK3* at this site. These two variants resulted in the recovery of gene expression by significantly altering the miRNA-mediated regulation of *NTRK3*. Their data provides evidence that miRNAs play a key role in posttranscriptional regulation, in this case allele-specific miRNA regulation of *NTRK3* in anxiety disorders (Muinos-Gimeno et al., 2009).

A cross-species approach is another interesting method that has been used to study anxiety and to identify genes that regulate anxiety-like behaviour. This approach has enabled researchers to identify a SNP (rs817782) in the 3'UTR of the aminolevulinate dehydratase gene (*ALAD*) that was shown to be associated with social phobia (Donner et al., 2011). The rare A allele of rs817782 generated a putative target site for both miR-211 and miR-204 within the ALAD 3'-UTR, as predicted by a miRNA target prediction program (http://www.patrocles.org) (Donner et al., 2011). The authors previously found that *ALAD* was expressed at a higher level in the hippocampus and periaqueductal grey of six inbred anxious mouse strains. These two brain regions together are part of the abnormally sensitive fear network that patients with PD suffer from. However, a direct link between this functional *ALAD* SNP, the putative miRNA target sites (for miR-211 and miR-204) and PD has yet to be established (Hovatta et al., 2005)."

Recent studies provide experimental evidence that indicate that miRNA biogenesis occurs locally in the vicinity of synapses. Especially, Drosha and DGCR8 (the microprocessor components) and their pri-miRNAs substrate are particularly enriched in postsynaptic densities (Lugli et al., 2012). This will provide an ideal position for miRNAs rapidly respond to synaptic activity and regulate translation. Findings from Lugli et al., (2005) support this hypothesis; they demonstrated that postsynaptic densities are highly enriched for Dicer and Argonaute 1 (the rate-limiting enzymes in mature miRNA production) and that neuronal activity modulate their levels (Lugli et al., 2005).

Researchers were also interested in the role of miRNAs in processes such as LTP. A recent study investigating this in rat hippocampus found that LTP induced differential expression of numerous miRNAs, with miRNA-188 exhibiting the largest upregulation. This result suggests that miR-188 is involved in the fine-tuning of synaptic plasticity in hippocampal neurons (Lee et al., 2012). Table 2.7 provides a list of miRNAs, as described in animal and human studies, that are possibly involved in anxiety disorders.

Table 2.7: Summary of microRNAs that are possibly involved in anxiety disorders (adapted from Malan-Müller et al., 2012)

MiRNA	Involvement with Anxiety Disorders	Species	Reference
Let-7a-1	Upregulated expression in the frontal cortex following acute stress Down regulated in amygdala after acute and chronic stress	*Mus musculus* *Rattus norvegicus*	Rinaldi et al. 2010 Meerson et al., 2010
Let-7b	Increased expression in the hippocampus due to treatment with lithium and sodium valproate	*Rattus norvegicus*	Zhou et al. 2009
Let-7c	Decreased expression in the hippocampus due to treatment with lithium and sodiumvalproate	*Rattus norvegicus*	Zhou et al. 2009
miR-1	Upregulated in amygdala under chronic stress and downregulated in the hippocampus under acute stress	*Rattus norvegicus*	Meerson et al., 2010
miR-9	Involved in neural lineage differentiation in ESCs	*Mus musculus* and *in vitro* cell line	Krichevsky et al., 2006
	Upregulated expression in the frontal cortex following acute stress	*Mus musculus*	Rinaldi et al., 2010

		Rattus norvegicus	
	Upregulated expression in the medial pre-frontal cortex following maternal separation		Uchida et al., 2010
miR-9-1	Pre-miRNA upregulated expression in the medial pre-frontal cortex following maternal separation	*Rattus norvegicus*	Uchida et al., 2010
	Down regulated in CA1 region of hippocampus under acute or chronic stress		Meerson et al., 2010
miR-9-3	Pre-miRNA upregulated expression in the medial pre-frontal cortex following maternal separation	*Rattus norvegicus*	Uchida et al., 2010
miR-17-5p	Upregulated in the hippocampus CA1 region under chronic stress	*Rattus norvegicus*	Meerson et al., 2010
	Controls neuronal development and differentiation	*in vitro* cell line	Hebert et al. 2009
miR-18a	Possible repressor of the glucorticoid receptor gene in the hypothalamic paraventricular nucleus regulating stress responses	*Rattus norvegicus*	Uchida et al et al.,2008 Vreugdenhil et al., 2009;
miR-21	Involved in the control of glial cell differentiation	*in vitro* cell line	Chan et al., 2005
miR-22	Associated with Panic Disorder - Repression of *RGS2*, *BDNF*, *HTR2C*, and *MAOA*	*Homo sapiens*	Muiños-Gimeno et al., 2011
miR-24a	Decreased expression in the hippocampus due to treatment with lithium and sodium valproate	*Rattus norvegicus*	Zhou et al., 2009
miR-26a/b	Upregulated expression in the frontal cortex following acute stress	*Mus musculus*	Rinaldi et al. 2010
miR-29a	Upregulated expression in the medial pre-frontal cortex following maternal separation	*Rattus norvegicus*	Uchida et al., 2010
miR-30c	Decreased expression in the hippocampus due to treatment with lithium and sodium valproate	*Rattus norvegicus*	Zhou et al., 2009
miR-34a	Correlation between differential expression of this miRNA and behavioural measures for exploration on the elevated plus-maze task	*Mus musculus*	Parsons et al.,2008
	Decreased expression in the hippocampus due to treatment with lithium and sodium Valproate	*Rattus norvegicus*	Zhou et al., 2009
miR-34c	Correlation between differential expression of this miRNA and behavioural measures for anxiety in mice. Upregulated by exposure to stress	*Mus musculus*	Parsons et al.,2008 Haramati et al., 2011
miR-124	Upregulated expression in the medial pre-frontal cortex following maternal separation	*Rattus norvegicus*	Uchida et al., 2010
miR-124-1	Pre-miRNA upregulated expression in the medial pre-frontal cortex following maternal separation	*Rattus norvegicus*	Uchida et al., 2010
	Down regulated in the hippocampus under acute stress. Controls neuronal development and differentiation		Meerson et al., 2010 Hebert et al. 2009
miR-124a	Involved in neural lineage differentiation in ESCs	*Mus musculus* and *in vitro* cell line	Krichevsky et al., 2006; Lim et al., 2005; Makeyev et al., 2007
	Down-Regulates glucocorticoid receptor	*Rattus norvegicus*	Vreugdenhil et al., 2009
miR-128	Association of an allelic variant in the target site for miR-128 in *NTRK3 (ss102661458)* with Panic Disorder - Reduction of *NTRK3* repression	*Homo sapiens*	Muiños-Gimeno et al., 2009
miR-128a	Decreased expression in the hippocampus due to treatment with lithium and sodium valproate	*Rattus norvegicus*	Zhou et al. 2009
miR-128b	Regulates formation of fear-extinction memory in the infralimbic pre-frontal cortex	*Mus musculus*	Lin et al., 2011
miR-132	One of the most highly inducible CREB targets, plays a role in	*in vitro* neural cell line	Vo et al., 2005

	neurite outgrowth and neuronal plasticity	*Rattus norvegicus*	
	Upregulated expression in the medial pre-frontal cortex following maternal separation		Uchida et al. 2010[87]
	Pre-miRNA upregulated expression in the medial pre-frontal cortex following maternal separation		
miR-134	Modulates synaptic plasticity in hippocampus	*Rattus norvegicus*	Hansen et al. 2007
	Upregulated expression in the central amygdala and hippocampus after acute stress.	*Rattus norvegicus*	Meerson et al., 2010
	Downregulated expression in the central amygdala and hippocampus after chronic stress	*Rattus norvegicus*	Meerson et al., 2010
miR-138-2	Associated with age at onset in Panic Disorder - Repression of *GABRA6*	*Homo sapiens*	Muiños-Gimeno et al., 2011
miR-144	Decreased expression in the hippocampus due to treatment with lithium and sodium valproate	*Rattus norvegicus*	Zhou et al., 2009
miR-148a	Associated with age at onset in Panic Disorder - Repression of *CCKBR*	*Homo sapiens*	Muiños-Gimeno et al., 2011
miR-183	Upregulated expression in the central amygdala following acute stress	*Rattus norvegicus*	Meerson et al., 2010
miR-186	Upregulated in the cerebellum of animals subjected to daily restrained stress compared to the naïve controls	*Rattus norvegicus*	Babenko et al., 2012
miR-204	Association of an allelic variant in the 3'UTR of *ALAD* with SP	*Homo sapiens*	Donner et al., 2008
miR-208	Upregulated in CA1 region of hippocampus under acute or chronic stress	*Rattus norvegicus*	Meerson et al., 2010
miR-211	Association of an allelic variant in the 3'UTR of *ALAD* with SP	*Homo sapiens*	Donner et al., 2008
miR-212	Pre-miRNA upregulated expression in the medial pre-frontal cortex following maternal separation	*Rattus norvegicus*	Uchida et al., 2010
miR-221	Decreased expression in the hippocampus due to treatment with lithium and sodium valproate	*Rattus norvegicus*	Zhou et al., 2009
miR-273	Plays a role in neuronal differentiation	*C.elegans*	Chang et al., 2004; Johnston et al., 2005; Johnston et al., 2003
miR-323	Correlation between differential expression of this miRNA and behavioural measures for anxiety in mice	*Mus musculus*	Parsons et al.,2008
miR-339	Associated with Panic Disorder	*Homo sapiens*	Muiños-Gimeno et al., 2011
miR-376	Upregulated in CA1 region of hippocampus under acute or chronic stress	*Rattus norvegicus*	Meerson et al., 2010
miR-378	Association between miRNA and behavioural measures (exploration, learning and memory) for anxiety in mice	*Mus musculus*	Parsons et al.,2008
miR-451	Association between miRNA and behavioural measures (exploration, learning and memory) for anxiety in mice	*Mus musculus*	Parsons et al.,2008
miR-381	Upregulated in the cerebellum of animals subjected to daily restrained stress compared to the naïve controls	*Rattus norvegicus*	Babenko et al., 2012
miR-485-3p	Significantly associated with hoarding subtype of OCD	*Homo sapiens*	Muiños-Gimeno et al., 2009
miR-488	Associated with Panic Disorder - Repression of *POMC*	*Homo sapiens*	Muiños-Gimeno et al., 2011
miR-491	Associated with Panic Disorder	*Homo sapiens*	Muiños-Gimeno et al., 2011
miR-509	Association of an allelic variant in the target site for miR-509 in *NTRK3* (ss102661458) with Panic Disorder - Reduction of *NTRK3* repression	*Homo sapiens*	Muiños-Gimeno et al., 2009
miR-709	Downregulated in the cerebellum of animals subjected to daily	*Rattus norvegicus*	Babenko et al., 2012

	restrained stress compared to the naïve controls		
miR-765	Association of an allelic variant in the target site for miR-765 in *NTRK3 (ss102661460)* with Panic Disorder - Reduction of *NTRK3* repression	*Homo sapiens*	Muiños-Gimeno et al., 2009
mmu-miR-1971	Downregulation of this miRNA in PFC is associated with therapeutic action of fluoxetine in shocked mice	*Mus musculus*	Schmidt et al., 2013

3'UTR – three prime untranslated region, CA1 - Cornu Ammonis region 1, ESCs - embryonic stem cells, CREB - cAMP response element-binding protein. Please refer to list of abbreviations for full gene names

2.3.2.4 MicroRNAs and pharmacotherapies for anxiety disorders

"The serotonin transporter (SERT) is an important neurotransmitter in the CNS that ensures the reuptake of serotonin at the synaptic cleft and regulates serotonin levels in the brain. Defective serotonergic neurotransmission has been associated with anxiety, OCD, depression, and suicidal behaviour (van Praag, 1996a, b). SERT is also a pharmacological target of selective SSRI antidepressants (Torres et al., 2003), one of the very effective treatments for various anxiety disorders. A study by Baudry et al. (2010) found that SERT is a target of miR-16. After chronically treating mice with the SSRI fluoxetine (Prozac), there was an increase in miR-16 levels in serotonergic raphe nuclei that resulted in reduced SERT expression (Baudry et al., 2010). These studies clearly confirm the important role of miRNAs in the pathophysiology of anxiety disorders. Furthermore, miRNAs presents a novel therapeutic strategy as targets for anxiolytic drugs. Since miRNAs play an essential role in regulating numerous stress response pathways, it is imperative that miRNAs be evaluated as potential drug targets for anxiety disorders.

Zhou et al. (2009) conducted one of the first studies that demonstrated that miRNAs and their effectors are targets of pharmacotherapeutic drugs. Lithium and valproate (VPA) have been found to be effective in treating bipolar disorder (BPD). Although not routinely used in the anxiety disorder setting, valproate in particular may be useful adjunct in treatment-refractory anxiety disorder patients as well as in those patients with a comorbid bipolar disorder and might enhance exposure-based cognitive therapy for anxiety disorders and PTSD (Kuriyama et al., 2011). Zhou et al. (2009) found fluctuating levels of various hippocampal miRNAs following chronic treatment with mood stabilizers, lithium and VPA. The miRNAs that they were able to confirm were let-7b, let-7c, miR-24a, miR-30c, miR-34a, miR-128a, miR-144 and miR-221. The predicted effectors of these miRNAs are involved in neurogenesis, neurite outgrowth and signalling of extracellular signal-regulated kinase (ERK), phosphatase and tensin homologue deleted from chromosome 10 (PTEN) and Wnt/ß-catenin pathways (Zhou et al., 2009). Treatment with mood stabilizers such as lithium and VPA has been found to increase the expression of genes encoding dipeptidyl-peptidase 10, metabotropic glutamate receptor 7 (GRM7) and thyroid hormone receptor ß *in vivo* (Zhou et al., 2009). Several of these effector-coding genes have previously been described as candidates for susceptibility to the development of BPD. The authors went on to investigate the effects of lithium and VPA on the expression of miRNAs and their effectors in primary cultures. Primary cultures that received treatments of lithium or VPA showed

lowered levels of miR-34a and elevated levels of GRM7 (a predicted effector of miR-34a). In addition, treatment with a miR-34a precursor decreased GRM7 levels and treatment with a miR-34a inhibitor increased GRM7 levels. These results confirm that endogenous miR-34a regulates the levels of GRM7, which may contribute to the therapeutic effects of lithium and VPA on GRM7 (Zhou et al., 2009). Valproate has been shown to be effective, particularly as an augmentation strategy, for a number of anxiety disorders, including PTSD, panic disorder, GAD and SAD (Zhou et al., 2009)."

2.4 Treatment of PTSD

Stress-related diseases, such as depression and anxiety disorders, place a heavy health and economic burden on society. However, there is a limited range of available pharmacotherapies to treat these disorders and the majority of treatments are suboptimal with regard to efficacy and tolerability (Holmes et al., 2003; Kessler et al., 2005; Kasper et al., 2010). Several forms of psychotherapy, especially CBT, form part of the current recommendations for the treatment of PTSD (Foa et al., 2000), as well as psycho-education and supportive measures (Cohen et al., 2004; Oflaz et al., 2008). Another important component in the comprehensive treatment of PTSD is the treatment of comorbid conditions, such as mood and substance use disorders.

Several of the current CBT for PTSD are based on regulating the processes of fear extinction (McNally, 2007; Otto et al., 2007). Exposure-based CBT is the most commonly used approach for PTSD treatment and relies on extinction-based methods (Norton and Price, 2007). The therapy is procedurally similar to extinction training in animal models of emotional learning (Thyer et al., 1988; Foa and Kozak, 1986; Zarate and Agras, 1994). This therapy involves repeatedly exposing the patient to an anxiety-producing stimulus in a controlled setting, thereby reducing the uncontrolled fear associated with the anxiety (Foa and Kozak, 1986). Although exposure-based CBT is effective in the initial phases of PTSD treatment, the CS-no US association that is acquired during the fear extinction process has been found to diminish over time. As a result, a large proportion of patients with PTSD undergoing this therapy are expected to relapse (Rescorla 2004). Cumulatively, these findings suggest that exposure-based CBT can, and should, be improved upon, as the inhibitory extinction learning seems to fade more rapidly than the excitatory conditioning learning.

Pharmacological strategies for the treatment of established PTSD that target the emotional response or other non-cognitive symptoms include SSRIs (Van der Kolk et al., 1994; Connor et al., 1999; Brady et al., 2000; Martenyi et al., 2002), other antidepressants (Davidson et al., 1990, 2006; Frank et al., 1988; Onder et al., 2006), adrenoceptor agonists and antagonists (Peskind et al., 2003; Raskind et al., 2003, 2007; Taylor et al., 2008) as well as anticonvulsants and antipsychotics (Hageman et al.,2001; Berlin, 2007) (for more detail see review by Steckler and Risbrough 2012, Bradley et al., 2013, Jonas et al., 2013 and Table 2.8).

Table 2.8: Pharmacotherapies that have been investigated and prescribed for PTSD treatment

Drug class	Drugs	References
Selective serotonin re-uptake inhibitors (SSRIs)	fluoxetine, sertraline	Marshall et al., 2001;Tucker et al., 2001; Davidson et al., 2001; van der Kolk et al., 2007; Xu et al., 2011; Panahi et al., 2011; Chen et al., 2013
Serotonin–norepinephrine reuptake inhibitors (SNRIs)	venlafaxine, nefazodone*, mirtazapine**, bupropion	Davidson et al., 2006; Davis et al., 2004; Becker et al., 2007; Sonne et al., 2013
Tricyclic and tetracyclic antidepressants (TCAs)	imipramine, amitriptyline, desipramine	Kosten et al., 1991; Dow et al., 1997; Davidson et al., 1990; Theeler et al., 2012; Petrakis et al., 2012
Monoamine oxidase inhibitors (MAOIs)	phenelzine, brofaromine***	Shestatzky et al., 1988; Baker et al., 1995; Weizman et al., 1996; Connor et al., 2001; Aurora et al., 2010
Anticonvulsants	lamotrigine, topiramate, tiagabine, divalproex	Hertzberg et al., 1999; Tucker et al., 2007; Davidson et al., 2007; Davis et al., 2008; Bajor et al., 2011; Kozarić-Kovačić et al., 2013
Benzodiazepines	alprazolam, temazepam	Braun et al., 1990; Mellman et al., 2002; Hermos et al., 2007
NMDAR modulators	D-cycloserine	Heresco-Levy et al., 2002; Litz et al., 2012; Philbert et al., 2013
Atypical antipsychotics (as an adjunctive therapy in combination with antidepressant therapy)	risperidone monotherapy, olanzapine,	Padala et al., 2006; Butterfield et al., 2001; Stein et al., 2002; Rothbaum et al., 2008; Reich et al., 2004; Hamner et al., 2003; Detweiler et al.,2011; Carey et al., 2012; Krishnamurthy et al., 2013
Adrenergic inhibitors	propranolol, prazosin,	Pitman et al., 2002; Stein et al., 2007; Hilakivi et al., 1984; Raskind et al., 2003; Taylor et al., 2008; Aurora et al., 2010; Poundja et al., 2012
Corticosteroids	Hydrocortisone	Schelling et al., 2001; Wingenfeld et al., 2013

SSRI - selective serotonin re-uptake inhibitors, SNRI - serotonin norepinephrine reuptake inhibitors, TCA - tricyclic antidepressants, MAOI - monoamine oxidase inhibitors, NMDA - N-Methyl-D-aspartate. *serotonin reuptake inhibitor and a 5-HT2A receptor antagonist, **α2-adrenergic antagonistwith 5-HT2- and 5-HT3-blocking properties, ***reversible MAOI.

2.4.1 *N*-methyl-D-aspartate receptors

The therapeutic target of interest in this PhD study is the NMDAR, one of the pharmacological targets for secondary prevention of PTSD and a major role player in the memory consolidation processes. Activation of the NMDARs requires the binding of both glutamate and the co-agonist glycine for efficient opening of the calcium channel. Upon opening of the channel, intracellular calcium concentrations increase which activates signal transduction pathways critical to the plasticity underlying fear extinction (Myers and Davis, 2002) (Fig. 2.4). NMDAR agonists have been investigated in the treatment of PTSD (Ledgerwood et al., 2003; 2005; Walker et al., 2002; Yang and Lu, 2005; Litz et al., 2012; Philbert et al., 2013).

Administration of NMDAR antagonists (a ligand that binds to the receptor and blocks or dampens agonist-mediated responses) shortly after predator stress exposure was found to interfere with anxiety-related behaviour in rats (Adamec et al., 1999) (Refer to Section 2.1.2.3 Fear conditioning and extinction and

Section 2.2.4.1 Gene expression analyses in PTSD animal models, for more detail). Furthermore, a preliminary, retrospective study in a group of US military soldiers who had sustained thermal injuries during deployment found that those treated with the NMDAR antagonist, ketamine, during hospitalization, had lower incidence of developing PTSD (McGhee et al. 2008). Whilst NMDAR antagonists impair memory formation and LTP, agonists enhance those processes (see Myers et al., 2011 for a review). These findings support the utility of NMDAR subunits as novel pharmacological targets for the treatment of PTSD.
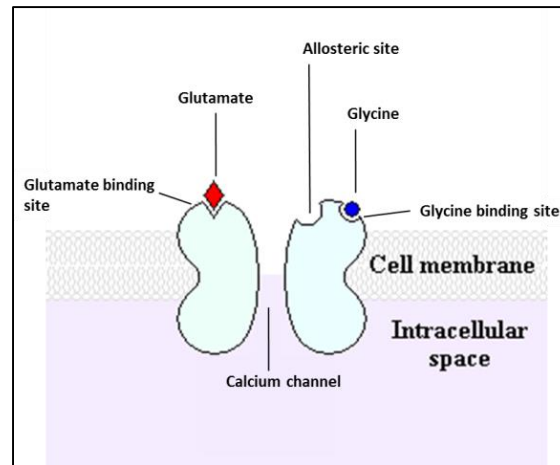
Figure 2.5: Diagram depicting an activated *N*-methyl-D-aspartate receptor. The receptor contains a glutamate and a glycine binding site, activation of the NMDAR requires the binding of both glutamate and a glycine for efficient opening of the calcium channel.

2.4.1.1 D-cycloserine

D-cycloserine (DCS) is an antibiotic and partial NMDAR1 agonist at the glycine site on the NMDAR1 receptor subunit and has been found to be effective in facilitating extinction learning in rats when administered before or immediately after extinction training (Ledgerwood et al., 2003; 2005; Walker et al., 2002; Yang and Lu, 2005; Philbert et al., 2013). DCS has also been shown to be effective in human trials of different anxiety disorders (Ressler et al., 2004; Hofmann et al., 2006; Kushner et al., 2007; Guastella et al., 2008; Wilhelm et al., 2008; Otto et al., 2010; Storch et al., 2010; Yamamoto et al., 2010; de Kleine et al., 2012). Administration of DCS has been found to result in generalized extinction of fear (Legderwood et al., 2005), a characteristic which could be of clinical benefit to PTSD, as the extinction of a single cue might generalize to other fear-associated cues simultaneously. This could greatly facilitate therapy for PTSD patients for whom many cues trigger fear responses.

DCS treatment has been found to augment exposure therapy (Smits et al., 2013), especially in patients suffering from more severe PTSD that require longer treatment (de Kleine et al., 2012). DCS has furthermore been shown to reduce the rate of relapse following successful exposure-based CBT (Richardson et al., 2004). Yamamoto et al. (2008) subjected rats to SPS, which is incorporated in an animal model of PTSD, and found that these rats exhibited impaired fear extinction relative to rats not subjected to SPS (sham rats). The study

also showed that DCS administration, in unison with extinction training, improved the impairment of fear extinction in SPS rats (Yamamoto et al., 2008).

Efficacy of DCS has especially been demonstrated in patients with maladaptive fear and rodents with prior stress exposure (Myers et al., 2011). Systemical injection of DCS as well as infusion into the mPFC, BLA and hippocampus of rats has shown to enhance fear memory extinction, suggesting regional specific effects (Myers et al., 2011). Interestingly, BDNF Met66 knock-in mice have demonstrated impaired NMDAR dependent synaptic plasticity (Ninan et al., 2010) as well as delayed fear extinction; systemic DCS administration reversed these effects (Yu et al., 2009). These results suggest that DCS may mediate its actions through the BDNF system (Andero and Ressler 2012).

In a study that investigated expression and extinction of fear memory in the lateral amygdala, found that fear conditioning resulted in an increase in AMPA/NMDA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/ *N*-methyl-D-aspartate) ratio as well as depression of paired-pulse facilitation (PPF) in neurons of the lateral nucleus of amygdala (Lin et al., 2010). The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) is a non-NMDA-type ionotropic transmembrane glutamate receptor that facilitates fast synaptic transmission in the CNS. An increase in the ratio of AMPAR to NMDARs (following high-frequency stimulation) is one of the key indicators of LTP induction. PPF occurs when postsynaptic potentials, evoked by an impulse, are increased when that impulse closely follows a prior impulse; PPF is thus a form of short-term synaptic plasticity (Zucker and Regehr 2002). Lin et al., 2010 found that extinction training did not affect the conditioning-induced changes in synaptic transmission. Administration of DCS before extinction training facilitated extinction and reversed the AMPA/NMDA ratio increase without changing the PPF depression. Extinction training significantly increased the amplitude and frequency of miniature inhibitory post-synaptic currents and DCS treatment didn't have an influence on these effects. DCS-induced reversal of AMPA/NMDA ratio and the facilitation of fear extinction was blocked by the disruption of AMPA receptor endocytosis with a synthetic peptide containing a short C-terminal sequence of GluR2 (869YKEGYNVYG877, GluR23Y). Extinction training appears to mainly increase inhibitory transmission without altering conditioning-induced excitatory association, whilst DCS (administered before extinction training) does not affect inhibitory transmission but reverses the conditioning-induced post-synaptic memory trace (Lin et al., 2010).

To gain more insight into the molecular function of drugs that potentiate GABAergic or glutamatergic systems during extinction, Leslie and Norwood (2013) compared operant extinction with re-extinction to investigate the neuropharmacological mechanisms with pre-session chlordiazepoxide (CDP) or post-session DCS. Both chlordiazepoxide and DCS facilitated extinction and re-extinction, with CDP showing an earlier effect during re-extinction. These results replicate earlier findings that DCS, administered post-session, facilitated extinction of food-reinforced behaviour (Shaw et al., 2009). DCS administration prior to extinction sessions failed to achieve extinction (Vurbic et al. 2011). The study by Leslie and Norwood

59

(2013) furthermore showed that DCS facilitated re-extinction to a similar extent as the initial extinction and that the effect was greater during re-extinction (as was also the case with CDP and re-extinction), although the mechanisms of actions of the two drugs on operant extinction are absolutely different (Leslie et al., 2012). These results of DCS facilitated re-extinction was contrary to other studies that found that re-extinction is not facilitated by the drug unless a different conditioned stimulus is used (Langton and Richardson, 2008, 2010). Leslie and Norwood (2013) hypothesised that after extinction sessions, DCS acts on a mechanism that is common to both extinction and re-extinction, such as the mediation of extinction of Pavlovian conditioning (Vurbic et al. 2011). These findings, indicating that operant re-extinction may be facilitated by DCS post-session administration, have important implications for human clinical use.

The precise mechanisms by which co-administration of DCS reduces the fear triggered by a traumatic context remain to be fully elucidated. It has been suggested that DCS may function by increasing the efficiency of exposure-based CBT (Norberg et al., 2008; Hofmann et al., 2006; Ressler et al., 2004). Another possibility is that when DCS activates the strychnine-insensitive glycine-binding site of the NMDAR, it facilitates NMDAR-mediated synaptic potentials and subsequently assists in learning and memory (Yamamoto et al., 2010).

2.4.2   Epigenetic drugs

Since the advent of the phrase 'personalised medicine' there have been high expectations that patient-specific pharmacogenetic data will improve treatment outcomes in neuropsychiatric disorders. However, owing to the complexity of transcriptional regulation and the influence of environmental factors and the epigenome, simple translation of individual genetic information into personalised treatment is not enough. How could pharmacogenetics explain the fact that monozygotic twins, who are both treated for major depression with the same drug, exhibit different clinical responses? Why do some patients who suffer from recurrent major depression not show the same response to drugs as they did during a previous episode? The answers might lie in epigenetics seeing that the dynamic nature of DNA-methylation patterns and histone acetylation provide plausible explanations for some of these puzzling pharmacogenetic questions (Holsboer 2008).

Most of the epigenetic therapies target DNA methylation and histone deacetylation enzymes and several of these drugs (mainly developed to treat cancer) have been tested in clinical trials (Szyf 2009). Some DNMT inhibitors have been approved for clinical therapy (such as Azacytidine, Decitabine), other are in phase one, such as 5-Fluoro-2′-deoxycytidine at phase 1, whilst Zebularine is still in the preclinical phase of development (Amatori et al., 2010). Another strategy to achieve demethylation in the brain involves the use of histone deacetylase inhibitors (HDACis) (Simonini et al., 2006, Tremolizzo et al., 2002).

MicroRNA research provided novel insights into the molecular mechanism underlying the therapeutic effects of certain anxiolytic drugs (Zhou et al., 2009; Baudry et al., 2010). In addition, miRNAs also presents novel therapeutic targets for the treatment of anxiety disorders, either through directly targeting the miRNA itself or by targeting the targets of those miRNAs that have been associated with anxiety disorders (Malan-Müller at al., 2012). Please refer Section 2.3.2.4 MicroRNAs and Pharmacotherapies for Anxiety Disorders.

3. Methods and Materials

Ethics approval was granted for this study by the Stellenbosch University Research Ethics Committee: Animal Care and Use (REC: ACU) (Ref: ACU/2010/006 (A1)). The animal behavioural model used in this project was based on the PTSD mouse model described by Siegmund and Wotjak (2007). The animal behavioural section of the methods was performed by Ms Lorren Fairbairn as part of her PhD project (PhD in Psychiatry, Stellenbosch University, South Africa). For the present study, brain tissue from the left dorsal hippocampus (LDH) and peripheral blood samples were utilised. Please refer to Figure 3.1 for an overview of the methods used during this project.
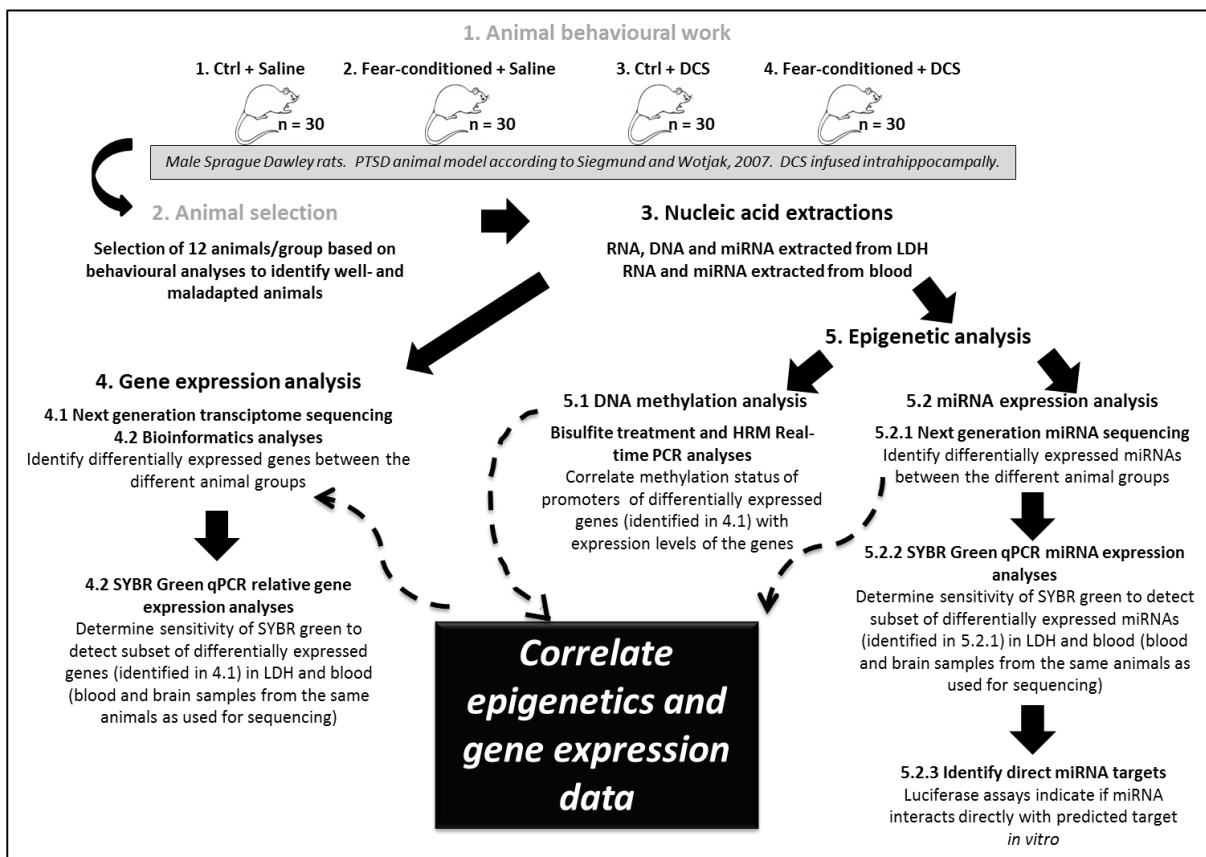
Figure 3.1: Methods overview flow diagram. Animal behavioural work (1) and animal selection (2) was performed by Ms L Fairbairn. n - sample number, ctrl – control, DCS – d-cycloserine, PTSD – posttraumatic stress disorder, LDH – left dorsal hippocampus, qPCR – quantitative polymerase chain reaction, miRNA – microRNA

3.1 Animal studies

3.1.1   Overview of the PTSD animal model

Exaggerated implicit fear memories underlying PTSD can be attributed to both associative fear conditioning and non-associative sensitization processes (Antelman, 1988; Charney et al., 1993; Sorg and Kalivas, 1995). The symptoms associated with the traumatic memory (e.g. flashbacks and avoidance of trauma-related cues)

are part of the associative fear component, while those symptoms not associated with the traumatic memory (e.g. hyperarousal and exaggerated startle) are the non-associative, sensitised fear component.

Animal models of PTSD, in addition to meeting the criteria for face, construct and predictive validity, should also demonstrate that animals exposed to trauma-related stimuli (associative fear) display conditioned responses, and that these conditioned (fearful) responses are also elicited following the presentation of a novel, non-trauma-related stimuli (thus eliciting non-associative fear). The PTSD animal model used in this study was based on a modified PTSD mouse model that was originally described by Siegmund and Wotjak (2007), incorporating the associative and non-associative properties of the PTSD phenotype. The model they described meets the criteria of construct and predictive validity, and is characterised by utility value (Siegmund and Wotjak, 2007). In the current study, ten repetitive footshocks (each lasting one second) over a period of one minute was used during fear conditioning instead of the single 2 sec electric footshock described by the original model (Siegmund and Wotjak, 2007). No further modifications were done to the PTSD evoking model (i.e. the administration of the electric footshocks), the only other modification was that male Sprague-Dawley rats were used instead of male C57BL/6N (B6N) or C57BL/6JOla (B6JOla) mice (as described by Siegmund and Wotjak (2007)). The main reason for this modification was the fact that the animal facility at the current research institution only bred Sprague-Dawley rats and these rats were successfully used in other behavioural research projects at the institution. It is therefore believed that the animal model still met the criteria of construct and predictive validity, and is characterised by utility value (Siegmund and Wotjak, 2007). Rats were bred in the central research facility at the University of Stellenbosch. The day of birth was designated as postnatal day (PND) 1. The pups were weaned at PND 21 and were subjected to regular manual handling to eliminate the possibility of introducing a novel stressor (manual handling) during later stages of the model. Animals were housed according to standard laboratory conditions as stipulated by the Ethical Guidelines of the University for Housing Experimental Animals. The housing room is separate from the location where the surgical procedure, behaviour recordings and dissections took place.

D-cycloserine (DCS) was injected intrahippocampally via a surgical procedure, 30 minutes before the fear extinction procedure took place. The surgical implantation was performed on PND 59. Briefly, rats were anaesthetised intraperitoneally with a combination of ketamine hydrochloride (Anaket-V, Bayer Healthcare, South Africa) and medetomide hydrochloride (Domitor, Pfizer, South Africa) at a dose of 0.1 ml/100 g. Anaesthetised rats were then placed in a stereotaxic instrument, and after exposing the skull, an opening was drilled at the precise coordinates corresponding to the target area, the dorsal hippocampus, according to co-ordinates given by the rat brain atlas (http://www.scribd.com/doc/22822097/Rat-Brain-Atlas). Bilateral infusions were done (i.e. both sides of the dorsal hippocampus therefore left and right dorsal hippocampus). An intracranial indwelling guide cannula was lowered into the opening for DCS or saline intrahippocampal infusion at a later stage, and was secured on the skull with dental cement. DCS (Aspen Pharmacare, Durban, South Africa) was prepared fresh daily (immediately before administration), by dissolving it in physiological

saline to a concentration of 10 μg (Walker et al., 2002). A needle was subsequently inserted into the guide cannula for the administration of DCS or saline. After infusion, the needle was left in place for a further two minutes to allow diffusion from the tip of the needle into the hippocampus. DCS and saline was administered for 30 minutes before initiation of each fear extinction protocol for six consecutive days (PND 62 – PND 67) (Walker et al., 2002), during the light phase of the light/dark cycle. The timeline followed for the PTSD animal model is shown below in Figure 3.2.
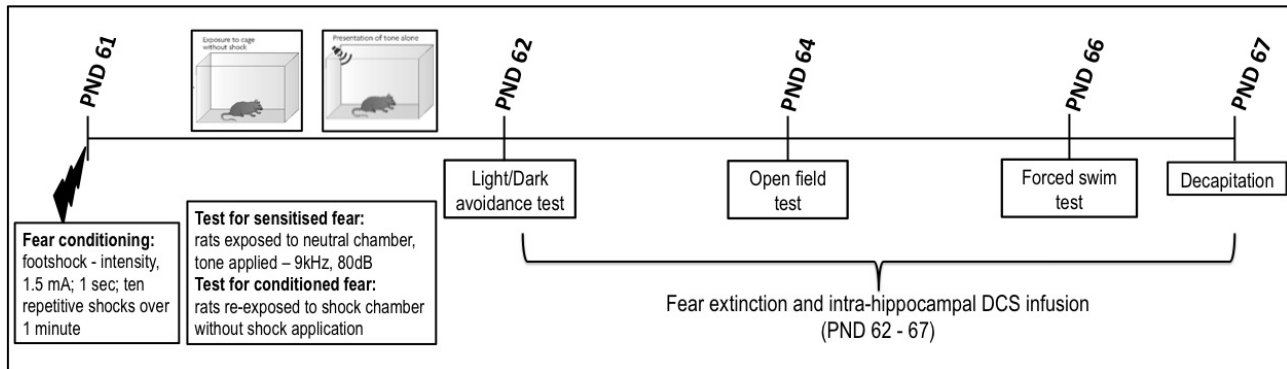
Figure 3.2: Experimental schedule for the PTSD animal model illustrating the behavioural procedures conducted at various time points. Fear conditioning was performed on PND 61 and consisted of administration of a series of 10 single electric footshocks, thereafter, to test for conditioned fear, rats were re-exposed to the shock chamber for another 3 min without stimulation. The rats were then placed in neutral chamber, after 3 minutes a tone was presented for 1 minute, thereafter rats remained in the chamber for another 60 seconds; this tested for sensitised or non-associative fear. For the next 6 days (PND 62 – PND 67) rats received intrahippocampal DCS infusion followed by a fear extinction paradigm, during which they were re-exposed to the shock chamber for 3 minutes without stimulation to test for conditioned fear. Behavioural tests were conducted to evaluate features and symptoms of fear conditioning (PND 62, PND 64 and PND 66) and rats were sacrificed on PND 67. DCS – D-cycloserine, dB – decibel, kHz – kilohertz, mA – milliampere, PND - postnatal day, sec – second.

The animals were divided into four experimental groups.

1. Control + Saline (CS): animals not subjected to fear conditioning and receiving intrahippocampal saline injections. ($n = 30$)

2. Fear-conditioned + Saline (FS): animals subjected to fear conditioning and receiving intrahippocampal saline injections. ($n = 30$)

3. Control + D-cycloserine (CD): animals not subjected to fear conditioning and receiving intrahippocampal DCS injections. ($n = 30$)

4. Fear-conditioned + D-cycloserine (FD): animals subjected to fear conditioning and receiving intrahippocampal DCS injections. ($n = 30$)

3.1.2   Fear conditioning, fear extinction and behavioural analyses

A single electric footshock (shock intensity, 1.5 mA; shock duration, 1 seconds (sec); ten repetitive shocks over the period of one minute (min)) was used during fear conditioning and served as the US. The footshock was administered via a chamber with a metal grid floor, connected to an electrical current supply. Following the footshock stimulation, rats remained in the chamber for an additional 60 sec, after which they were returned to their home cages. Control animals were placed in the shock chamber, but did not receive the electric footshock. Fear conditioning commenced on PND 61 (Fig. 3.2).

To test for conditioned or associative fear, the animals were re-exposed to the shock chamber for three minutes, without shock application or tone presentation, after which they were returned to their home cages. Animals were then placed in a neutral test chamber for three minutes, and a neutral tone (80 dB, 9 kHz) was administered; this also tested for sensitised or non-associative fear. The animals remained in the neutral chamber for another 60 seconds, before being returned to their home cages. The fear extinction procedure was carried out for six consecutive days (from PND 62 – PND 67). On PND 62, 64 and 66, the fear extinction protocol was performed prior to the behavioural tests (Fig. 3.2).

The core features of PTSD were assessed by measuring freezing behaviour during:

(1) re-exposure to the shock context (associative fear memory)
(2) exposure to a neutral tone in a novel context (non-associative fear memory).

Freezing movements are defined as immobility displayed by the animal, except for respiratory movements (Holahan and White, 2002).

Additional symptoms of PTSD include emotional blunting, social withdrawal or avoidance (DSM-5, APA 2013), levels of comorbid depression have also been described (Cryan and Mombereau 2004). The PTSD animal model therefore also tested for signs of social withdrawal and stress-coping strategies by using the following tests:

(1) the light/dark (L/D) avoidance test
(2) the forced swim test (FST)
(3) the open field test (OF test)

All behavioural assessments were performed between 10h00 and 13h00.

3.1.2.1 Light/dark avoidance test

The L/D avoidance test was performed on PND 62; the test is based on rodents' instinctive aversion of brightly lit areas and their spontaneous exploratory behaviour in response to mild stressors, i.e. the novel environment and light (Crawley and Goodwin, 1980). Upon exposure to an unfamiliar environment or to

novel objects, the animal is faced with a natural conflict situation between the initial tendency to avoid the unfamiliar (neophobia) and the tendency to explore. An increase in locomotion and time spent in the light compartment is typical of anxiolytic behaviour. Decreased locomotion and more time spent in the dark compartment are indicative of anxiogenic behaviour (Bourin and Hascoët, 2003). For the purpose of this study, the test arena was split in half, resulting in one illuminated and one dark compartment, connected by an open doorway. The animals had a habituation time of 5 minutes to eliminate the stress of a new environment. Rats were then placed in the middle of the dark compartment and during the 10 minute test, end-parameters, such as movement and exploration, were recorded by video camera and analysed with the EthoVision XT programme (Noldus, Wageningen, The Netherlands). After completion of the test, animals were returned to their home cages. Between each trial, the floor of the arena was cleaned with soap water.

The following parameters were measured:

- Horizontal locomotion (total distance travelled)
- Exploration (number of rearings)
- Relative time spent in dark compartment
- Relative distance moved in dark compartment

3.1.2.2 Open field test

The open field (OF) test was performed on PND 64. The test measures behaviours elicited by the placing the animal in a novel open space, surrounded by a wall (to prevent escape). The OF test is based on conflicting innate tendencies of animals to avoid open and brightly lit spaces and the tendency to explore a novel environment. The OF test can also be used to assess basal animal locomotor activity and exploration. Animals prefer to spend time around the edges, since they are fearful of the exposed open area, which would render them vulnerable to predators. Freezing behaviour and avoiding the centre of the field is indicative of "anxiety-like" behaviours (Cryan and Sweeney, 2011), however, numerous other parameters, such as distance covered, escape attempts, latency and field area visited can also be measured as a proxy for locomotion, exploration and anxiety. In this model, the rat was placed in the bottom right quadrant of the board and was left in the novel context for a period of 10 minutes. Behaviour was recorded with a video camera and was analysed with the EthoVision XT programme (Noldus Wageningen, The Netherlands).

The following parameters were measured:

- Time spent in zones
- Locomotion

3.1.2.3 Forced swim test

The forced swim test (FST) was performed on PND 66. The FST assesses levels of depression in animal models; this model is commonly used to measure the effect of antidepressant drugs on behaviour. It usually

comprises of perspex cylinders (about 18 cm in diameter), filled to a height of about 15 cm with ambient (25 °C) water. Rats are gently placed into the water for a period of 10 min, whereafter they are placed in a warm room to dry and returned to their cages. The duration of floating (lack of movement except for necessary movement to keep the head above the water), swimming (forward motion through the water, forepaws do not break through water surface) and struggling (upright position in the water, forepaws break through water surface) can be scored.

3.1.2.4 Harvesting of rat tissues for use in genetic analyses

After completion of the behavioural tests on PND 66, the rats were sacrificed on PND 67 by decapitation by means of a guillotine. This method inflicts the least amount of stress in the animals and also limits subsequent effects on mRNA and epigenetic expression in the brain. Decapitation was also necessary in order to obtain the brain tissue on which the genetic and epigenetic analyses were done. The left hippocampal tissue was then divided into left ventral and left dorsal hippocampal tissues (LVH and LDH, respectively). The LDH tissue from all 30 animals from each of the abovementioned groups as well as whole blood from each animal was used in the present study for genetic and epigenetic analyses. Due to the high level of N-methyl-D-aspartate receptors (NMDARs) (DCS is a partial NMDAR agonist) and the fact that it plays a pivotal role in fear extinction and retention processes (Fredrikson et al., 1976; Quirk and Mueller 2008), the hippocampus was selected as the brain region for direct DCS infusion. The LDH region was used in genetic and epigenetic analyses in order to determine the effects of intra-hippocampal DCS administration in this brain region.

3.1.3   Statistical analyses of behavioural data

In order to determine whether the fear conditioning model was effective in establishing fearful behaviours in fear-conditioned animals, statistical analyses were used to determine if there was a significant difference between the different treatment groups for each of the following behavioural measurements:

(1) time spent in the open field and outer regions

(2) time spent in the light and dark compartments

(3) duration of freezing in response to the tone in the neutral box

(4) duration of freezing in the open field test.

In order to determine which treatment groups were significantly different, a one-way analysis of variance (ANOVA) and least square (LS) differences (LSD) *post-hoc* analysis comparing the four treatment groups were performed using Statistica v11 (StatSoft, Tulsa, USA). Based on the relative sensitivity of the behavioural measurements in detecting successful fear conditioning, animals were selected for behavioural subgrouping as described below (see Section 3.1.4).

3.1.4 Animal selection based on behavioural data

Animals display a range of behavioural responses to stressors, with varying degrees of behavioural disturbances. It is, therefore, important to distinguish between mal- and well-adapted animals (Cohen et al., 2003, 2004). Behavioural data were used in subsequent experiments to distinguish between animals that were fear-conditioned and exhibited PTSD-like behaviour (maladapted) and animals that were fear-conditioned, but did not exhibit PTSD-like behaviour (well-adapted).

In the L/D avoidance test, animals in the FS group spent more time in the dark compartment compared to the CS group ($p = 0.035$), thus indicating that the fear conditioning model was effective in eliciting a fearful response. Furthermore, there was a significant difference in the FS group (animals spent more time in the dark compartment, typical anxiogenic behaviour) compared to the FD group (animals spent more time exploring the light compartment, typical anxiolytic behaviour) ($p < 0.001$), suggesting that DCS was effective in reducing anxiogenic behaviour in fear-conditioned animals (refer to the Section 4.1 for detailed behavioural results).

Animals were thus subgrouped, for genetic and epigenetic analyses, based on these results. In addition, the selection of animals also took into account the available quantity and quality of RNA that would be sufficient for downstream analyses. Twelve animals were selected from the CS group, with a time spent in the light closest to the group's mean time spent in the light value of 311 sec; twelve animals were selected from the CD group with a time spent in the light closest to the group's mean time spent in the light value of 344.07 sec. Selecting animals with behavioural test measures closest to the group mean values ensured that animals with extreme behaviours were not selected in the control group, ensuring the 'normality' of the control groups. For both the FS and the FD groups, six animals that spent the most time in the light compartment were selected as the well-adapted group and six animals that spent the least amount of time in the light compartment were selected as maladapted. In addition, an RNA concentration of at least 75 ng/μl was required as this is the minimum concentration for next generation RNA sequencing purposes.

Statistical tests were used to determine if there was a significant difference between the FS maladapted (FSM) and FD well-adapted (FDW) groups in the L/D avoidance test measurements (since not only the animals with extreme behavioural results were selected, but RNA quantity and quality was also considered). A one-way analysis of variance (ANOVA) was performed with least square differences (LSD) *post-hoc* analysis to compare the LS means of the L/D avoidance test measurements between the FSM and FDW groups.

The 120 animals were divided into four subgroups according to the behavioural test results. For the control groups, twelve animals per group were selected (with L/D avoidance test values closest to the group mean) and for each of the fear-conditioned groups, six well- and six maladapted animals were selected for further

genetic analyses. These groups consisted of 12 CS, 12 CD, 6 FS well-adapted (FSW), 6 FSM, 6 FDW and 6 FD maladapted (FDM) animals.

3.2 Nucleic acid isolation

Animals were decapitated on PND 67 (adulthood), at which time the LDH was dissected and whole blood collected in EDTA tubes. 500 µl of whole blood was added to 1.3 ml of RNA*later*™ (Ambion, Inc., Austin, Texas). Left dorsal hippocampal brain tissue was placed into 1.2 ml of RNA*later*. These samples were immediately stored at 4 °C for 24 hours whereafter the samples were stored at -80 °C (to enable long term usaage). RNA*later*™ (Ambion, Inc., Austin, Texas) prevents RNA degradation ex vivo and halts further changes in gene expression once blood is obtained.

RNA, micro RNA (miRNA) and DNA fractions were isolated simultaneously but in separate fractions from the LDH using the RNeasy Plus Mini Kit (Qiagen, Hilden Germany) in conjunction with the RNeasy MinElute Cleanup Kit (Qiagen, Hilden Germany) according to the manufacturer's instructions. Brain tissue samples were lysed by placing the LDH tissues in 2 ml lysing matrix tubes (matrix D, 1.4 mm ceramic spheres) (MP Biomedicals, Ohio, USA) that contained 400 µl buffer RLT Plus (lysis buffer) (Qiagen, Hilden Germany) and 4 µl β-mercaptoethanol (MERCK, Darmstadt, Germany). Samples were homogenised in the FastPrep FP120 cell disrupter (MP Biomedicals, Ohio, USA) for 30 seconds at a time. This was repeated three times, and after each 30 second homogenisation step, the tubes were placed on ice.

Total RNA (tRNA) (also containing miRNA within the same fraction) was isolated from rat blood samples using the Mouse RiboPure™-Blood RNA Isolation Kit (Ambion®, Texas, USA). Blood samples were utilized to correlate gene expression and epigenetic results between brain tissue and blood.

3.2.1   Nucleic acid quantity and quality assessment

DNA

The quantity and quality of the DNA extracted from the LDH was assessed using the NanoDrop™ 1000 Spectrophotometer V 3.7 (Thermo Scientific, Delaware, USA). The software calculates the concentration based on the 260 nm measurement and reports the concentration in ng/µl. The 260/280 nm ratio is used to assess the purity of the DNA sample. A ratio of about 1.8 is indicative of pure DNA, values below 1.8 indicate possible contamination by a protein, phenol or other contaminants that absorb strongly at or near 280 nm.

RNA

The quantity and quality of RNA extracted from both the LDH brain tissue and blood was assessed with the RNA Nano 6000 kit (Agilent Technologies, California, USA) on the 2100 Bioanalyzer platform (Agilent Technologies, California, USA) according to the manufacturer's instructions. The Bioanalyzer is a

microfluidics-based platform for size determination, quantification and quality control of DNA, RNA, proteins and cells. The software displays the RNA quantity in ng/µl. An RNA integrity number (RIN) is used to assess the integrity or quality of RNA. RIN values range from 10 (intact) to 1 (totally degraded). An RNA sample with a RIN value higher than 7 is regarded as good quality (Thompson et al., 2007).

miRNA

The quantity and quality of miRNA (within the purified small RNA fraction) extracted from both the LDH brain tissue and blood was assessed with the Agilent small RNA kit (Agilent Technologies, California, USA) on the 2100 Bioanalyzer platform (Agilent Technologies, California, USA) according to the manufacturer's instructions. The software displays the percentage of miRNAs within the small RNA fraction and provides the concentration as pg/µl.

3.3 Gene expression analyses

3.3.1   Next generation RNA sequencing

Next generation RNA sequencing was performed on the selected 48 LDH RNA samples. Extracted and quantified RNA samples were sent to Novartis Institute for Bio-Medical Research, department of Biomarker Development (Basel, Switzerland). One microgram (µg) of RNA was used as starting material for the sample preparation with the TruSeq RNA Sample Preparation Kit v2 (Illumina, California, USA). The mRNA was purified using poly-A selection, followed by chemical fragmentation and complementary DNA (cDNA) conversion with random hexamer primers. Second strand synthesis generated double stranded (ds) cDNA (Fig. 3.3) for use in TruSeq library construction (Fig. 3.4).
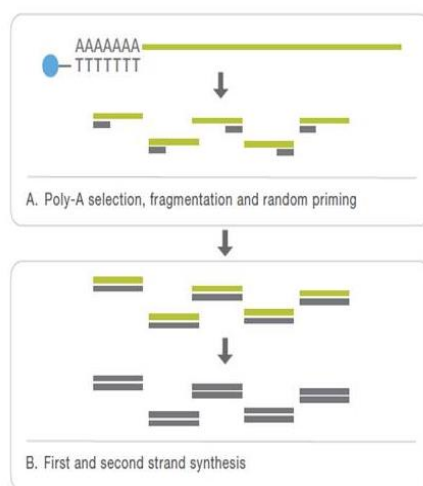
Figure 3.3: RNA sample preparation with Illumina TruSeq kit. Starting from large RNA fraction (tRNA minus small RNA) ds cDNA products ready for library preparation are created (Adapted from http://www.illumina.com)

The ds cDNA (generated during RNA sample preparation [Fig. 3.3]) was used to generate blunt-end DNA fragments by using different fill-in reactions and exonuclease activity (Fig. 3.4 A). An adenine (A) base was added to the blunt ends of each strand (Fig. 3.4 B) in preparation for ligation to the sequencing adapters. The 3' end of each adapter has a thymine (T) overhang, which provides a complementary overhang for adapter ligation (Fig. 3.4 C) to the A-tailed fragmented cDNA. The adapter sequence contains the full complement of the sequencing primer hybridization sites for paired-end multiplexed reads. Purified DNA fragments were denatured and PCR-amplified (Fig. 3.4 D) using primers complementary to the adapters. This generates enriched libraries that can be pooled and used for cluster generation on the cBot (Illumina, California, USA) (an automated system used for clonal cluster generation from single molecule DNA templates in preparation for sequencing by synthesis on a next generation sequencing platform) (Fig. 3.5).
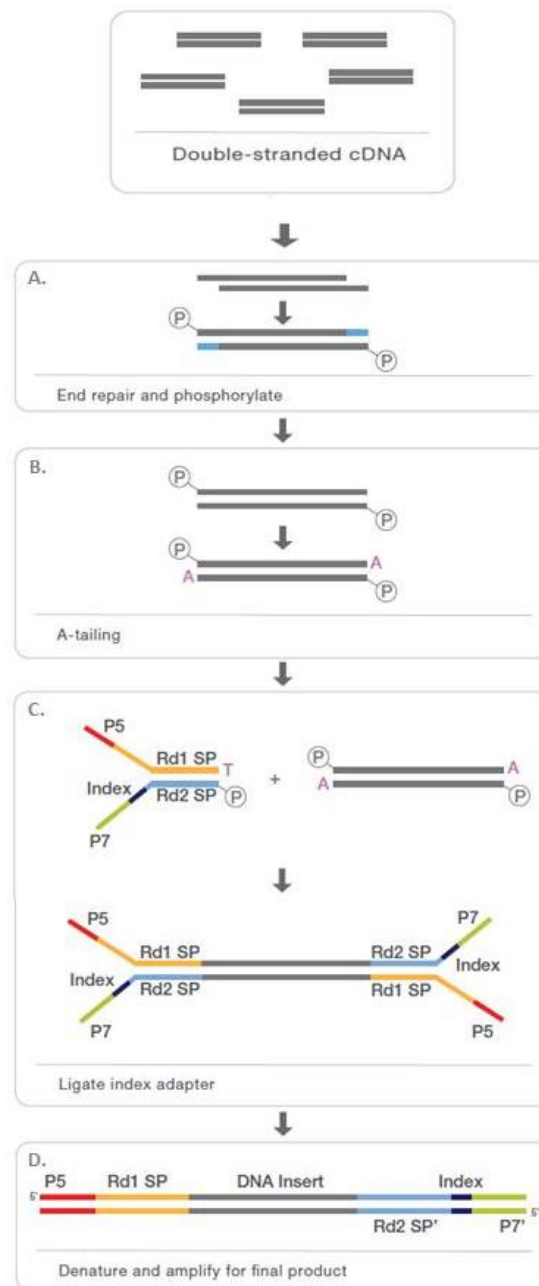
Figure 3.4: Adapter ligation and library construction. Double stranded cDNA (Fig. 3.3) was used to generate adapter ligated libraries that were used for cluster generation (Adapted from http://www.illumina.com)

Clusters were generated on the cBot (Illumina, California, USA) on paired end TruSeq v3 flow cells (Illumina, California, USA) using the TruSeq PE (*Paired-End*) Cluster v3-cBot-HS kit (Illumina, California, USA) according to the manufacturer's instructions. During this process, adapter-ligated DNA fragments bind to complementary adapter oligomers grafted on the flow cell's surface (Fig. 3.5 A). Starting from the hybridized primer, the templates are copied using 3' extension by incubation with free nucleotides and a high fidelity DNA polymerase (*Bst*), in a process called bridge amplification (Fig. 3.5 B). Thereafter, formamide denatures the double-stranded DNA fragments, and the process is repeated (Shendure and Ji, 2008) (fig 3.5 C). Bridge amplification generates clonal clusters of identical DNA fragments of about 1000 copies each (Fig. 3.5 C). The DNA fragments are denatured during the last cycle and a sequencing primer is annealed to

each DNA strand (Fig. 3.5 D) (Shendure and Ji, 2008; Tucker et al., 2009). The kit also contains reagents that allow cluster resynthesis of the reverse strand, regenerated by bridge amplification within the paired-end flow cell. Following resynthesis of the reverse strand, the original forward strand is cleaved and the reverse strand is sequenced for the second read. The DNA fragments are denatured during the last cycle and a sequencing primer is annealed to a universal sequence that flanks the region of interest (Fig. 3.5 D) (Shendure and Ji, 2008; Tucker et al., 2009).
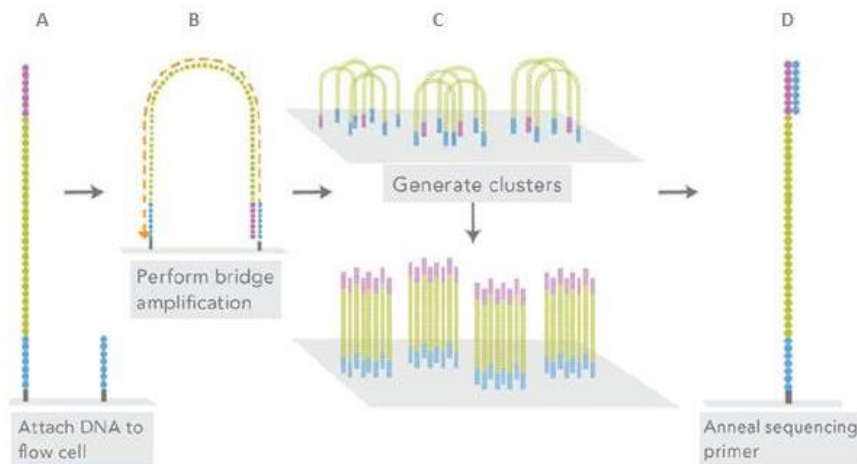
Figure 3.5: Cluster generation. Single DNA fragments are bound to the flow cell and bridge amplification is performed to generate clusters of identical molecules that can be sequenced (Adapted from http://www.illumina.com)

The flow cell, containing the DNA clusters, is subjected to sequencing on the Illumina HiSeq 2000 platform. The Illumina platform uses a cyclic reversible termination (CRT) method that involves three steps: nucleotide incorporation, fluorescence detection and cleavage. The nucleotides are chemically modified to contain a cleavable reverse terminator at their 3'-OH group that only allows single-base incorporation to occur in each cycle. The nucleotides also contain one of four fluorescent labels (Fig. 3.6). After each cycle, excess reagents are removed during a washing step and an image is taken of the clusters to identify the incorporated fluorescent labelled nucleotide (Fig. 3.6 D). This is followed by unblocking the 3'-ends of the nucleotides and cleaving of the fluorescent labels; the nucleotide incorporation cycle is then repeated (Fig. 3.6 B - D). Each flow cell has eight independent lanes and each lane contains 300 tiles, which are individually photographed. The images are captured by a camera and two lasers detect the four fluorescent colours. After the sequencing run, the images are analysed to identify the nucleotides incorporated at each position and to determine the sequence of the DNA fragment in each cluster (Fig. 3.6 D). One base is read per sequencing cycle, thus the number of cycles equals the read length (Ansorge, 2009; Metzker, 2010; Pettersson et al., 2009).
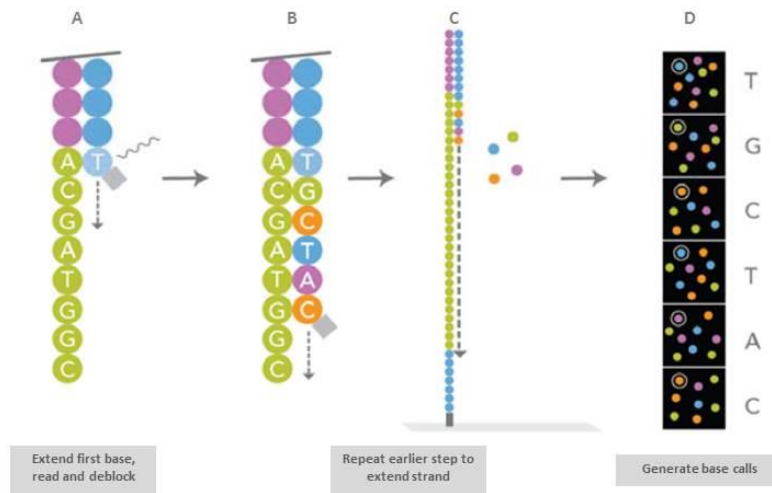
Figure 3.6: Sequencing by synthesis. The system applies a cyclic reversible termination (CRT) method, three steps are involved, namely nucleotide incorporation, fluorescence detection and cleavage (Adapted from http://www.illumina.com).

In the present study, two multiplex libraries were created for the 48 samples, with each multiplex library containing 24 samples. Each multiplex library was loaded onto four lanes of two separate flow cells (therefore eight lanes per multiplex library of 24 samples and 16 lanes in total for the 48 samples) and the 48 RNA samples were paired end sequenced on the Illumina HiSeq 2000 platform at a read length of 101 bp.

3.3.2   Differential gene expression analysis

In order to identify differentially expressed genes, the following comparisons were made:

a. FSM vs. FDW – to identify differentially expressed genes associated with fear extinction induced by DCS

b. CS vs. FSM – to identify differentially expressed genes associated with the process of fear conditioning

c. FSM vs. FSW – to identify differentially expressed genes associated with fear conditioning as well as susceptibility and resilience to developing anxiety disorders

d. FDM vs. FDW - to identify differentially expressed genes associated with efficacy of the drug or the mechanism of effective action

e. FDW vs. FSW - to identify differentially expressed genes associated with DCS-induced fear extinction compared to the "natural" process of fear extinction

The aim of the current study was to determine the molecular mechanisms whereby DCS facilitates fear extinction. For this reason, the comparison groups FSM vs. FDW formed a major focus of the current study as genes that are differentially expressed between these two groups may be associated with the fear extinction process specifically induced by DCS.

Bioinformatics analyses were performed in collaboration with the South African National Bioinformatics Institute (SANBI), University of the Western Cape (UWC). A pipeline was created to analyse the gene expression data. The following programs were implemented in the running of the pipeline:

1. *TopHat* (http://tophat.cbcb.umd.edu/): a splice junction mapper for RNA-sequencing reads that sequentially aligns the sequencing reads to known RefSeq mRNAs. The program then predicts combinations of known exons, and finally the remainder of the reference genome of interest, in this case the *Rattus novergicus* rn4 rat reference genome (http://support.illumina.com/sequencing/sequencing_software/igenome.ilmn) by using the ultra-high-throughput short read aligner *Bowtie*. The mapping results are then analysed by *TopHat* to identify splice junctions between exons.

2. *Bowtie* (http://bowtie-bio.sourceforge.net/index.shtml): a short read aligner that aligns short DNA sequences (sequencing reads) to the reference genome of interest.

3. *Cuffdiff* (http://cufflinks.cbcb.umd.edu/): calculates differential expression within the RNAseq samples and between different treatment groups. It assembles the alignments into a parsimonious set of transcripts from the aligned RNA-sequencing reads and estimates the relative abundance of these transcripts based on how many reads support each one (allowing for biases in library preparation protocols).

4. The *Bio-Ontological Relationship Graph* (*BORG*) database (designed by Dr. Gamieldien from SANBI, UWC, South Africa) identified biologically relevant genes associated, or predicted to be associated, with, fear, anxiety and memory (any factors or phenotypes associated with anxiety or PTSD) within the large set of differentially expressed genes identified by *Cuffdiff*. The *BORG* database integrates hundreds of thousands of curated facts about genes and their known functions, disease and phenotype associations, and pathway membership into a large knowledge structure known as a semantic network. At the heart of the database are human, rat and mouse genes, which are linked to each other and to relevant terms in several bio-ontologies, resulting in a large on-disk concept map-like structure. A custom semantic model for PTSD was built into the database by linking between terms in different ontologies so that the disorder is described by its known features. This ensured that any gene previously annotated as being involved in a phenotype, cellular process, or pathway known or hypothesized to be related to PTSD would be prioritised through a novel concept called 'guilt by indirect association'. For example, a rat gene whose counterpart in the mouse causes an anxiety phenotype (or one of its sub-phenotypes) when knocked out, was selected as a candidate in view of the anxiety concept being linked to the PTSD concept in the semantic model. The *BORG* semantic discovery protocol produced a shortlist of differentially expressed genes predicted to be involved in fear extinction and PTSD that could be utilised in downstream investigations (Fig. 3.7).
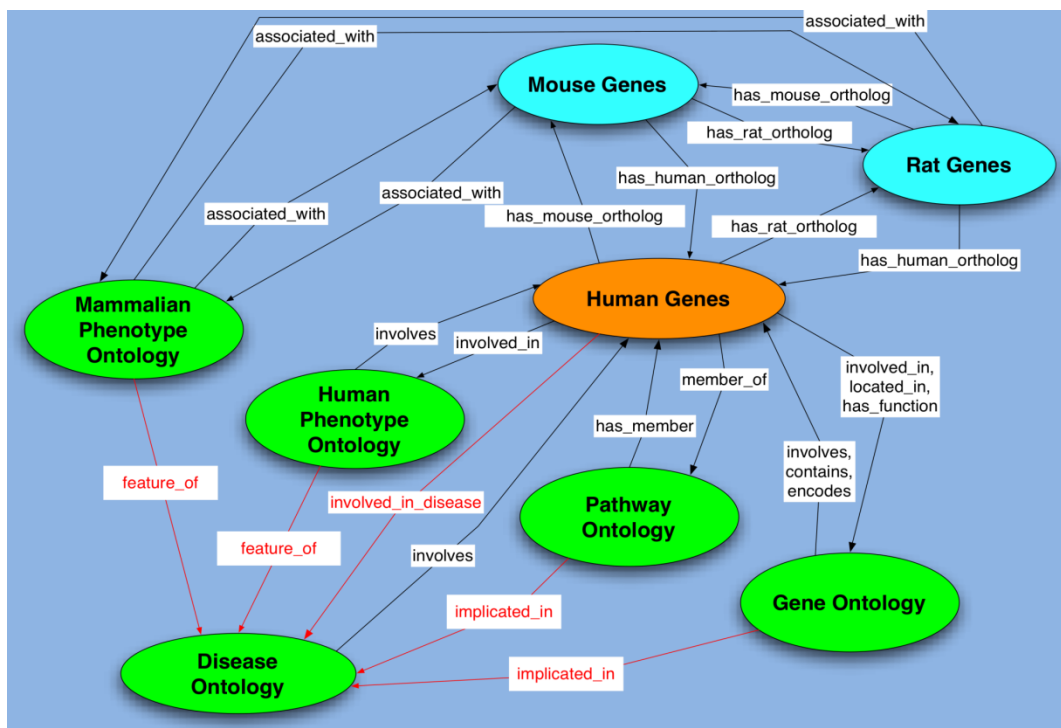
Figure 3.7: The Bio-Ontological Relationship Graph (*BORG*) database. Diagram illustrating how the biological evidence or knowledge is linked in the *BORG* database and how the semantic model of the disease is built using features of the disease (red links).

3.3.3   Gene enrichment analyses and clustering

The 42 biologically relevant, differentially expressed genes produced by *BORG* (fold change ≥ 1.3 and $p <$ 0.05) were used to perform gene enrichment analyses using the Comparative Toxicogenomics Database (CTD) (http://ctdbase.org/tools/analyzer.go). Biological interpretation of large sets of differentially expressed genes can be improved by grouping genes together based on their functional similarity (Cline et al., 2007). Genes were selected as the input type and the analyses options include '*enriched diseases*', '*enriched GO functional annotations*' or '*enriched pathways*'. For the '*enriched GO functional annotations*' *Section*, genes can be classified according to '*biological process*', '*molecular function*' or '*cellular component ontologies*'. Categories were considered overrepresented if $p < 0.05$. Cytoskape v2.8.3 (http://www.cytoscape.org) was used to generate visual models of the molecular interaction networks and biological pathways. Cytoscape is an open source software platform that can be used to visualize biological pathways and molecular interaction networks and integrate these networks with gene expression profiles, annotations and other data.

3.3.4   SYBR Green real-time quantitative PCR gene expression analysis

From the subset of 42 biologically relevant genes identified by the *BORG* analyses, nine were selected (based on function and fold change) (Table 3.1) for relative real-time qPCR analyses using SYBR Green technology on the ABI 7900HT platform (Applied Biosystems, Foster City, CA, USA). This was done in

order to determine whether real-time qPCR is a sensitive enough tool to detect these differences in gene expression.

Table 3.1: Genes investigated in the SYBR Green real-time qPCR differential expression analysis. Nine biologically relevant differentially expressed genes (selected based on fold change and function) used in the SYBR Green real-time qPCR differential expression analysis

Gene	Name	Fold change	Function
SPP1	Secreted phosphoprotein 1	-6.0	Demyelination
IL1RN	Interleukin 1 receptor antagonist	-4.2	Learning or memory
TRH	Thyrotropin releasing hormone	-2.8	Anxiety-related response
CYBB	Cytochrome b-245, beta polypeptide	-2.5	Abnormal learning or memory; nervous system physiology
MT2A	Metallothionein 2A	-2.1	Abnormal learning or memory
CXCL13	Chemokine (C-X-C motif) ligand 13	-5.2	Elevation of cytosolic calcium ion concentration
S100A4	S100 calcium binding protein A4	-3.5	Neuron projection; calcium ion binding; calcium-dependent protein binding
MMP9	Matrix metallopeptidase 9	-2.4	Impaired contextual conditioning behaviour
NPY	Neuropeptide Y	-1.3	Increased anxiety-related response; abnormal depression-related behaviour

Please refer to list of abbreviations for full gene names

The RNA extracted from the LDH and blood was reverse transcribed to cDNA by means of the QuantiTect® Reverse Transcription Kit (Qiagen, Hilden Germany) according to the manufacturer's instructions. A starting amount of 300 ng of RNA was used for reverse transcription (RT). cDNA was evaluated on the NanoDrop™ 1000 Spectrophotometer v3.7 (Thermo Scientific, Delaware, USA) to get a rough estimate of the concentration of cDNA. A calibrator sample was prepared by pooling equal amounts of cDNA from each sample for the construction of a standard curve. The calibrator cDNA sample was serially diluted 2 fold per dilution, to produce a seven-point standard curve, with cDNA concentrations ranging from 800 ng to 12.5 ng. Each of the LDH and blood cDNA samples was diluted to 200 ng for use in subsequent gradient PCR reactions (for annealing temperature optimisation) and real-time qPCR reactions.

Each 25 µl reaction consisted of 1 x KAPA SYBR®FAST Master Mix ABI Prism™ (KAPA Biosystems, Massachusetts, USA) and forward and reverse primers (IDT, Iowa, USA) (see Table 2.2 for concentrations). Primers were designed and evaluated using the IDT PrimerQuest http://eu.idtdna.com/PrimerQuest/Home/Index) and OligoAnalyser tools (http://eu.idtdna.com/analyzer/Applications/OligoAnalyzer/). All primers spanned intron-exon boundaries to eliminate amplification of possible DNA contaminants. Reference gene primer sequences for β-actin (ACTB), cyclophilin A (Cyc A), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were described by

Bonefeld et al. (2008), and primers for phosphoglycerate kinase (Pgk) were described by Langnaese et al. (2008). Reference genes were also validated using geNorm and Normfinder, both of which are included in GenEx software package v5.4.3 (GenEx [www.multid.se]) (MultiD, Gothenburg, Sweden).

Each sample was analysed in triplicate in order to eliminate technical variability. Following the amplification of serial dilutions, a linear plot of the quantification cycle (Cq) versus the log value of the input amount of DNA (standard curve) was constructed using ABI's SDS v.2.3 software (Applied Biosystems, California, USA). The PCR efficiency was subsequently calculated from the standard curve of reaction for each individual primer set. Software-determined default threshold and baseline values were used.

All Cq values for each primer set were corrected for the PCR efficiency of the particular primer set and normalised to the aforementioned reference genes using GenEx software (http://www.gene-quantification.de/datan.html) (MultiD, Gothenburg, Sweden). Statistical analyses were performed using GenEx [www.multid.se] (MultiD, Gothenburg, Sweden) and Statistica v11 (StatSoft, Tulsa, USA). One-way ANOVA was performed to determine if there was a significant difference between the least square (LS) means of the gene expression fold change between the FDW and FSM animals for each of the primer sets. A p-value of ≤ 0.05 was regarded as significant.

Table 3.2: Primers for SYBR Green real-time qPCR differential expression analysis. Primer sequences, melting temperatures (Tm), annealing temperatures (Ta) and concentration of primers in each 20 µl SYBR Green real-time qPCR reaction.

Gene	Primer sequences (5' – 3')	Tm (°C)	Ta (°C)	Final primer concentrations (µM)
SPP1 F	TGTGTCCTCTGAAGAAACGG	54.6	54	0.12
SPP1 R	GGTGAGATTCGTCAGATTCATCC	55.2		
IL1RN F	GGGATACTAACCAGAAGACC	51.7	56	0.2
IL1RN R	CGAAAGTCAATAGGCACC	50.7		
TRH F	CCTAACTGGTATCCCTGAATCC	54.3	61	0.4
TRH R	GATGCTGGCGTTTCTCAG	53.8		
CYBB F	CCAGGTATCCAAGCTAGAGTG	53.8	54	0.2
CYBB R	GTCACAATATTTGTACCAG	45.6		
MMP9 F	CCTGTACCGCTATGGTTACACTC	56.9	58	0.2
MMP9 R	GATGACAATGTCTGCTTCGAGC	56.2		
MT2A F	GAACTCTACAGCGATCTCTCG	54.4	54	0.12
MT2A R	CGAAGCCTCTTTGCAGATG	53.9		
CXCL13 F	CTGGACCAAGGCCAAGAAAGC	58.8	61	0.6
CXCL13 R	CGAGCAGGGATTAAGAAAGGGTG	57.7		
S100A4 F	CACAAATACTCAGGCAACGAGG	56.0	58	0.6
S100A4 R	GCCCAACACTTCATCTGAGGAG	57.7		

NPY F	CAGCCCTGAGACACTGATTTC	55.6	56	0.2
NPY R	CAACGACAACAAGGGAAATGG	54.6		
ACTB F*	TGTCACCAACTGGGACGATA	55.7	54	0.2
ACTB R*	GGGGTGTTGAAGGTCTCAAA	55		
CYC A F*	TATCTGCACTGCCAAGACTGAGTG	58.7	54	0.2
CYC A R*	CTTCTTGCTGGTCTTGCCATTCC	58.6		
GAPDH F*	ACCACAGTCCATGCCATCAC	57.7	55	0.12
GAPDH R*	TCCACCACCCTGTTGCTGTA	58.6		
PGK F*	ATGCAAAGACTGGCCAAGCTAC	58.0	54	0.2
PGK R*	AGCCACAGCCTCAGCATATTTC	57.6		

*Primers designed to amplify regions of the reference genes *ACTB*, *CYC A*, *GAPDH* and *PGK*. Please refer to the list of abbreviations for full gene names

3.4 Epigenetic analysis

The study of epigenetics investigates the stable alterations in gene expression that are not attributable to DNA sequence changes (Bjornsson et al., 2004). We investigated whether DNA methylation and miRNAs were driving the differential gene expression detected with the RNA sequencing, and to what extent.

3.4.1   DNA methylation analysis

High resolution melt (HRM) analysis was used to investigate DNA methylation status of CpG islands of a subset of biologically relevant differentially expressed genes identified with RNA sequencing. The approach described by Wojdacz and Dobrovic (2007) was followed. Approximately 300 ng of DNA extracted from LDH as well as 300 ng DNA from rat pre-mixed methylation controls (EpigenDx, Massachusetts, USA) were subjected to bisulfite conversion using the EZ DNA Methylation-Lightning™ Kit (Zymo Research, California, USA), according to the manufacturer's instructions. Bisulfite modification of DNA converts unmethylated cytosine residues to uracil, while the methylated cytosines in CpG islands remain unconverted. The subset of genes for which real-time qPCR detected differential expression in the LDH (Results Section 4.2.4, Table 4.10) was analysed using EMBL EMBOSS Cpgplot (http://www.ebi.ac.uk/Tools/seqstats/emboss_cpgplot/) to determine which of these genes contained CpG islands. We selected these genes for DNA CpG island methylation analysis seeing as real-time qPCR was able to detect differential expression in two of these genes, these genes have functions that could contribute to PTSD disease pathology and if DNA methylation mediated the differential expression of these genes, these combined results would form a strong unit. The nine genes that were investigated for differential expression using SYBR Green real-time qPCR, were analysed with EMBL EMBOSS Cpgplot (http://www.ebi.ac.uk/Tools/seqstats/emboss_cpgplot/) in order to identify which of the selected genes contained CpG islands. CpG island methylation levels were therefore investigated in *MT2A*, *TRH* and *NPY* (Table 3.3). *NPY* contained two predicted CpG islands, the island closest to the transcription start site (TSS)

was investigated, as DNA methylation close to the TSS has been shown to prevent transcription factors and RNA polymerase from accessing the DNA, resulting in silencing of the gene (Strathdee and Brown, 2002; Turner, 2001; Hashimshony et al., 2003; Kass et al., 1997; Venolia and Gartler 1983). Primers for the amplification of bisulfite converted DNA were designed using Bisulfite Primer Seeker 12S (Zymo Research) (http://www.zymoresearch.com/tools/bisulfite-primer-seeker), a free online primer design tool created specifically for methylation analyses. This program designs primers specifically for bisulfite-converted sequences (see Table 3.4 for DNA methylation primers).

Table 3.3: CpG island chromosomal positions for *MT2A*, *TRH* and *NPY*

Gene	CpG island position	
MT2A	Chromosome 19: 11, 284, 267 – 11, 285, 166	
TRH	Chromosome 4: 189, 149, 064 – 189, 149, 408	
NPY	Chromosome 4: 144, 235, 298 – 144, 235, 513	Chromosome 4: 144, 235, 974 – 144, 236, 905

MT2A - metallothionein 2A, *NPY* – neuropeptide Y, *TRH* - thyrotropin releasing hormone

Real-time qPCR amplification and HRM analyses were carried out sequentially on the Rotor-Gene™ 6000 (Corbett Research, Mortlake, Australia). Each 25 µl qPCR reaction consisted of the following: 1x Kapa 2G Robust HotStart ReadyMix (KAPA Biosystems, Massachusetts, USA), 640 nM of the relevant forward and reverse primers (Integrated DNA Technologies (IDT), Iowa, USA), 4 µM SYTO®9 (Invitrogen, California, USA), and 2.5 ul (theoretical concentration of 20 ng/ul) of bisulfite-converted LDH DNA and calibration standards. Each sample was analysed in triplicate in order to eliminate technical variability. The bisulfite-treated rat calibration standards (including 100%, 75%, 50%, 25%, 10%, 5% and 0% methylated DNA) (EpigenDx, Massachusetts, USA) were included in all the reactions in order to determine the level of CpG island methylation within the investigated regions for each sample. The 36-sample rotor on the Rotor-Gene™ was used and samples were analysed in triplicate, thus the standards were amplified in the first run and re-melted in the HRM analyses for the 2nd and 3rd runs with the rest of the samples. The real-time qPCR cycling conditions were as follows: 95 °C for 5 min followed by 40 cycles of 95 °C for 10 sec, *annealing temperature* for 30 sec (see Table 2.4 for annealing temperatures of each primer set) and 72 °C for 25 sec. HRM analyses were performed at the optimised temperature ramping (see Table 2.3) and fluorescence acquisition settings were used as recommended by the manufacturer, with the temperature rising by 0.1 °C/2 sec.

During the real-time qPCR reaction, the intercalating dye, SYTO®9 (Invitrogen, California, USA), emits fluorescent light when it intercalates with the double stranded DNA (dsDNA) as the DNA amplifies exponentially during each cycle of the PCR, until it reaches a plateau. The real-time qPCR is followed by a HRM step. HRM exploits the differential melting temperature that exists between methylated and unmethylated alleles where the change in fluorescence is sequence-specific. The degree of methylation is

represented by the *C* to *T* content in the amplicon and this will determine the rapidity of melting and release of the intercalating dye. Thus, methylation is assessed across the amplified region of the CpG island as a whole, instead of at specific sites. The dsDNA products are melted from dsDNA into single-stranded DNA (ssDNA) during the HRM step resulting in a high emission of fluorescence from the SYTO®9 dye (Invitrogen, California, USA), which slowly decreases as the temperature increases and the dsDNA is melted into ssDNA. Normalised melting profiles were generated by the Rotor-Gene™ 6000 (Corbett Research, Mortlake, Australia) software (by calculating the 'line of best fit' for the region between two normalization regions, representing the melting of the PCR product). The algorithm enables direct comparison between samples with different starting fluorescence levels. The melting profiles of the LDH DNA samples were compared to the profiles of the calibration standards (EpigenDx, Massachusetts, USA) in order to determine the percentage methylation of the CpG islands for each sample.

Table 3.4: Primers for DNA methylation analyses. Primers designed for DNA methylation analyses of selected CpG islands, primer sequences, annealing temperatures, HRM conditions and amplicon sizes are indicated

Gene	Sequence	Annealing Temperature	Amplicon size	HRM conditions
*MT2A*_Forward	TAGGAGAAAGGGGGTGTGATTTAG	56 °C	347 bp	75 °C – 83 °C
MT2A _Reverse	TTACCTATAACACAAAAACAATTAAAATCCATAAC			
*TRH*_Forward	GGGACGTTTTTTTTTTTTTTGTTTTTTAGTTAGATG	53 °C	350 bp	72°C – 82 °C
TRH _Reverse	TACCTACGAAATAAAAACTCTACAAAACTTTC			
*NPY*_Forward	GGAGATTAGTAGGTTTAGTAGGTTTAGTAGGTTTAAG	56 °C	250 bp	71 °C -89 °C
NPY _Reverse	AATCCCRCTACCAAAAAAAAAAAATAAAAC			

MT2A - metallothionein 2A, *NPY* – neuropeptide Y, *TRH* - thyrotropin releasing hormone

3.4.2   MicroRNA expression analysis

3.4.2.1 Small RNA library preparation

The purified small RNA fraction (containing miRNAs and other small RNAs smaller than 200 nt) extracted from the LDH was used for cDNA library preparation with the TruSeq Small RNA Sample Preparation kit (Illumina, California, USA) according to the manufacturer's instructions**,** with modifications. The TruSeq Small RNA Sample Preparation kit protocol was followed for the 3' and 5' adapter ligation and reverse transcription steps. Normally a minimum of 1 µg of tRNA starting material (or the equivalent amount of miRNA found within 1 µg of tRNA) is required for cDNA library preparation. Due to the small size of rat LDH brain samples and the fact that we had initially separated the small and large RNA fractions, we had limited amounts of miRNA starting material (between 2 ng – 7 ng of miRNA). Subsequently, the PCR amplification section of the protocol was modified in order to generate sufficient product for use in the sequencing reaction. Instead of the TruSeq Small RNA Sample Preparation kit, we used a high fidelity polymerase, Velocity DNA polymerase (Bioline, London, UK), and increased the PCR cycle numbers from

the suggested 11 cycles to 20 cycles. Velocity DNA polymerase was chosen for a number of reasons: first, it has a high thermostability as well as 3'- 5' proofreading exonuclease activities; second, the polymerase has an error-rate of 4.4 x 10^{-7}, thus providing a 50-fold higher fidelity than *Thermus aquaticus* DNA polymerase (as determined by Mo (1991) and Fujii (1999)); third, Velocity exhibits high amplification rates (up to 66 bp/s or 15s/kb) and produces higher yields than most commercially available enzymes. The TruSeq PCR mix (PML) was replaced with 2.5 U Velocity DNA polymerase (Bioline, London, UK), 1 x HI-Fi buffer (containing 10mM Mg^{2+}) (Bioline, London, UK) and 1.4 mM deoxynucleotide triphosphates (dNTPs) (Bioline, London, UK). RNA PCR primer (RP1) and RNA PCR indexes from the TruSeq Small RNA Sample Preparation Kit (Illumina, California, USA) were used in the PCR reaction as indicated by the manufacturer's instructions. This adapted protocol was developed and optimised by using real-time qPCR and SYTO®9 (Invitrogen, California, USA) to determine the optimum PCR cycle number when DNA amplification is within the exponential phase.

Adapted PCR protocol for small RNA library preparation

After attempting amplification of the limited starting amount of small RNAs with the PCR mix (PML) from the TruSeq Small RNA Sample Preparation kit (Illumina, California, USA)**,** another high fidelity real-time qPCR mix was investigated. The TruSeq PCR mix (PML) was replaced with 2.5 U Velocity DNA polymerase (Bioline, London, UK), 1 x HI-Fi buffer (containing 10mM Mg^{2+}) (Bioline, London, UK) and 1.4 mM dNTPs (Bioline, London, UK) and 4 µM SYTO®9 (Invitrogen, California, USA). RP1 and RNA PCR indexes from the TruSeq Small RNA Sample Preparation kit (Illumina, California, USA) were used in the PCR reaction as indicated by the manufacturer's instructions. The sample 1 contained 1.3 ng and sample 2 contained 24.3 ng of miRNA starting material which was used and amplified with the adapted protocol on the Rotor-Gene^{TM} 6000 (Corbett Research, Mortlake, Australia) with the following cycle conditions: 98 °C for 2 min, 98°C for 30 sec, followed by 45 cycles of 60°C for 30 sec and 72°C for 20 sec, with final elongation at 72°C for 10 min. The Cq value of sample 1 was 15 and sample 2 had a Cq value of 12, where sample 1 reached the exponential phase of amplification around cycle 18 whereas sample 2 at cycle 14 (Fig. 3.8)

3.4.2.2 MicroRNA sequencing

The amplified libraries were size-selected (to ensure that the miRNA fraction was captured) using the Pippin Prep DNA size selection system with a 2% agarose gel (Sage Science, Massachusetts, USA). The Pippin Prep is a preparative electrophoresis platform that separates and extracts user-defined sizes of DNA fragments. The size of the adapters are 118 bp in total and miRNAs range in size from 18 bp – 25 bp, thus a final size of between 136 bp - 143 bp is expected for miRNA libraries. The size selection range of 132 bp – 156 bp was set slightly beyond these minimal ranges to ensure all possible miRNAs were included). The 12 size-selected library preparations were loaded onto the MiSeq® System (Illumina, California, USA), where

cluster generation and sequencing was performed over three consecutive single-read runs (all 12 samples were sequenced on three machines) using MiSeq Reagent Kits V2 for 50 cycles (Illumina, California, USA).
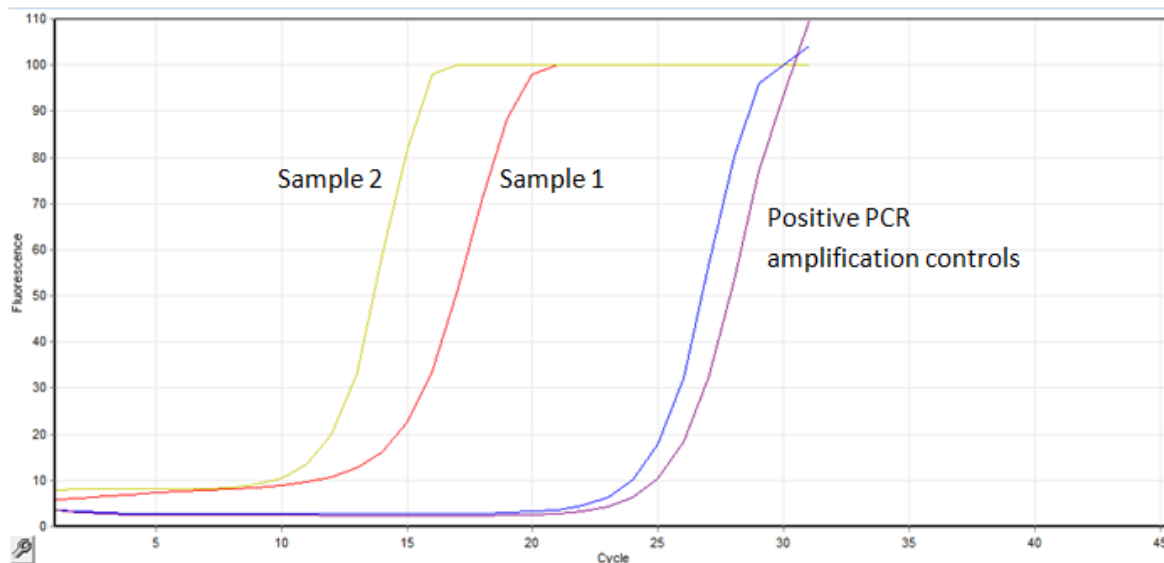
Figure 3.8: Real-time qPCR amplification curves generated during adapted PCR protocol for small RNA library preparation. Real-time qPCR amplification curves of test sample 1 and 2 using Velocity DNA polymerase, 1 x HI-Fi buffer and SYTO®9 dye

3.4.2.3 Bioinformatics analyses to identify differentially expressed miRNAs

Bioinformatics analyses were performed in collaboration with SANBI, UWC. A pipeline was created to analyse the miRNA expression data to identify differentially expressed miRNAs between the FDW and FSM groups, consisting of the following programs and analysis tools:

1. Novoalign (novocraft.com) was used to trim adapter sequences and to map the short reads to the rn4 *Rattus   novergicus*   reference   genome (http://support.illumina.com/sequencing/sequencing_software/igenome.ilmn)

2. The generalized fold change count facility (GFOLD) (Feng et al., 2012) was used to count the reads that mapped to known miRNA gene coordinates (from miRbase [http://www.mirbase.org/]). 100 000 iterations were performed during the sampling phase and a significant p-value cut-off of 0.05 was selected in order to identify differentially expressed miRNAs with GFOLD

3.4.3   Identifying mRNA targets of the differentially expressed miRNAs

The next step was to determine whether the significantly differentially expressed miRNAs targeted any of the differentially expressed genes identified following RNAseq analysis, in order to determine if the miRNAs mediated differential gene expression. Ingenuity Pathway Analysis (IPA) (http://www.ingenuity.com/) software was used for target enrichment analysis. First, functions of the differentially expressed miRNAs were predicted based on the functions of the genes they targeted. Second, IPA used the differential miRNA and gene expression data to predict if any of the differentially expressed genes were targeted by differentially expressed miRNAs. IPA utilizes miRecords

(http://mirecords.umn.edu/miRecords/), TargetScan human (http://www.targetscan.org/) and TarBase (http://www.microrna.gr/tarbase) to predict mRNA targets of the differentially expressed miRNAs. Additional manual searches were performed on databases that included *Rattus norvegicus* in the species list (these databases were MicroCosm, microrna.org and DIANA lab). Both MicroCosm and microrna.org tools use the miRanda algorithm to identify potential miRNA binding sites within a genomic sequence. The MicroCosm algorithm uses a weighted scoring system that rewards complementarity at the 5' end of the microRNA. The algorithm demands strict complementarity between the miRNA and target mRNA at the miRNA seed region and also estimates thermodynamic stability of the target region. Finally, every potential 3'UTR target site was investigated to determine if the site is conserved in orthologous transcripts from other species. For target a site to be conserved, it must be detected by a miRNA (of the same family) at the same position in a cross-species untranslated region (UTR) alignment in at least two other species than the species of interest (the complete process is depicted in Fig. 3.9).
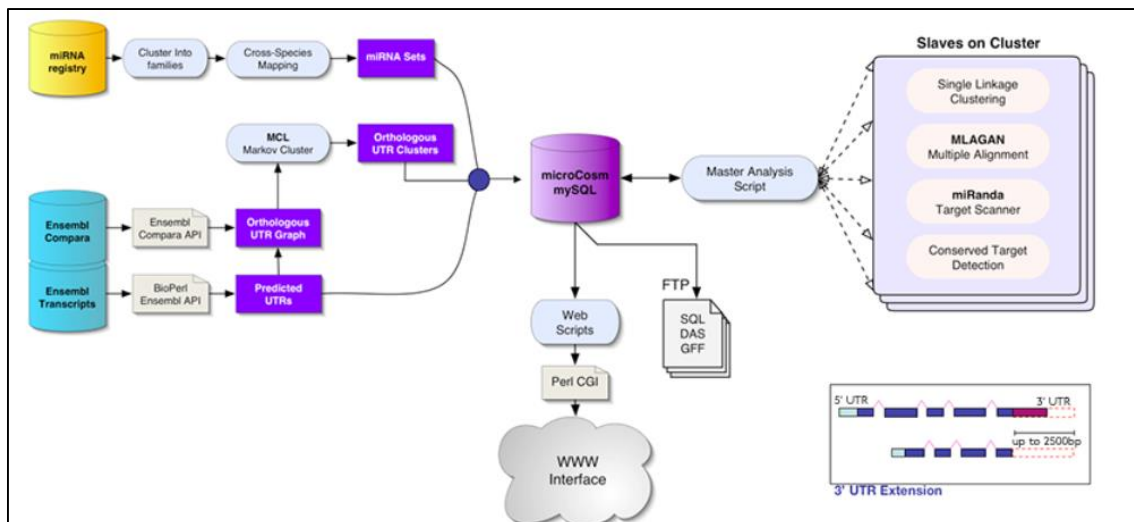
Figure 3.9: The complete computational prediction protocol incorporated in the MicroCosm prediction tool (http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/info.html)

MicroRNA.org uses miRSVR (miRNA support vector regression) (Betel at al., 2010) as a scoring tool. MiRSVR is a regression method that predicts the likelihood that a target mRNA will be down-regulated based on sequence and structure features in microRNA/mRNA predicted target sites. MiRSVR thus ranks microRNA target sites by a down-regulation score. miRanda, in combination with mirSVR (miRanda-mirSVR), predicts the extent of miRNA-induced down-regulation at the mRNA or protein levels. Importantly, the method identifies a significant number of experimentally determined non-canonical and non-conserved sites. MicroCosm thus produced predicted conserved miRNA targets and microRNA.org allowed identification of those miRNA targets that were not conserved (Betel at al., 2010).

3.4.4   SYBR Green real time qPCR expression analysis of rno-miRNA-31a-5p in LDH brain and blood

Rno-miRNA-31a-5p was one of the significant differentially expressed miRNAs identified with miRNA sequencing; furthermore, this miRNA was predicted to target *IL1RN* mRNA. This was significant to the present study, as *IL1RN* has previously been shown to be involved in learning or memory processes (Palin et al., 2004). In the FDW group, *IL1RN* was downregulated 4-fold compared to the FSM group, and real-time qPCR was also sensitive enough to detect this differential expression in the LDH and therefore this miRNA was selected to be used in SYBR Green real-time qPCR relative expression analysis as well as functional target verification luciferase assays.

To investigate if real-time qPCR analysis could be used to detect differential expression of rno-miRNA-31a-5p between the FDW and FSM group (in miRNAs extracted from LDH samples as well as tRNA extracted from rat blood samples), the All-in-OneTM miRNA qRT-PCR Detection Kit (GeneCopoeia, Maryland, USA) in combination with an All-in-OneTM miRNA qPCR primer was used (GeneCopoeia, Maryland, USA). The kit quantitatively measures miRNAs with the of use real-time qPCR technology. The experimental procedure includes three main steps, namely (1) poly-A tail addition: poly-A polymerase adds poly-A tails to 3' miRNA ends; (2) cDNA Synthesis: M-MLV RTase and a unique Oligo-dT Adaptor Primer is used to reverse-transcribe the poly-A miRNAs; (3) qPCR: SYBR Green qPCR specifically amplifies and detects the reverse transcribed miRNA (the miRNA-specific forward primer is used with the Universal Adaptor Primer).

The small nuclear RNA (snRNA) component of U6 small nuclear ribonucleoprotein (snRNP) was used as internal reference for normalisation of the miRNA expression data. It is an RNA-protein complex that combines with other snRNPs, unmodified pre-mRNA, and various other proteins to assemble a spliceosome. It is commonly used for normalisation of miRNA expression analysis due to its stable expression (Takamizawa et al. 2004; Choonget al. 2007; Corney et al. 2007; Pineles et al. 2007) and was supplied with the All-in-OneTM miRNA qPCR Primer. This real-time PCR method is more specific and sensitive, requires less sample material and is less time-consuming compared to traditional hybridization-based miRNA detection methods, such as Northern blot analysis.

The 25µl reverse transcription (RT) reaction consisted of 2.5 U Poly A Polymerase, 1µl RTase Mix, 1 x PAP/RT Buffer (all aforementioned products were from GeneCopoeia, Maryland, USA) and 200 ng of LDH miRNA or 200 ng of blood tRNA. After cDNA conversion, a calibrator sample was prepared (a separate calibrator sample for LDH and blood samples) by pooling equal amounts of cDNA from each sample for the construction of a standard curve (this was done separately for the LDH cDNA and the blood cDNA). The calibrator cDNA samples were serially diluted 2 fold per dilution, to produce a seven-point standard curve, with cDNA concentrations ranging from 750 ng to 11.7 ng. Amplification of rno-mir-31a-5p and snRNA U6 internal reference gene was performed separately. Each 10µl qPCR reaction consisted of 1 x KAPA

SYBR®FAST Master Mix ABI Prism™ (KAPA Biosystems, Massachusetts, USA), 0.2 µM All-in-One miRNA-31a-5p qPCR Primer or 0.2 µM snRNA U6 internal reference gene primer (miRNA-31a-5p and snRNA U6 were amplified in separate reactions) (GeneCopoeia, Maryland, USA), 0.2 µM Universal Adaptor PCR Primer (GeneCopoeia, Maryland, USA) and first-strand cDNA (200 ng). The miRNA-31a-5p - specific forward primer (GeneCopoeia, Maryland, USA) and snRNA U6 internal reference (GeneCopoeia, Maryland, USA) was designed by GeneCopoeia.

MiRNA-31a-5p and snRNA U6 qPCR amplification were performed during the same run on the ABI 7900HT platform (Applied Biosystems, Foster City, CA, USA). Each sample was analysed in triplicate in order to eliminate technical variability. Following the amplification of the serially diluted samples, a linear plot of the threshold cycle (Cq) versus the log value of the input amount of DNA (standard curve) was constructed for each primer pair using ABI's SDS v2.3 software (Applied Biosystems, California, USA). The efficiency of each reaction (per primer set) was also determined from the standard curve of that reaction. Default threshold and baseline values were used as determined by the SDS v2.3 software. All Cq values, for each primer pair, were corrected for the calculated PCR efficiency and normalised to the reference gene, snRNA U6 using GenEx software v5.4.3 (http://www.gene-quantification.de/datan.html) (MultiD, Gothenburg, Sweden). Statistical analyses were performed using the appropriate statistical software, such as GenEx [www.multid.se] (MultiD, Gothenburg, Sweden) and Statistica v11 (StatSoft, Tulsa, USA). One-way ANOVA was performed to determine if there was a significant difference between the least square (LS) means of the gene expression fold change between the FDW and FSM animals for each of the primer sets. A p-value of ≤ 0.05 was regarded as significant.

3.4.5   Functional analysis of miRNA-target interaction

It is important to determine if a particular miRNA elicits a direct or indirect effect on the mRNA target as predicted by different software programs. In order to determine whether miR-31a-5p interacts directly with the predicted target gene, *IL1RN*, a luciferase reporter assay was performed, using the GLuc-ON™ Promoter Reporter Clones assay kit (GeneCopoeia, Maryland, USA). Each pEZX-MT05 promoter clone contains a ~1-1.5 kb insert that corresponds to the 5'-flanking sequence located approximately 1-1.5 kb upstream of 3' UTR sequences of the target gene, in this case *IL1RN*. This insert is placed downstream of the Gaussia Luciferase (GLuc) reporter gene (Fig. 3.10). According to the manufacturer-provided user manual, the putative *cis*-acting enhancer elements are expected to exist in the cloned promoter region. The luciferase activity observed during the reporter assay should thus closely resemble actual promoter regulation of these genes within human cells. Secreted Alkaline Phosphatase (SEAP), located on the same vector as GLuc, is a secondary reporter gene used for transfection normalization. Following co-transfection of the miRNA precursor clone (Fig. 3.11) and the promoter reporter clone, a decrease in luciferase activity is expected if the miRNA interacts with the predicted mRNA target. Co-transfection with the negative controls (scrambled mRNA target sequence and scrambled miRNA sequence) should not result in lowered luciferase activity.
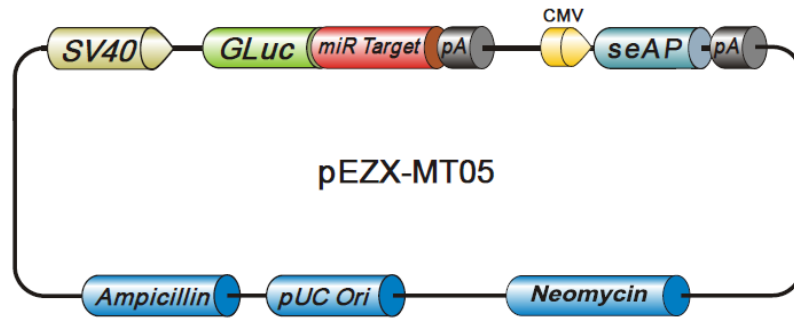
Figure 3.10: The pEZX-MT05 GLuc-ON^{TM} Promoter Reporter Clone. A 3' UTR region of *IL1RN* was inserted downstream of the GLuc reporter gene (in the region depicted as miR Target in the diagram). A chimeric mRNA was subsequently transcribed, driven by SV40 promoter, consisting of the GLuc and the *IL1RN* 3' UTR target sequence. In addition to GLuc, a SEAP reporter, driven by a CMV promoter, is also cloned into the same vector (pEZX-MT05) and serves as the internal control. The dual-reporter vector system enables transfection-normalization for accurate across-sample comparison. 3'UTR – 3' untranslated region, Ampicillin – ampicillin resistance gene, CMV – cytomegalovirus, GLuc - Gaussia Luciferase, *IL1RN* - interleukin 1 receptor antagonist, Neomycin - neomycin resistance gene, SEAP - Secreted Alkaline Phosphatase, SV40 - Simian virus 40, pUC Ori- pUC plasmid origin of replication
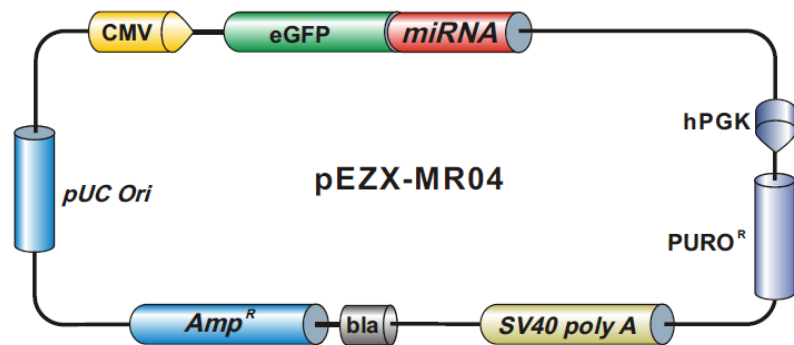
Figure 3.11: The pEZX-MR04 GFP miRNA precursor clone. The precursor of rno-mir-31a-5p was cloned downstream of an eGFP construct and a CMV promotor. AmpR – ampicillin resistance gene, bla – beta- lactamase, CMV – cytomegalovirus, eGFP - enhanced green fluorescent protein, hPGK – human phosphoglycerate kinase I promoter (for puromycin selection), miRNA – miRNA precursor sequence, pUC Ori- pUC plasmid origin of replication, PUROR - puromycin resistance gene, SV40 poly A - Simian virus 40 poly A

Human Embryonic Kidney 293 (HEK 293) cells (obtained from our laboratory) were maintained in Dulbecco's Modification of Eagle's Medium (DMEM) (Lonza, Basel Switzerland) supplemented with 1 % nonessential amino acids, 10 % fetal bovine serum (Sigma, Missouri, USA), 100 U/mL penicillin and 100 mg/mL streptomycin (Lonza, Basel Switzerland) (Appendix I). The cells were maintained at 37 °C with 5 % CO_2 and subcultured every other day. Cells between passage 15 and 18 were used for luciferase experiments. The GLuc-ON^{TM} Promoter Reporter Clone containing the *IL1RN* mRNA target region specific for rno-mir-31a-5p as well as the precursor sequence of rno-mir-31a-5p was purchased from GeneCopoeia.

The day before the transfection assay, 1 x 10^{4}cells were seeded into a 6-well culture tray containing 3 ml DMEM (complete media) (Appendix I) per well, the cells were grown for 24 hours to reach a confluency of 70–75%, cells were co-transfected with 150 ng GLuc-ON^{TM} Promoter Reporter Clone for *IL1RN* (GeneCopoeia, Maryland, USA) and 150 ng miR-31a-5p precursor clone (GeneCopoeia, Maryland, USA) in serum-free media (Appendix I) in a 6-well culture tray using HiPerFect Transfection Reagent (Qiagen, Hilden Germany) and following the traditional protocol for transfection of adherent cells with miRNA, according to the manufacturer's instructions (Qiagen, Hilden Germany). Negative control assays included the following co-transfections: (1) 150 ng of the miR-31a-5p precursor clone and 150 ng of a scrambled control reporter clone; (2) 150 ng of a scrambled miR precursor control and 150 ng of the *IL1RN* reporter clone; (3) 150 ng of *IL1RN* reporter clone and no miR-31a-5p precursor clone (negative control to test if any endogenous miRNAs are targeting the *IL1RN* mRNA target region). A positive control (EF1A-PG04 media) was provided in the kit by the manufacturer (GeneCopoeia, Maryland, USA). After about 24 hours the media was removed and replaced with fresh DMEM (complete media) (Appendix I). About 72 hours after transfection, cell culture media was collected and assayed for both GLuc and SEAP luciferase signal, simultaneously on the same white 96-well plate and according to the manufacturer's instructions (GeneCopoeia, Maryland, USA). For the GLuc assay, the GL-S Buffer was used for more stable activity. Each sample was analysed in duplicate and the experiment was repeated to ensure reproducibility.

When comparing GLuc activities of multiple transfected cell samples, signal normalization is necessary. SEAP signal was used as an internal standard control and signal normalization (ratio of GLuc and SEAP activities) eliminated the impact of transfection efficiency variations; thus the normalized GLuc activities of the different samples more accurately reflected the true biological events. The ratio of luminescence intensities (RLU, Relative Light Unit) of the GLuc over SEAP was calculated and the normalized GLuc activity (GLuc/SEAP ratio) was used to compare the different co-transfections (as described in the paragraph above).

In order to determine whether rno-miR-31a-5p targeted *IL1RN in vitro*, the co-transfection of the rno-miR-31a-5p precursor clone and the *IL1RN* promoter reporter clone had to show significantly lower luciferase activity compared to the negative control co-transfections. A mixed model repeated measures ANOVA was performed using Statistica v11 (StatSoft, Tulsa, USA) to determine if there were statistically significant differences.

4. Results

4.1 PTSD animal model

Animals were subjected to several behavioural tests following fear conditioning and fear extinction, to evaluate their anxiety-like phenotypes (refer to Methods Sections 3.1.2 and 3.1.3) and to group the animals in well-adapted (WA) and maladapted (MA) groups (refer to Methods Sections 3.1.4). One of these tests was the L/D avoidance test. Statistical analysis (ANOVA F-test) of behavioural data showed that there were significant group differences in the time spent in the dark ($p < 0.05$) (Fig. 4.1 A). *Post-hoc* analysis revealed that the FS group spent significantly more time in the dark compartment (indicative of fearful behaviour) compared to the CS and the FD groups that spent more time in the light compartment (indicative of non-fearful behaviour) ($p < 0.05$) (Fig. 4.1 B) (Table 4.1). These results from the L/D avoidance test indicate that fear conditioning was effective in eliciting a fearful response in FS group and that a fear extinction effect was evident in the FD group. The difference in the time spent exploring the light compartment was also statistically significant between the CS and CD groups. This suggested that in the absence of fear conditioning, the drug still elicited an effect on behaviour. However, after sub-group selection for the CD and CS groups, whereupon 12 animals with behavioural values closest to the group mean were selected, there were no statistically significant group differences, suggesting that outliers may have caused this spurious result.

We therefore concluded that the L/D avoidance test was the most sensitive behavioural test, compared to the OF and FST, to detect the effects of fear conditioning and fear extinction in the present study. Since 30 animals per treatment group were used in the behavioural analysis, it provided more statistical power as well as confidence in the data. Behavioural data from the L/D avoidance test were subsequently used as selection criteria for the WA and MA subgroups within the FS and FD groups.

After subgrouping the FD and FS animals into WA and MA groups, statistical analyses (ANOVA F-test) were performed on the L/D avoidance test behavioural data to determine if there was indeed a significant difference between the WA and MA groups and between the FSM and FDW groups. We also wanted to confirm that these subgroups were appropriate for downstream genetic and epigenetic analyses. There was a statistically significant difference in the time spent in the dark compartment between the WA and MA groups in the L/D avoidance test (p < 0.05) (Fig. 4.1 C): WA animals spent significantly less time in the dark compartment compared to MA animals. *Post-hoc* analysis indicated that there was a statistically significant difference in the time spent in the dark compartment between the two focus groups, FDW and FSM, as well as among all other subgroups, except between FSW and FDM (Table 4.2). The *post-hoc* analysis accounts for multiple comparisons.

Furthermore, a significant interaction effect between treatment group (FS or FD) and WA or MA status was found (p = 0.00087) (Fig. 4.1 D). This implies that the combination of these factors results in more extreme

values than if a single factor had been considered. Figure 4.1 D shows that the combination of DCS administration and WA status resulted in less time spent in the dark in the L/D avoidance test, compared to what would have been expected if group status (DCS/saline) or adaption status (MA/WA) were independently considered.
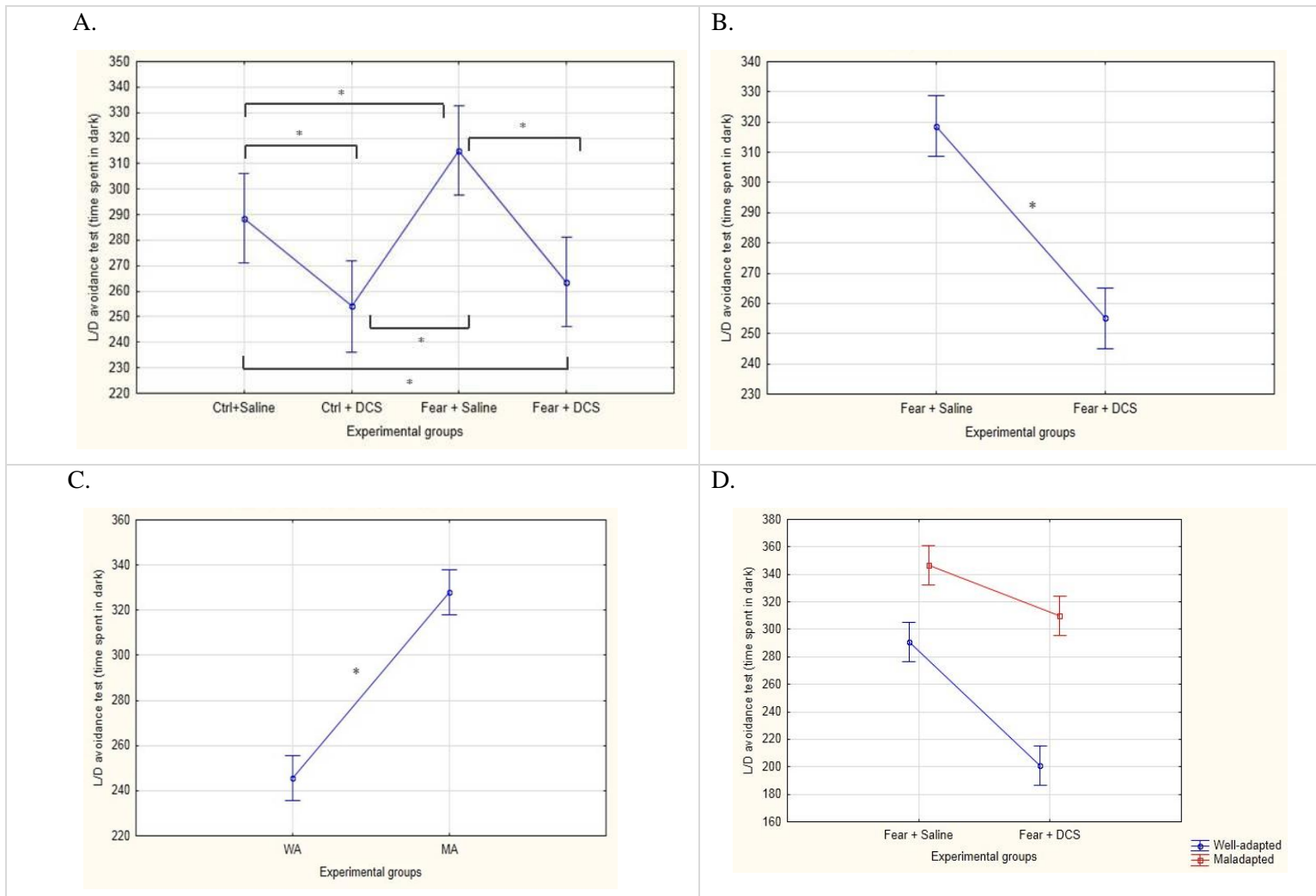
Figure 4.1: Statistical analyses of L/D avoidance test results. (A) There was a statistically significant group difference in the time spent in the dark compartment for the four treatment groups (p = 0.01). One-way ANOVA results for the L/D avoidance test shows the time spent in the dark compartment (*y-axis*) for all treatment groups (*x-axis*). **(B) A statistically significant difference was evident in the time spent in the dark compartment between the FS group compared to the FD group (p < 0.001).** One-way ANOVA and *post-hoc* test results for the L/D avoidance test shows the time spent in the dark compartment (*y-axis*) for the FS and the FD groups (*x-axis*). **(C) There was a statistically significant difference in the time spent in the dark compartment between the WA and MA subgroups (p = 0.00**). One-way ANOVA results for the L/D avoidance test shows the time spent in the dark compartment (*y-axis*) for the WA and MA subgroups (*x-axis*). **(D) An interaction effect was evident between the MA or WA status and group status (FS or FD) which had an effect on the L/D avoidance test results (p < 0.001).** F-test results for the L/D avoidance test shows the time spent in the dark compartment (*y-axis*) for the FSW, FDW, FSM, FDW groups (*x-axis*). ANOVA – analysis of variance, L/D – light/dark, FS – fear-conditioned + saline, FD – fear-conditioned + DCS, WA – well-adapted, MA – maladapted, FSM - fear-conditioned + saline maladapted, FDW - fear-conditioned + DCS well-adapted. * indicate significant p-values (p < 0.001)

Table 4.1: LSD *post-hoc* analysis test results for the L/D avoidance test for the four treatment groups. P-values <
0.05 in red show statistically significant differences in mean values for the time spent in the dark compartment for each
treatment group. Statistically significant differences were evident between all groups, except between CD and FD

	Group	{1} 288.63	{2} 254.07	{3} 315.10	{4} 263.60
1	CS		0.007020	0.036150	0.047284
2	CD	0.007020		0.000004	0.450616
3	FS	0.036150	0.000004		0.000070
4	FD	0.047284	0.450616	0.000070	

LSD - Least Significant Difference, L/D – light/dark, CS – control + saline, CD – control + DCS, FS – fear-conditioned + saline, FD
– fear-conditioned + DCS

Table 4.2: LSD *post-hoc* analysis test results for the L/D avoidance test for the fear-conditioned subgroups. P-
values < 0.05 in red show statistically significant differences in mean values for the time spent in the dark compartment
for each of the WA and MA subgroups

	Group	Status	{1} - 290.67	{2} - 346.50	{3} - 200.67	{4} - 309.67
1	FS	WA		0.000011	0.000000	0.062203
2	FS	MA	0.000011		0.000000	0.001049
3	FD	WA	0.000000	0.000000		0.000000
4	FD	MA	0.062203	0.001049	0.000000	

LSD - Least Significant Difference, L/D – light/dark, FS – fear-conditioned + saline, FD – fear-conditioned + DCS, WA – well-
adapted, MA - maladapted

4.2 Gene expression analyses

4.2.1   Next generation RNA sequencing

The paired-end sequencing data from the two flow cells sequenced on the Illumina HiSeq 2000 platform
yielded an average of about 60 million reads per sample. The quality of the sequencing data was very high as
illustrated by the phred scores that were mostly ≥ 30 (Fig. 4.2). Phred score (Q) is the log transformation of
error rate (P) at each base calling position. $Q = -10\log_{10}P$. A phred score of 30 thus denotes 1 error per 1000
nucleotides and a score of 20 denotes 1 error per 100 nucleotides. An average of 99.96% of the reads could
be   mapped   to   the   *Rattus   novergicus*   rn4   rat   reference   genome
((http://support.illumina.com/sequencing/sequencing_software/igenome.ilmn).   Among   the   mapped
sequences, approximately 56.90% of the read pairs were uniquely mapped to the reference genome. These
reads were used to estimate transcript expression of all 48 samples (consisteing of 12 CS, 12 CD, 6 FSM, 6
FSW, 6 FDW and 6 FDM animals). *Cufflinks* measures transcript abundances in Fragments Per Kilobase of
exon per Million fragments mapped (FPKM). Of the 16 491 annotated genes, 13 676 (82.93%) transcripts
had FPKM values higher than 0 and 7 479 (45.35%) transcripts had FPKM values higher than 5. In order to
identify a gene or transcript as differentially expressed, *Cuffdiff* tests the observed log-fold-change in its
expression against the null hypothesis of no change (i.e. the true log-fold-change is zero). Genes with log2

(fold change values) $\geq$ 1.3 and p < 0.05 were considered as significantly up-regulated while those with log2 (fold change values) $\leq$ 0.5 and p < 0.05 were regarded as significantly down-regulated and investigation of these genes was thus taken further.

A. Flow cell one

B. Flow cell two
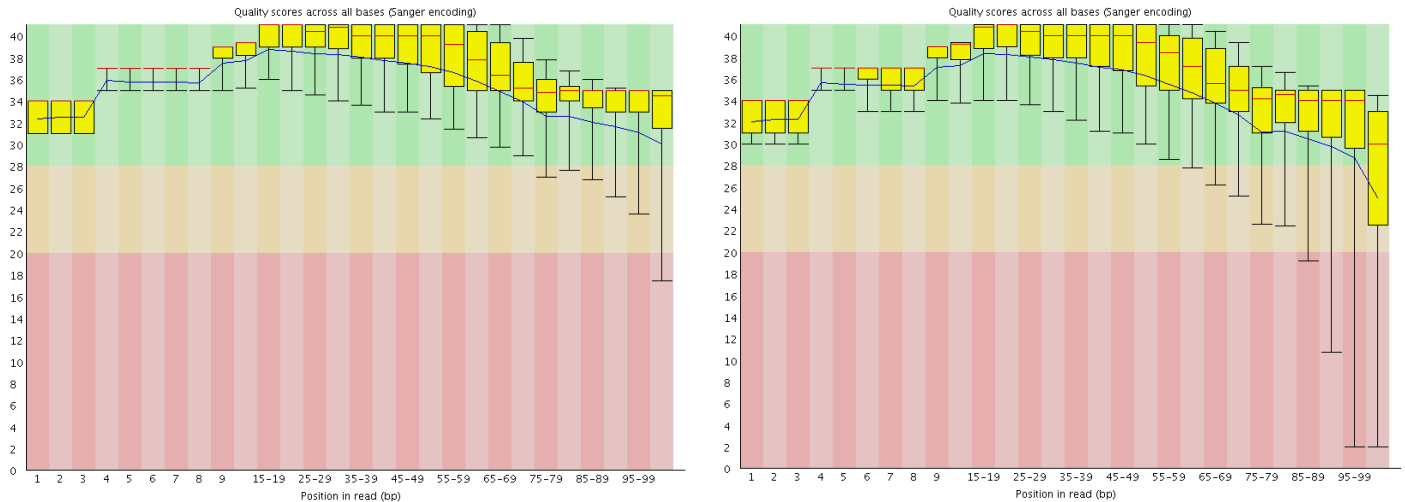
Figure 4.2: Distribution of phred (Q) score in reads for flow cell one (A) and flow cell two (B). The *x-axis* indicates the bp position in the 101 bp read and the *y-axis* indicates the phred score at each position. The phred scores are usually lower at the start and end points and should be trimmed if the per base quality score is below 20. Due to the high quality of the sequencing data, no end trimming was required

4.2.2 Differential gene expression analyses

Bioinformatics analyses of the RNAseq data detected a total 93 genes that were statistically significant downregulated in the FDW compared to the FSM group, of which 42 were predicted to have biologically relevant functions by the *BORG* analysis tool. Only one gene, cysteine-rich angiogenic inducer 61 (*CYR61*) was found to be overexpressed in the FDW group compared to the FSM group, however it was not assessed as being biologically relevant by the *BORG* analysis tool in the present study. Table 4.3 provides a summary of the genes that were differentially expressed between the different subgroups; it also indicates how many genes were predicted to be biologically relevant by *BORG* analysis. Table 4.4 contains the 42 biologically relevant genes that were differentially expressed between FDW and FSM animals, the gene expression fold changes (negative fold changes indicate that genes were down-regulated in the FDW group compared to the FSM group) as well as functions of the differentially expressed genes as predicted by the *BORG* analyses. Refer to Appendix II, Table II.1 for the differentially expressed genes between the other treatment groups (mentioned in Table 4.3).

Table 4.3: Summary of differential gene expression results for the different treatment groups. The total number of overexpressed (fold change $\geq$ 1.3 and p < 0.05) and under-expressed (fold change $\leq$ 0.5 and p < 0.05) genes identified with *Cuffdiff* as well as biologically relevant differentially expressed genes identified with *BORG* analyses among the various treatment groups

	FDW vs FSM	FSM vs CS	FSW vs FSM	FDW vs FDM	CS vs CD	FDW vs. FSW
Total over-expressed genes	1	0	6	5	0	0
BORG over-expressed genes	0	0	3	3	0	0
Total under-expressed genes	93	2	1	1	0	364
BORG under-expressed genes	42	1	0	0	0	95

FSM - fear-conditioned + saline maladapted, FDW – fear-conditioned + DCS well-adapted, CS – control saline, FSW – fear-conditioned + saline well-adapted, FDM – fear-conditioned + DCS maladapted, CD – control + DCS

Table 4.4: Biologically relevant differentially expressed genes. The 42 biologically relevant genes (pertaining to fear, anxiety and PTSD, as identified by *BORG* analyses tool) that were significantly differentially expressed between the FDW compared to the FSM group; negative fold changes indicate that genes were down-regulated in the FDW group compared to the FSM group. Functions were predicted by the *BORG* analyses tool

Gene	Name	Fold change	Function
SPP1	Secreted phosphoprotein 1	**-6.00**	Demyelination (Chiocchetti et al., 2005) (http://www.ncbi.nlm.nih.gov/pubmed/15885319)
CXCL13	Chemokine (C-X-C motif) ligand 13	**-5.21**	Elevation of cytosolic calcium ion concentration (GO:0007204) / Immune response (GO:0006955) (http://www.ncbi.nlm.nih.gov/pubmed/22075990)/ Inflammatory response (Camon et al., 2004)
CLEC7A	C-type lectin domain family 7, member A	**-5.20**	Cytokine regulation (Camon et al., 2004)
IL1RN	Interleukin 1 receptor antagonist	**-4.19**	Learning or memory (http://www.ncbi.nlm.nih.gov/pubmed/15050649) (Palin et al., 2004)
C6	Complement component C6	**-4.15**	Neurodegenerative disease pathway (http://www.ncbi.nlm.nih.gov/pubmed/19594883)
CD8A	CD8a molecule	**-3.96**	Abnormal myelination (http://www.ncbi.nlm.nih.gov/pubmed/22075990)
MSR1	Macrophage scavenger receptor 1	**-3.80**	Amyloid beta deposits (http://www.ncbi.nlm.nih.gov/pubmed/22075990)
S100A4	S100 calcium binding protein A4	**-3.47**	Neuron projection (GO:0043005) (http://www.ncbi.nlm.nih.gov/pubmed/15101091) / calcium-dependent protein binding (GO:0048306) (http://www.ncbi.nlm.nih.gov/pubmed/8878885) / calcium ion binding (GO:0005509) (http://www.ncbi.nlm.nih.gov/pubmed/2357224) (http://www.ncbi.nlm.nih.gov/pubmed/15101091)
S100A9	S100 calcium binding protein A9	**-3.45**	Calcium ion binding(GO:0005509) / antioxidant activity (GO:0016209) (Camon et al., 2004) /chronic inflammatory response (GO:0002544) (http://www.ncbi.nlm.nih.gov/pubmed/8343166)
F10	Coagulation factor X	**-3.42**	Calcium ion binding (GO:0005509) (Camon et al., 2004) / coagulation cascade pathway (PW:0000474) (http://www.ncbi.nlm.nih.gov/pubmed/10048754) (http://www.ncbi.nlm.nih.gov/pubmed/12356487) / complement system pathway (PW:0000502) (Petri et al., 2011)
GPNMB	Glycoprotein (transmembrane) nmb	**-3.13**	Neuron degeneration (http://www.ncbi.nlm.nih.gov/pubmed/22075990)
LBP	Lipopolysaccharide binding protein	**-3.04**	Cytokine production (Camon et al., 2004)
HMOX1	Heme oxygenase (decycling) 1	**-2.99**	Negative regulation of neuron apoptotic process (GO:0043524) (http://www.ncbi.nlm.nih.gov/pubmed/19177228) / response to oxidative stress (GO:0006979)

			(http://www.ncbi.nlm.nih.gov/pubmed/17020887) / increased inflammatory response (MP:0001846) (http://www.ncbi.nlm.nih.gov/pubmed/22075990)
ST14	Suppressor of tumorigenicity 14 protein	**-2.81**	Abnormal neural tube morphology/development (http://www.ncbi.nlm.nih.gov/pubmed/22075990)
TRH	Thyrotropin releasing hormone	**-2.80**	Abnormal pituitary gland development (http://www.ncbi.nlm.nih.gov/pubmed/22075990)
ITGAL	Integrin, alpha L	**-2.74**	Abnormal inflammatory response (MP:0001845) (Camon et al., 2004) / Positive regulation of calcium-mediated signalling (GO:0050850) (http://www.ncbi.nlm.nih.gov/pubmed/8117278)
LYZ2	Lysozyme 2	**-2.64**	Altered response to CNS ischemic injury (http://www.ncbi.nlm.nih.gov/pubmed/22075990)
A2M	Alpha-2-macroglobulin	**-2.53**	Neurodegenerative disease pathway (Blacker et al., 1998)
CYBB	Cytochrome b-245, beta polypeptide	**-2.51**	Oxidative stress (Datla and Griendling 2010; Tyagi et al., 2011) / Nervous system physiology (GO:0030425) (http://www.ncbi.nlm.nih.gov/pubmed/17027166) (GO:0043025) (http://www.ncbi.nlm.nih.gov/pubmed/17027166)
MMP9	Matrix metallopeptidase 9	**-2.42**	Impaired contextual conditioning behaviour (http://www.ncbi.nlm.nih.gov/pubmed/22075990)
NCF1	Neutrophil cytosolic factor 1	**-2.24**	Abnormal spatial learning / impaired cued conditioning behaviour (http://www.ncbi.nlm.nih.gov/pubmed/22075990)
S100A3	S100 calcium binding protein A3	**-2.21**	Calcium ion binding (GO:0005509) (Camon et al., 2004)
C1QC	Complement C1q subcomponent subunit C	**-2.18**	Neurodegenerative disease pathway (http://www.ncbi.nlm.nih.gov/pubmed/19594883)
RRM2	Ribonucleoside-diphosphate reductase subunit M2	**-2.13**	p53/stress response signalling pathway (Petri et al., 2011)
MT2A	Metallothionein 2A	**-2.10**	Abnormal learning/memory/conditioning (http://www.ncbi.nlm.nih.gov/pubmed/22075990)
PTPRC	Protein tyrosine phosphatase receptor type, C	**-2.02**	Abnormal myelination (http://www.ncbi.nlm.nih.gov/pubmed/22075990)
TSPO	Translocator protein	**-1.97**	Response to pain (http://www.ncbi.nlm.nih.gov/pubmed/19555675)
RAC2	Ras-related C3 botulinum toxin substrate 2	**-1.92**	Axon guidance (GO:0007411) (Camon et al., 2004)
VIM	Vimentin	**-1.87**	Increased anxiety-related response / abnormal nervous system morphology (http://www.ncbi.nlm.nih.gov/pubmed/22075990)

Gene	Description	Value	Pathway / Function
C1QB	Complement C1q subcomponent subunit B	**-1.87**	Neurodegenerative disease pathway (http://www.ncbi.nlm.nih.gov/pubmed/19594883)
CTSC	Cathepsin C	**-1.79**	Decreased prepulse inhibition (http://www.ncbi.nlm.nih.gov/pubmed/22075990)
CP	Ceruloplasmin (glycoprotein)	**-1.76**	Abnormal neuron morphology (http://www.ncbi.nlm.nih.gov/pubmed/22075990)
C1S	Complement component 1, s subcomponent	**-1.64**	Calcium ion binding (Camon et al., 2004)/ glial cell differentiation (http://www.ncbi.nlm.nih.gov/pubmed/9524231)
CD4	Cd4 molecule	**-1.64**	Demyelination / brain inflammation (http://www.ncbi.nlm.nih.gov/pubmed/22075990)
CHRDL1	Chordin-like 1	**-1.61**	Neuron differentiation (GO:0030182) (http://www.ncbi.nlm.nih.gov/pubmed/14684875)
C1QA	Complement component 1, q subcomponent, A chain	**-1.60**	Neurodegenerative disease pathway (Petri et al., 2011) and seizures (http://www.ncbi.nlm.nih.gov/pubmed/22075990)
FCER1G	Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide	**-1.57**	Cytokine regulation (Camon et al., 2004)
CD44	Cd44 molecule	**-1.55**	Abnormal spinal nerve morphology, abnormal synaptic transmission (http://www.ncbi.nlm.nih.gov/pubmed/22075990)
CD74	Cd74 molecule, major histocompatibility complex, class II invariant chain	**-1.48**	Cytokine regulation (Camon et al., 2004)
LCP1	Ceruloplasmin	**-1.41**	Calcium ion binding (GO:0005509) (http://www.ncbi.nlm.nih.gov/pubmed/2378651) / T cell activation involved in immune response (GO:0002286) (Camon et al., 2004)
LGALS3BP	Lectin, galactoside-binding, soluble, 3	**-1.34**	Inflammatory response (Wang et al., 2011)
NPY	Neuropeptide Y	**-1.30**	Increased anxiety-related response (http://www.ncbi.nlm.nih.gov/pubmed/22075990), neuropeptide Y metabolic pathway (http://www.ncbi.nlm.nih.gov/pubmed/16046456), abnormal depression-related behaviour (http://www.ncbi.nlm.nih.gov/pubmed/14757324)

FDW – fear-conditioned + DCS well-adapted, FSM - fear-conditioned + saline maladapted, *BORG - Bio-Ontological Relationship Graph*

4.2.3   Gene Ontology enrichment analyses for differentially expressed genes

The set of 42 biologically relevant genes differentially expressed between the FDW compared to the FSM group were further analysed to identify shared or common functional categories between these genes, based on gene ontology terms. Statistically significant (p<0.05) over-expressed GO terms and pathways associated with the downregulated genes were identified using the Comparative Toxicogenomics Database (CTD) functional enrichment tool (http://ctdbase.org/tools/analyzer.go) (Davis et al., 2013). Integrative network diagrams were constructed with Cytoskape 2.8.3 (http://www.cytoscape.org) to create a more insightful view of the relationships between annotation categories. Functional categories of biological process (Table 4.5, Figs. 4.3, 4.4, 4.5), disease (Table 4.6, 4.7, Figs. 4.6 – 4.8), molecular function (Table 4.8, Figs. 4.9, 4.10) and enriched biochemical pathways (including KEGG and REACTOME biochemical pathways terms) (Table 4.9, Figs. 4.11, 4.12) were identified, only for the 42 biologically relevant genes.

4.2.3.1 Biological processes associated with biologically relevant differentially expressed genes

Biological processes that were overrepresented in the differentially expressed gene set include immune response, positive regulation of response to stimulus, responses to stress, developmental processes, cellular responses to stimulus and signal transduction. Other important associated processes that may be involved in anxiety-related pathologies include locomotion, complement activation, behaviour and negative regulation of glutamate secretion. Table 4.5 indicates the sets of genes associated with each process. Figure 4.3 indicates the number of genes associated with each process, and Figures 4.4, 4.5 indicate genes that share roles in certain processes.

Table 4.5: Biologically relevant differentially expressed genes in the LDH of FDW vs. FSM animals that were associated with the gene ontology (GO) biological process terms (p < 0.05) (as determined by CTD)

Biological process	Number of genes	Corrected p-value	Genes
Immune response	24	9.21E-21	*A2M, C1QA, C1QB, C1QC, C1S, C6, CD4, CD44, CD74, CD8A, CLEC7A, CTSC, CXCL13, CYBB, FCER1G, HMOX1, IL1RN, ITGAL, LBP, LCP1, MT2A, NCF1, PTPRC, S100A9*
Positive regulation of response to stimulus	22	7.61E-18	*A2M, C1QA, C1QB, C1QC, C1S, C6, CD4, CD44, CD74, CD8A, CLEC7A, CXCL13, F10, FCER1G, HMOX1, IL1RN, ITGAL, LBP, NPY, PTPRC, S100A4, S100A9*
Response to stress	28	7.48E-17	*A2M, C1QA, C1QB, C1QC, C1S, C6, CD4, CD44, CD74, CD8A, CLEC7A, CXCL13, CYBB, F10, FCER1G, HMOX1, IL1RN, ITGAL, LBP, LGALS3BP, MT2A, NCF1, PTPRC, RAC2, S100A9, SPP1, TRH, TSPO*
Developmental process	24	3.49E-08	*A2M, C1QB, C1QC, C6, CD4, CD44, CD74, CD8A, CHRDL1, CTSC, CXCL13, GPNMB, HMOX1, IL1RN, LCP1, MMP9, MSR1, NPY, PTPRC, RAC2, S100A4, SPP1, TSPO, VIM*
Locomotion	14	2.48E-07	*CD4, CD74, CXCL13, F10, FCER1G, HMOX1,*

97

			ITGAL, LBP, MMP9, PTPRC, RAC2, S100A9, SPP1, TSPO
Complement activation	6	3.53E-07	*A2M, C1QA, C1QB, C1QC, C1S, C6*
Cellular response to stimulus	24	6.23E-07	*A2M, CD4, CD44, CD74, CD8A, CHRDL1, CLEC7A, CXCL13, F10, FCER1G, HMOX1, IL1RN, ITGAL, LBP, LGALS3BP, MT2A, NPY, PTPRC, RAC2, S100A4, S100A9, SPP1, TRH, TSPO*
Signal transduction	22	7.35E-07	*A2M, CD4, CD44, CD74, CD8A, CHRDL1, CLEC7A, CXCL13, F10, FCER1G, HMOX1, IL1RN, ITGAL, LBP, LGALS3BP, MT2A, NPY, PTPRC, RAC2, S100A4, S100A9, TRH*
Response to chemical stimulus	18	3.36E-06	*CD44, CD74, CLEC7A, CTSC, CXCL13, FCER1G, HMOX1, IL1RN, LBP, MSR1, MT2A, NPY, PTPRC, RAC2, S100A9, SPP1, TRH, TSPO*
Cell differentiation	16	1.09E-04	*A2M, C1QC, CD4, CD74, CD8A, CHRDL1, GPNMB, MMP9, MSR1, NPY, PTPRC, RAC2, S100A4, SPP1, TSPO, VIM*
Ion homeostasis	7	4.67E-03	*CP, CXCL13, HMOX1, MT2A, PTPRC, S100A9, TSPO*
Positive regulation of calcium-mediated signalling	3	6.18E-03	*CD4, CD8A, ITGAL*
Behavior	7	6.29E-03	*CD74, CXCL13, IL1RN, LBP, NPY, TRH, TSPO*
Immune system development	7	1.35E-02	*C1QC, CD4, CD74, CD8A, CXCL13, MMP9, PTPRC*
Negative regulation of response to stimulus	8	1.48E-02	*A2M, CD44, CD74, CXCL13, HMOX1, IL1RN, PTPRC, SPP1*
Reactive oxygen species metabolic process	4	3.06E-02	*CYBB, NCF1, RAC2, TSPO*
Negative regulation of glutamate secretion	2	4.15E-02	*IL1RN, TRH*

LDH – left dorsal hippocampus, FDW – fear-conditioned + DCS well-adapted, FSM – fear-conditioned + saline maladapted, CTD – Comparative Toxicogenomics Database. Refer to Table 4.4 for full gene names

Figure 4.3: Main biological process gene ontology (GO) terms associated with the biologically relevant differentially expressed genes. The *x-axis* shows the main biological processes and the *y-axis* indicates the number of genes enriched for each biological process

Integrative network analysis was originally performed with all the above-mentioned biological processes depicted in the network (Fig. 4.4), however, due to the large number of genes associated with certain processes (immune response, response to stress, developmental process etc.), this network was visually complex. A subset of the biological processes (which included some of the more relevant biological processes that had fewer associated genes) was, therefore, selected to construct a simpler integrative network (Fig. 4.5). Refer to Table 4.5 for details regarding all genes associated with specific biological processes.

Biological processes
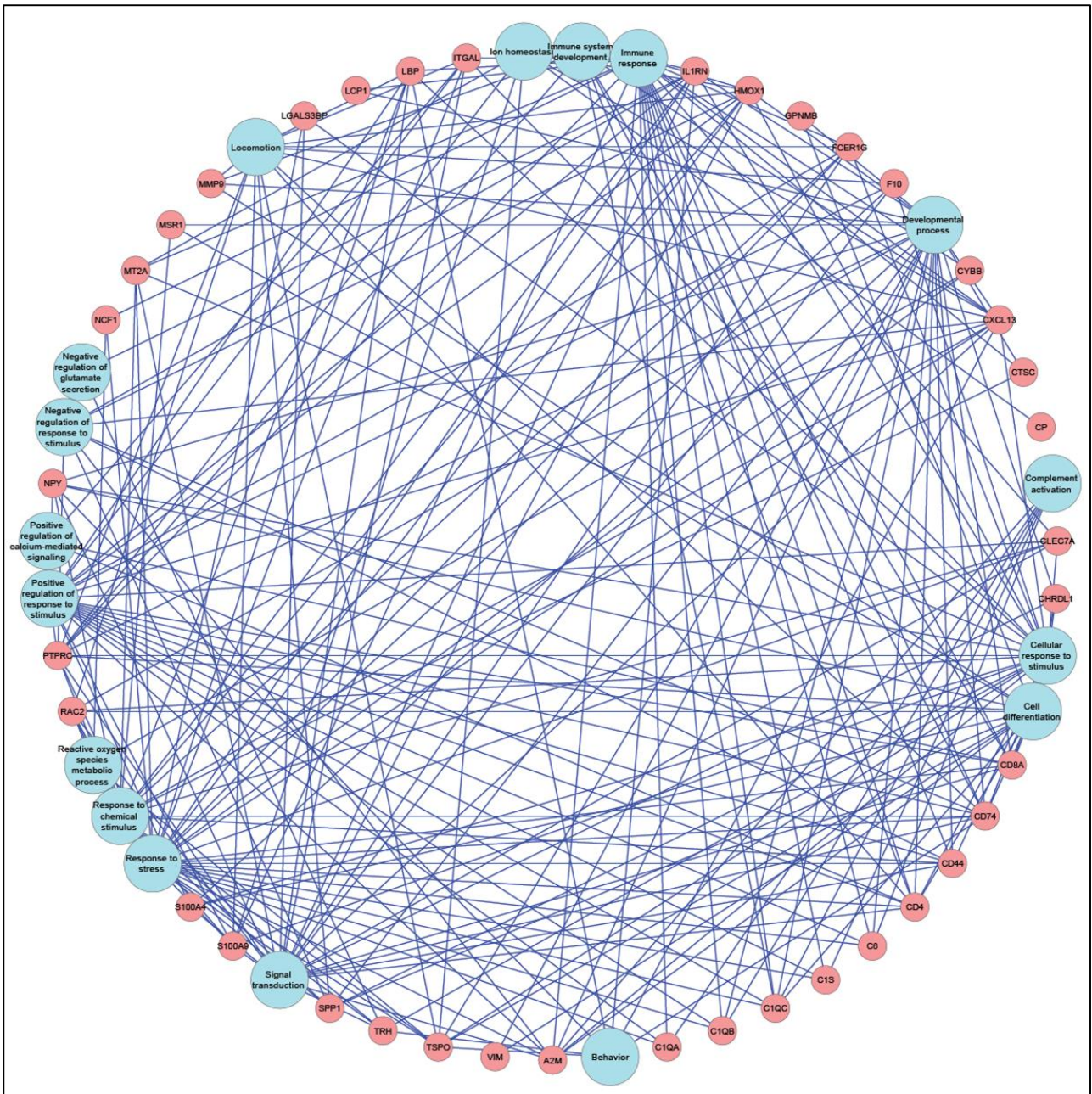
Figure 4.4: Integrative network diagram depicting the common biological processes associated with, and shared between, the biologically relevant differentially expressed genes in the FDW vs. FSM groups. Biological processes are represented using larger blue circles and the genes are represented by smaller pink circles

Biological processes
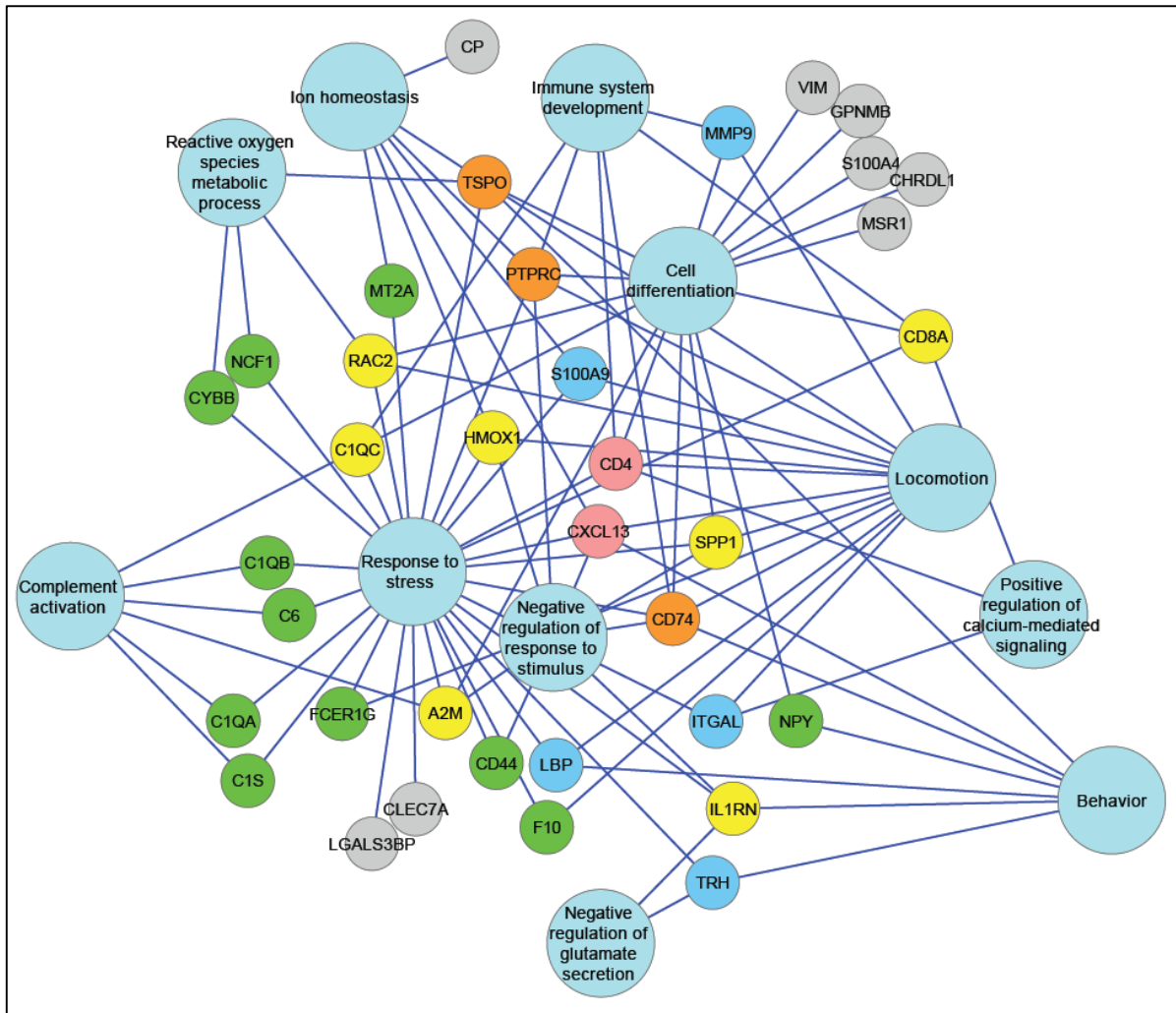
Figure 4.5: Integrative network diagram depicting a selected subset of the common biological processes associated with, and shared between, the biologically relevant differentially expressed genes in the FDW vs. FSM groups. Biological processes are represented using large blue circles; genes associated with one, two, three, four, five and six processes are represented using grey, green, blue, yellow, pink and orange circles, respectively. Refer to Table 4.4 for full gene names and Table 4.5 and Fig. 4.3 for biological process GO terms not included in the network. FDW – fear-conditioned + DCS well-adapted, FSM – fear-conditioned + saline maladapted

4.2.3.2 Diseases associated with biologically relevant differentially expressed genes

In order to identify differentially expressed genes that could play a role in PTSD or anxiety disorders, a search for significantly associated disease terms was conducted. CTD gene–disease relationships include curated and inferred relationships. Curated gene-disease relationships are based on published literature curated by CTD or derived from the Online Mendelian Inheritance in Man (OMIM) database. Inferred relationships are those based on CTD-curated chemical–gene interactions, for example, where gene A directly interacts with chemical B, and chemical B has a curated relationship with disease C, gene A will have an inferred relationship with disease C (via chemical B).

The biologically relevant differentially expressed genes, identified with RNAseq, were imported into CTD to identify the significantly associated GO disease terms. Significantly associated disease terms included, but were not limited to, nervous system diseases, mental disorders, cognitive disorders and neurodegenerative disorders, digestive system diseases and metabolic diseases. Genes that are associated with nervous system diseases, mental disorders, cognitive disorders and neurodegenerative disorders could be involved in fear extinction and fear conditioning processes, as well as anxiety related pathologies. In addition, other overrepresented disease terms, such as cardiovascular diseases, digestive system diseases and metabolic diseases could facilitate our understanding of co-morbidity and indicate which genes may explain certain disorders co-morbid with PTSD. Table 4.6 shows the subsets of genes significantly associated with different disease terms.

Table 4.6: Biologically relevant differentially expressed genes in the LDH of FDW vs. FSM animals that were associated with the gene ontology (GO) disease terms (p<0.05) (as determined by CTD)

Diseases	Number of genes	Corrected p-value	Genes
Immune System Diseases	17	4.02E-14	*C1QA, C1QB, C1S, C6, CD4, CD8A, CP, CTSC, CYBB, FCER1G, HMOX1, IL1RN, MMP9, NCF1, PTPRC, RAC2, SPP1*
Cardiovascular Diseases	18	1.02E-13	*C1QB, C1S, C6, CTSC, CYBB, F10, HMOX1, IL1RN, ITGAL, MMP9, MT2A, NCF1, NPY, RAC2, S100A4, SPP1, TRH, VIM*
Digestive System Diseases	17	9.06E-11	*A2M, C1S, CD44, CD74, CP, CTSC, GPNMB, HMOX1, IL1RN, LBP, MMP9, MT2A, PTPRC, RRM2, S100A4, S100A9, SPP1*
Central Nervous System Diseases	12	1.72E-06	*A2M, CP, CYBB, HMOX1, IL1RN, MMP9, NCF1, NPY, S100A4, SPP1, TRH, VIM*
Nervous System Diseases	16	2.28E-06	*A2M, CD4, CD44, CP, CYBB, HMOX1, IL1RN, MMP9, NCF1, NPY, PTPRC, S100A4, SPP1, TRH, TSPO, VIM*
Brain Diseases	11	7.06E-06	*A2M, CP, CYBB, HMOX1, IL1RN, MMP9, NCF1, NPY, S100A4, SPP1, TRH*
Hypersensitivity	7	1.59E-05	*C1QA, C1S, CTSC, FCER1G, HMOX1, MMP9, SPP1*
Diabetes Mellitus	7	1.63E-05	*CP, CYBB, HMOX1, IL1RN, MMP9, NCF1, SPP1*
Metabolic Diseases	11	4.00E-05	*C6, CP, CYBB, HMOX1, LBP, MMP9, NCF1, PTPRC, SPP1, TRH, VIM*
Autoimmune Diseases	7	8.36E-05	*C1QA, C1S, CP, IL1RN, MMP9, NCF1, PTPRC*
Delirium, Dementia, Amnestic, Cognitive Disorders	5	6.61E-04	*A2M, CP, HMOX1, NPY, TRH*
Pain	4	2.42E-03	*IL1RN, MMP9, TRH, VIM*
Pancreatic Diseases	4	5.43E-03	*CD44, HMOX1, MMP9, SPP1*
Mental Disorders	8	1.83E-02	*A2M, CP, HMOX1, IL1RN, LCP1, MMP9, NPY, TRH*
Nerve Degeneration	3	4.61E-02	*CD4, CD44, CP*

LDH – left dorsal hippocampus, FDW – fear-conditioned + DCS well-adapted, FSM – fear-conditioned + saline maladapted, CTD – Comparative Toxicogenomics Database. Refer to Table 4.4 for full gene names

Figure 4.6 indicates the number of genes associated with each disease term, with the highest number of genes being associated with cardiovascular diseases, immune system diseases, nervous system diseases, and digestive system diseases.

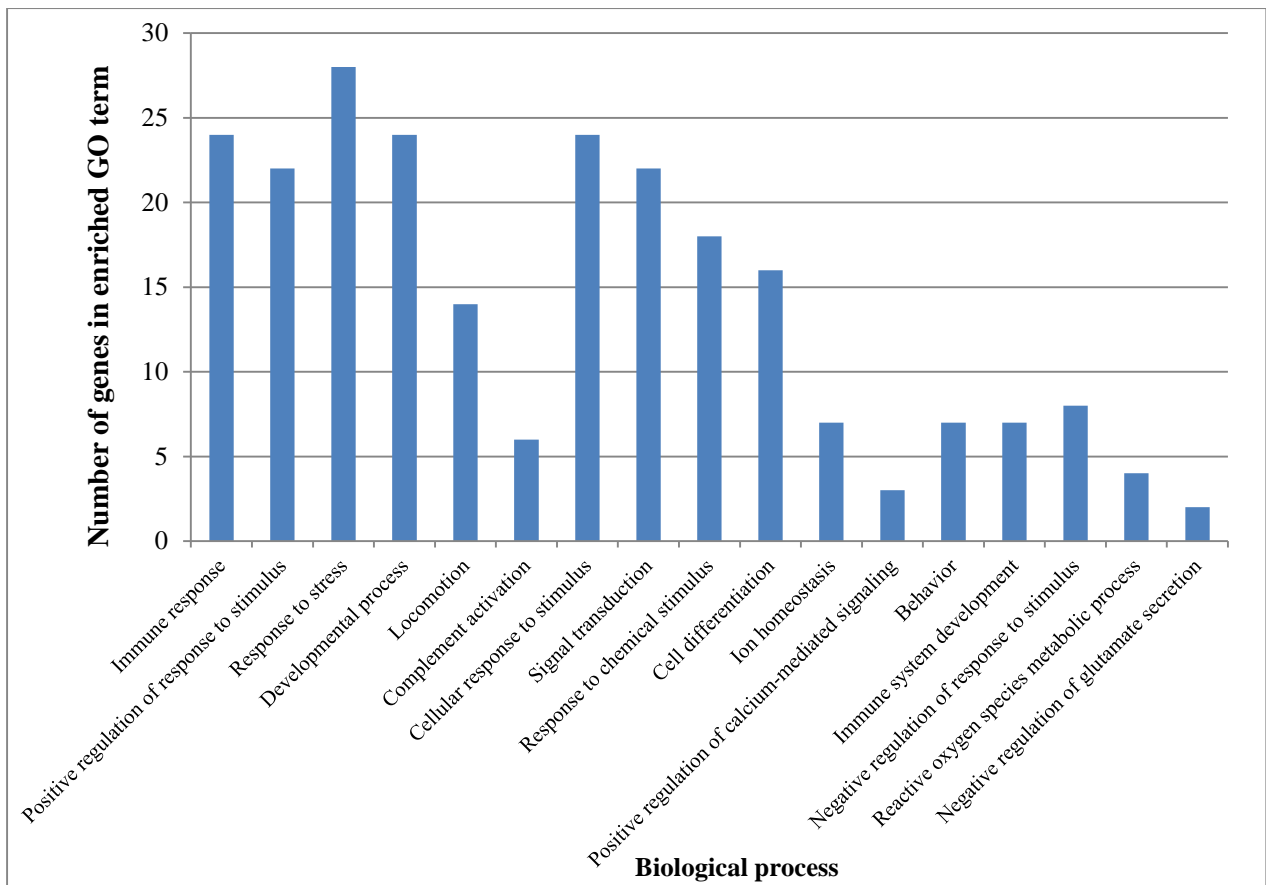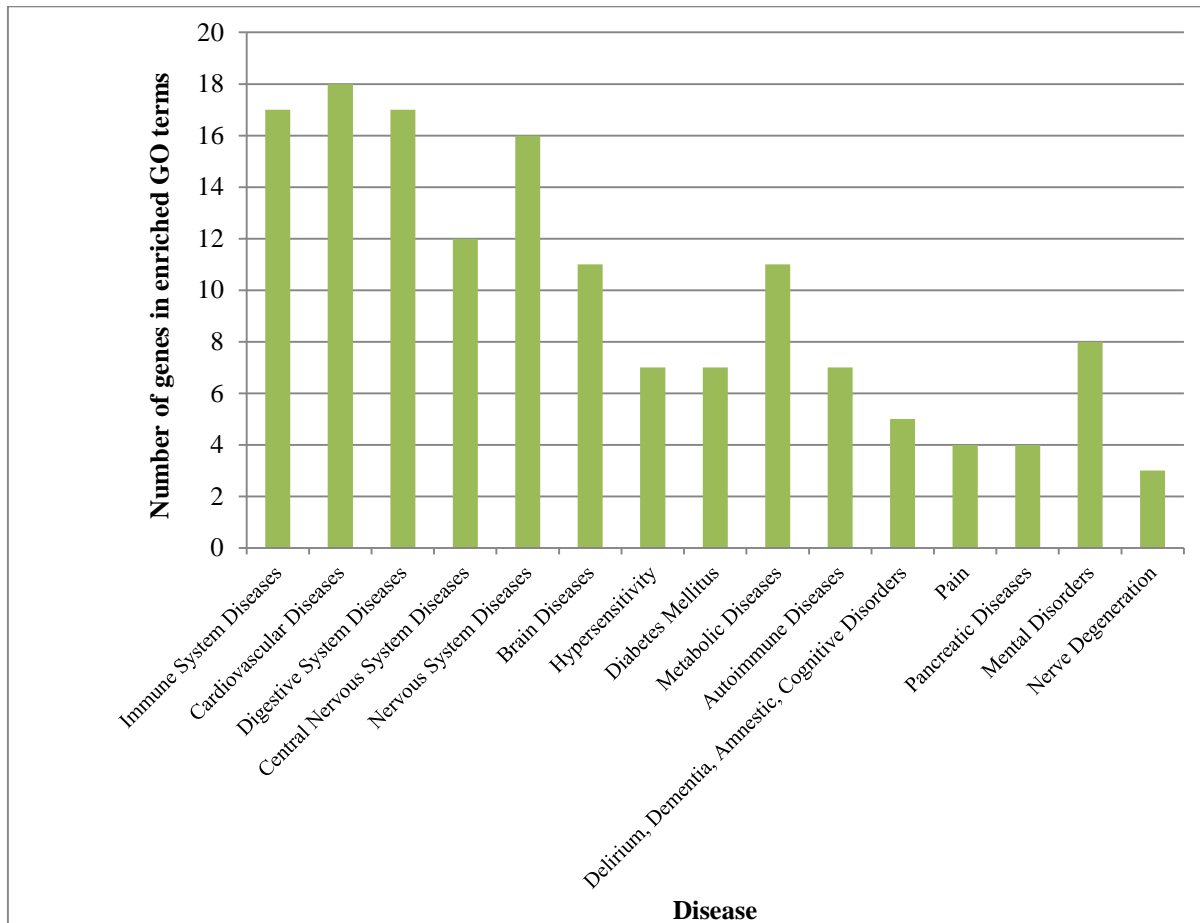**Figure 4.6: Main disease gene ontology (GO) terms associated with the biologically-relevant differentially expressed genes.** The *x-axis* represents the main GO disease terms and the *y-axis* indicates the number of genes enriched for each disease

Figure 4.7 illustrates which group of genes are associated with each disease term, showing sets of genes that are common between the diseases.

Diseases
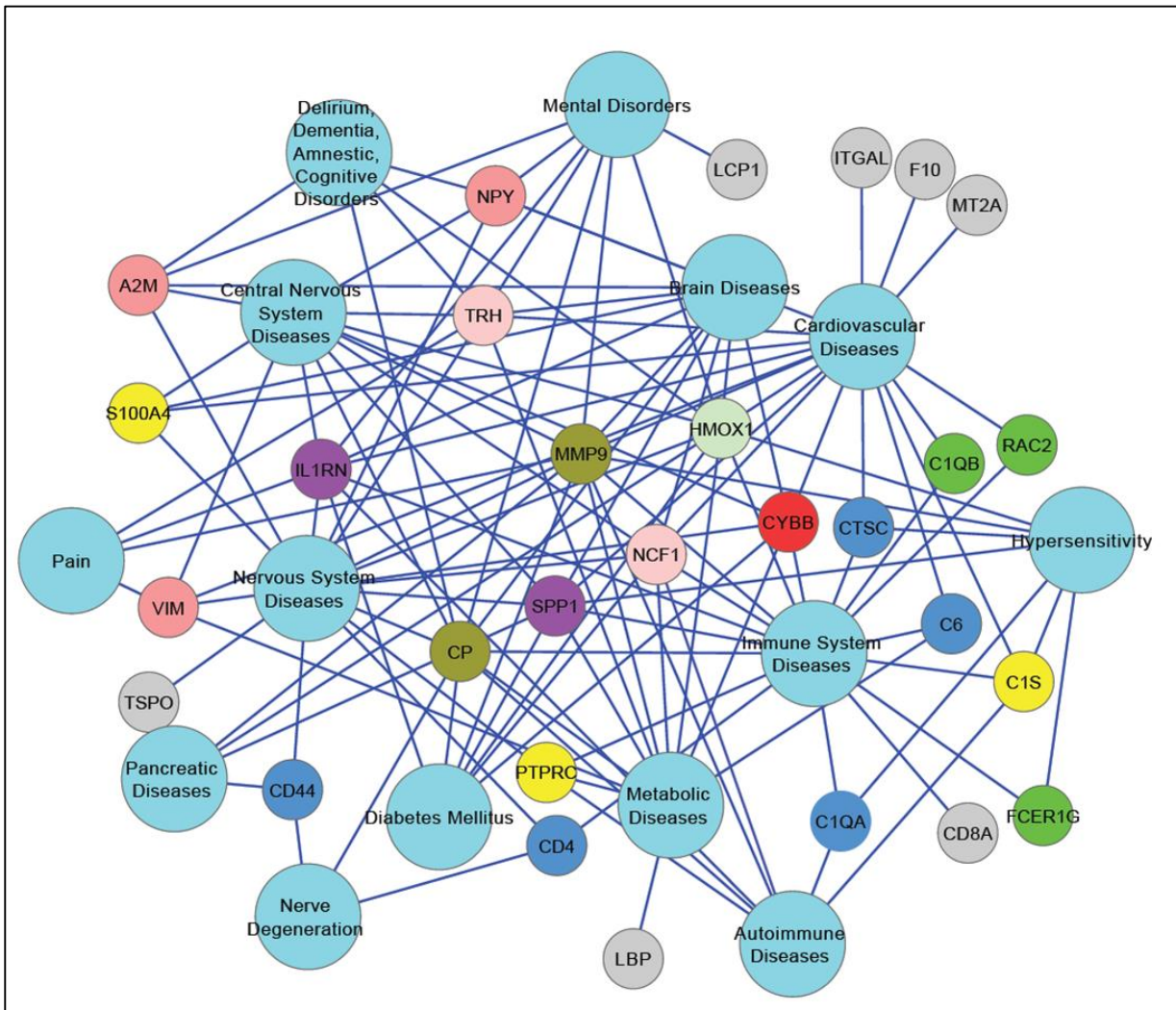
Figure 4.7: Integrative network diagram depicting a selected subset of the common diseases associated with and shared between the biologically-relevant differentially expressed genes in the FDW vs. FSM groups. Diseases are represented using large blue circles; one, two, three, four, five, six, seven, eight, nine, eleven and twelve diseases are represented using grey, green, blue, yellow, pink, orange, red, light pink, purple mint green and olive green respectively. Refer to Table 4.4 for full gene names and Table 4.6 and Fig. 4.6 for disease terms not included in the network

Venn diagrams were also constructed to compare the differentially expressed gene list to genes with inferred relationships (based on CTD-curated chemical–gene interactions, refer to Section 3.3.3 for a more detailed explanation) with anxiety disorders and PTSD (Fig. 4.8). From the 42 biologically relevant differentially expressed genes identified, 21 genes had inferred associations with anxiety disorder and 21 genes had inferred associations with both anxiety disorders and PTSD (Fig. 4.8, Table 4.7).
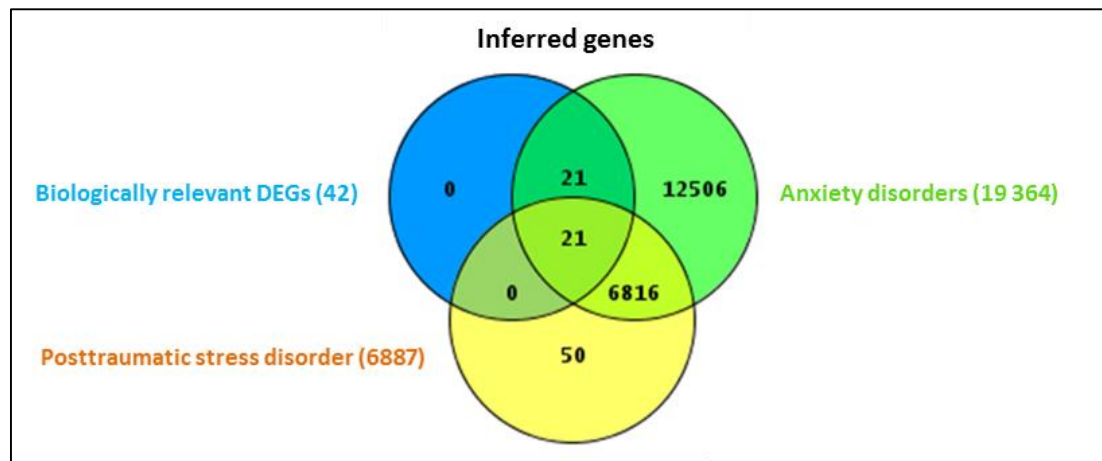
Figure 4.8: Venn diagram depicting the biologically relevant differentially expressed genes (between FDW and FSM groups) that had inferred relationships with PTSD, anxiety disorders or both.

DEG – differentially expressed genes, PTSD - posttraumatic stress disorder, CTD - Comparative Toxicogenomics Database

Table 4.7: Genes with inferred relationships with anxiety disorders alone, and with both anxiety disorders and PTSD (as determined by CTD)

Disorders	Genes
Anxiety disorders	*A2M*, *C1QA*, *C1QB*, *C1QC*, *C1S*, *CD4*, *CHRDL1*, *CLEC7A*, *CXCL13*, *FCER1G*, *GPNMB*, *IL1RN*, *ITGAL*, *LBP*, *LCP1*, *MSR1*, *PTPRC*, *RAC2*, *S100A3*, *S100A4*, ST14
Anxiety disorders and PTSD	*C6*, *CD44*, *CD74*, *CD8A*, *CP*, *CTSC*, *CYBB*, *F10*, *HMOX1*, *LGALS3BP*, *LYZ2*, *MMP9*, *MT2A*, *NCF1*, *NPY*, *RRM2*, *S100A9*, *SPP1*, *TRH*, *TSPO*, *VIM*

PTSD - posttraumatic stress disorder, CTD - Comparative Toxicogenomics Database. Refer to Table 4.4 for full gene names

4.2.3.3 Molecular functions associated with biologically relevant differentially expressed genes

Gene enrichment analysis was also conducted to identify the molecular functions associated with and shared between the 42 biologically relevant genes that were downregulated in the FDW group. Table 4.8 show the subsets of genes and their associated molecular functions.

Table 4.8: Biologically relevant differentially expressed genes in the LDH of FDW vs. FSM animals that were associated with the gene ontology (GO) molecular function terms (p<0.05) (as determined by CTD)

Molecular function	Number of genes	Corrected p-value	Genes
Protein binding	34	1.34E-12	*A2M, C1QA, C1QB, C1S, C6, CD4, CD44, CD74, CD8A, CLEC7A, CP, CTSC, CXCL13, CYBB, F10, FCER1G, GPNMB, HMOX1, IL1RN, ITGAL, LBP, LCP1, MMP9, MSR1, MT2A, NCF1, NPY, PTPRC, RRM2, S100A4, S100A9, SPP1, TRH, VIM*
Binding	37	8.77E-10	*A2M, C1QA, C1QB, C1S, C6, CD4, CD44, CD74, CD8A, CLEC7A, CP, CTSC, CXCL13, CYBB, F10, FCER1G, GPNMB, HMOX1, IL1RN, ITGAL, LBP, LCP1, MMP9, MSR1, MT2A, NCF1, NPY, PTPRC, RAC2, RRM2, S100A3, S100A4, S100A9, SPP1, TRH, TSPO, VIM*
Receptor binding	13	9.65E-07	*CD4, CD74, CD8A, CLEC7A, CXCL13, GPNMB, IL1RN, LBP, NPY, S100A4, S100A9, SPP1, TRH*
MHC protein binding	4	2.00E-05	*CD4, CD74, CD8A, CLEC7A*
Identical protein binding	11	2.29E-05	*C1QB, C1S, CD4, CD74, CD8A, CTSC, HMOX1, LCP1, MMP9, S100A4, VIM*
Receptor activity	11	2.03E-03	*CD4, CD44, CD74, CD8A, CLEC7A, FCER1G, LGALS3BP, MSR1, NPY, PTPRC, TSPO*
Molecular transducer activity	11	3.56E-03	*CD4, CD44, CD74, CD8A, CLEC7A, FCER1G, HMOX1, NPY, PTPRC, S100A9, TSPO*
Signal transducer activity	11	3.56E-03	*CD4, CD44, CD74, CD8A, CLEC7A, FCER1G, HMOX1, NPY, PTPRC, S100A9, TSPO*
Ion binding	20	7.93E-03	*C1S, CD4, CLEC7A, CP, CTSC, CXCL13, CYBB, F10, GPNMB, HMOX1, ITGAL, LCP1, MMP9, MT2A, NCF1, RAC2, RRM2, S100A3, S100A4, S100A9*
MHC class II protein binding	2	2.77E-02	*CD4, CD74*
Signaling receptor activity	9	3.62E-02	*CD4, CD44, CD74, CD8A, CLEC7A, FCER1G, NPY, PTPRC, TSPO*

LDH – left dorsal hippocampus, FDW – fear-conditioned + DCS well-adapted, FSM – fear-conditioned + saline maladapted, CTD – Comparative Toxicogenomics Database. Refer to Table 4.4 for full gene names

Figure 4.9 illustrates the number of genes for each enriched molecular function. The highest number of genes were associated with binding, protein binding, ion binding and receptor binding.
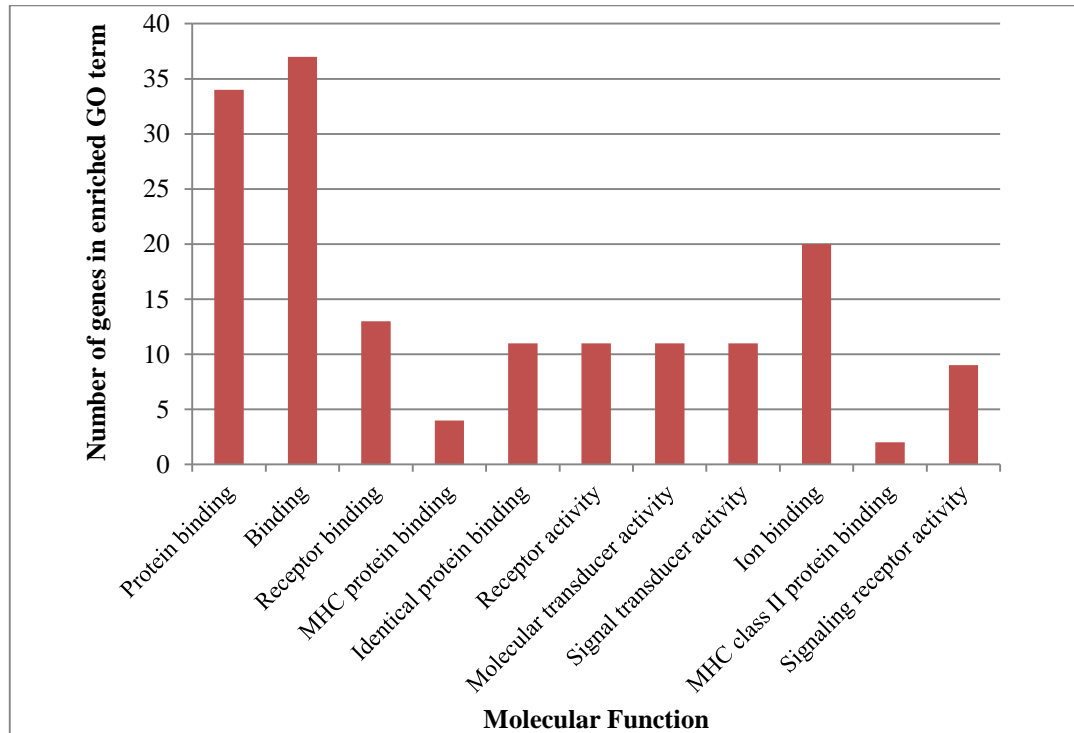
Figure 4.9: Main molecular function GO terms associated with the biologically relevant differentially expressed genes. The *x-axis* represents the main molecular functions and the *y-axis* indicates the number of genes enriched for each molecular function

Figure 4.10 indicates which subsets of genes share a common molecular function. The *CD4* gene was found to be associated with ten of the eleven molecular functions.

Molecular Function
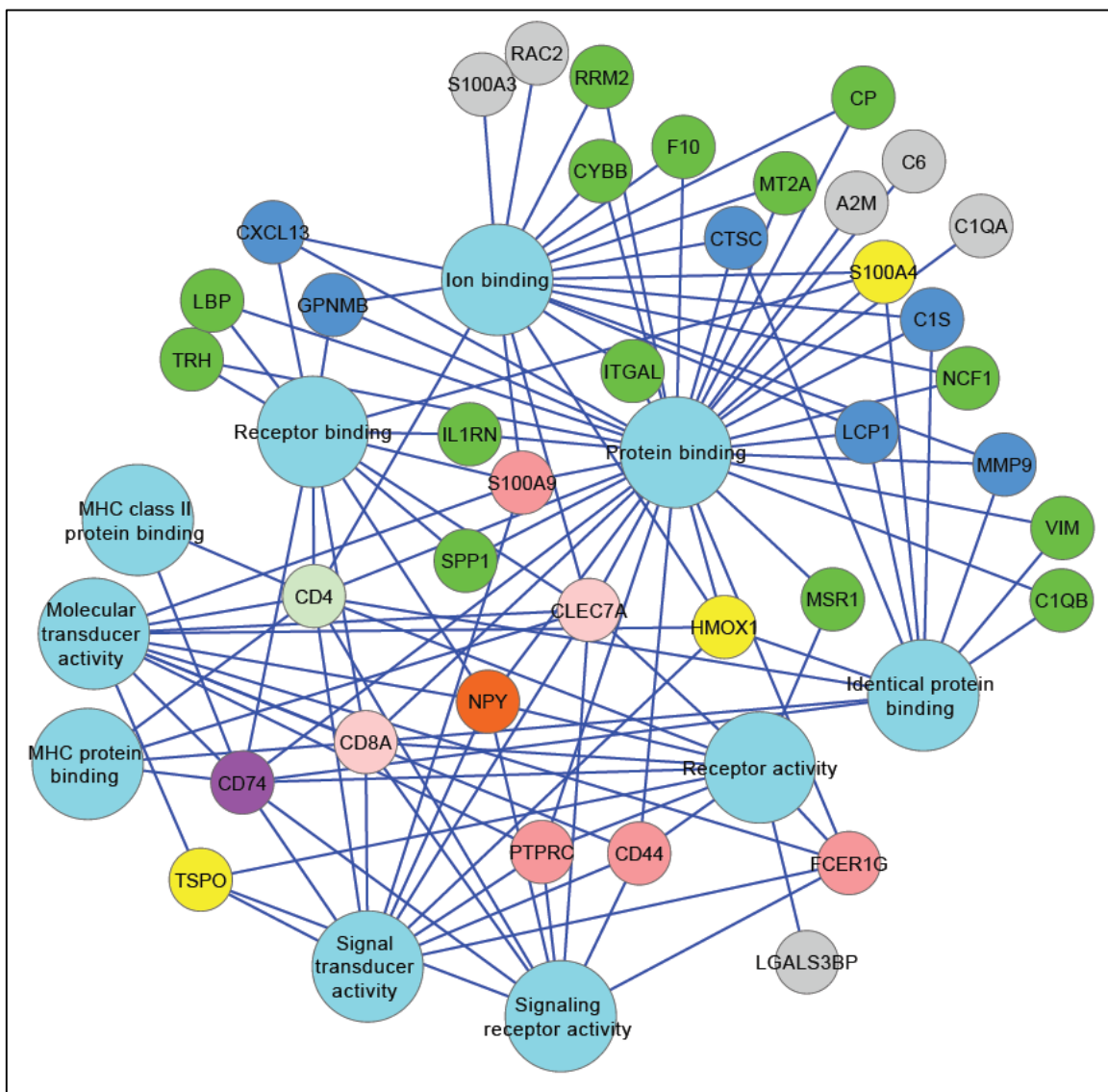
Figure 4.10: Integrative network diagram depicting selected subset of the molecular functions that are shared between the biologically relevant genes that were differentially expressed between the FDW vs. FSM groups. Molecular functions are represented using square blue blocks; one, two, three, four, five, six, eight, nine and ten molecular functions are represented using grey, green, blue, yellow, pink, orange, purple and mint green circles, respectively. Refer to Table 4.4 for full gene names and Table 4.8 for molecular function GO terms not included in the network. FDW – fear-conditioned + DCS well-adapted, FSM – fear-conditioned + saline maladapted

4.2.3.4 Biochemical pathways associated with biologically relevant differentially expressed genes

The comparative toxicogenomics database (CTD) contains KEGG and REACTOME pathway data that represent the current knowledge on the molecular interaction and reaction networks. These networks are integrated with genes, chemicals and diseases in CTD to provide insights into molecular networks that may be associated with different genes. Table 4.9 contains subsets of biologically relevant differentially expressed genes associated with different pathways.

Table 4.9: Biologically relevant differentially expressed genes in the LDH of FDW vs. FSM animals that were associated with KEGG and REACTOME biochemical pathways (p<0.05)

Pathways	Number of genes	Corrected p-value	Genes
Immune System	18	1.24E-14	*C1QA, C1QB, C1QC, C1S, C6, CD4, CD44, CD74, CD8A, CTSC, CYBB, FCER1G, IL1RN, ITGAL, LBP, MT2A, NCF1, PTPRC*
Complement and coagulation cascades	7	1.37E-10	*A2M, C1QA, C1QB, C1QC, C1S, C6, F10*
Staphylococcus aureus infection	5	4.22E-07	*C1QA, C1QB, C1QC, C1S, ITGAL*
Prion diseases	4	6.22E-06	*C1QA, C1QB, C1QC, C6*
Leukocyte transendothelial migration	5	1.83E-05	*CYBB, ITGAL, MMP9, NCF1, RAC2*
Systemic lupus erythematosus	5	4.13E-05	*C1QA, C1QB, C1QC, C1S, C6*
Phagosome	5	1.05E-04	*CLEC7A, CYBB, MSR1, NCF1, RAC2*
Primary immunodeficiency	3	8.16E-04	*CD4, CD8A, PTPRC*
Cell adhesion molecules (CAMs)	4	1.44E-03	*CD4, CD8A, ITGAL, PTPRC*
Tuberculosis	4	4.22E-03	*CD74, CLEC7A, FCER1G, LBP*
Antigen processing and presentation	3	7.12E-03	*CD4, CD74, CD8A*
Hematopoietic cell lineage	3	1.05E-02	*CD4, CD44, CD8A*
Fc gamma R-mediated phagocytosis	3	1.40E-02	*NCF1, PTPRC, RAC2*
Signal Transduction	9	1.57E-02	*A2M, CHRDL1, CXCL13, ITGAL, MMP9, NPY, RAC2, SPP1, TRH*
Chagas disease (American trypanosomiasis)	3	1.67E-02	*C1QA, C1QB, C1QC*
Hemostasis	5	1.79E-02	A2M, F10, FCER1G, ITGAL, RAC2
T cell receptor signaling pathway	3	1.97E-02	CD4, CD8A, PTPRC
Natural killer cell mediated cytotoxicity	3	4.32E-02	FCER1G, ITGAL, RAC2

KEGG - Kyoto Encyclopedia of Genes and Genomes, LDH – left dorsal hippocampus, FDW – fear-conditioned + DCS well-adapted, FSM – fear-conditioned + saline maladapted, CTD – Comparative Toxicogenomics Database. Refer to Table 4.4 for full gene names

Figure 4.11 illustrates the number of genes associated with each enriched pathway. Pathways involved in the immune system, signal transduction, complement and coagulation cascades as well as hemostasis (the process which causes bleeding to stop) had the biggest number of associated genes. Figure 4.12 indicates the subsets of genes that share a common biological pathway.
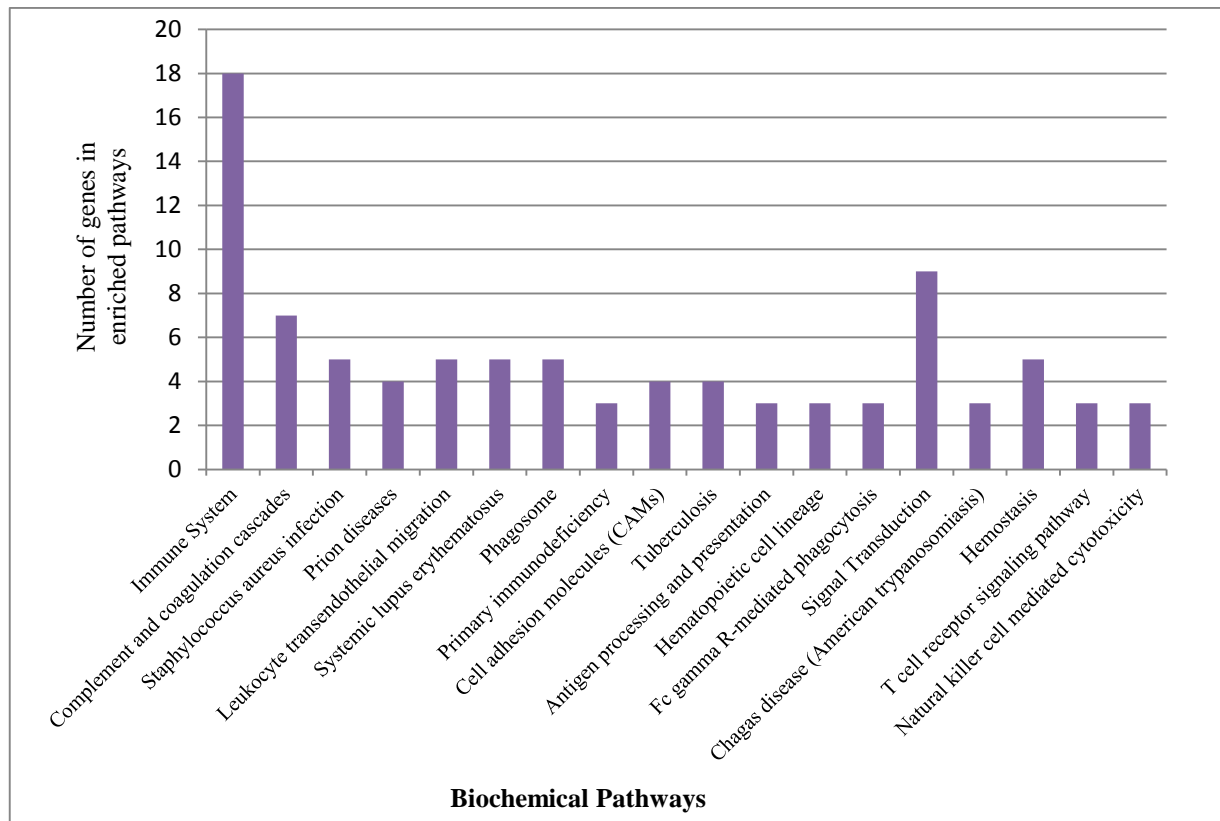
Figure 4.11: Main biochemical pathways associated with the biologically relevant differentially expressed genes based on KEGG and REACTOME search results. The *x-axis* shows the main pathways and the *y-axis* indicates the number of genes enriched for each pathway. KEGG - Kyoto Encyclopedia of Genes and Genomes

Biochemical pathways
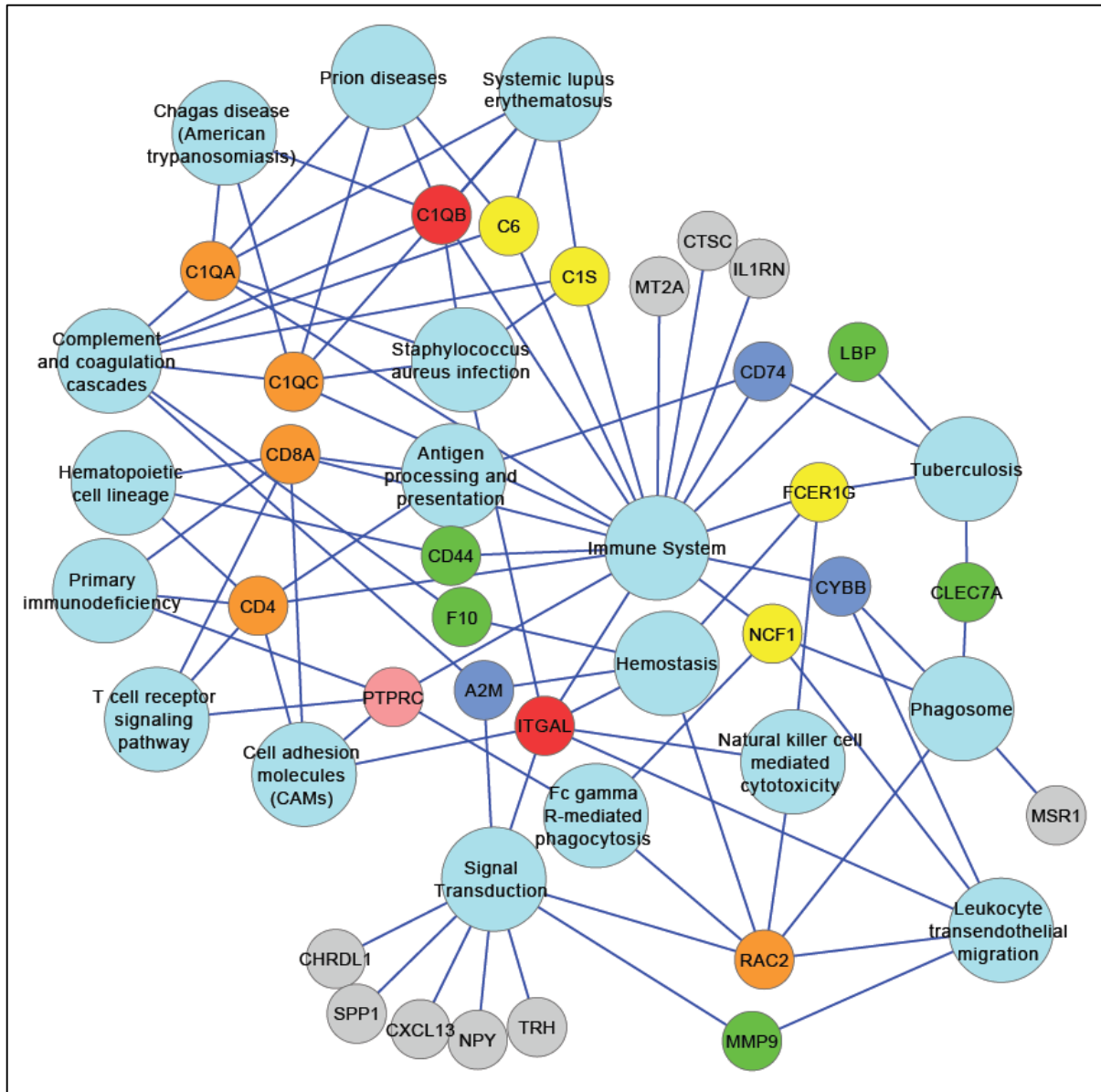
Figure 4.12: Integrative network diagram depicting the pathways that are shared between the biologically relevant genes that were differentially expressed between the FDW vs. FSM groups. Different pathways are represented using large blue circles and one, two, three, four and five, six and seven pathways are represented using grey, green, blue, yellow, pink, orange and red circles, respectively. FDW – fear-conditioned + DCS well-adapted, FSM – fear-conditioned + saline maladapted. Refer to Table 4.4 for full gene names

4.2.4 SYBR Green real-time quantitative PCR gene expression analyses

SYBR Green real-time quantitative PCR (qPCR) gene expression analysis was performed to determine if this technique would be sensitive enough to detect differential expression of a subset of the statistically significant differentially expressed genes identified during the RNAseq analysis. The same LDH cDNA that was used for RNAseq, as well as cDNA derived from blood samples were used in the analysis. Real-time qPCR analysis was able to detect significant downregulation of six genes (of the nine genes tested) in the LDH of FDW compared to the FSM animals (Table 4.10). Metallothionein 2A (*MT2A*) showed a trend towards significant downregulation in the LDH cDNA FDW group with a p-value of 0.06.

Table 4.10: The nine genes (selected based on fold change and function) investigated with SYBR Green real-time qPCR for differential expression in the LDH and blood between FSM and FDW animals

Gene	Name	ANOVA F-test p-values	Function
Differential expression in left dorsal hippocampus			
SPP1	Secreted phosphoprotein 1	0.1	Demyelination
CXCL13	Chemokine (C-X-C motif) ligand 13	0.04	Elevation of cytosolic calcium ion concentration / Immune response
CYBB	Cytochrome b-245, beta polypeptide	0.03	Oxidative stress / Nervous system physiology
IL1RN	Interleukin 1 receptor antagonist	0.05	Learning or memory
S100A4	S100 calcium binding protein A4	0.02	Neuron projection / Calcium ion binding / Calcium-dependent protein binding
TRH	Thyrotropin releasing hormone	0.05	Abnormal pituitary gland development
MMP9	Matrix metallopeptidase 9	0.33	Impaired contextual conditioning behaviour
MT2A*	Metallothionein 2A	0.06 *	Abnormal learning/memory/conditioning
NPY	Neuropeptide Y	0.91	Increased anxiety-related response, abnormal depression-related behaviour
Differential expression in blood			
MMP9	Matrix metallopeptidase 9	0.02	Impaired contextual conditioning behaviour

P-values for six significant differentially expressed genes in the LDH, and one gene in the blood (ANOVA F-tests $p < 0.05$) are indicated in red. Only data for the significantly differentially expressed gene in blood, *MMP9* is indicated. LDH – left dorsal hippocampus, FSM – fear-conditioned + saline maladapted, FDW – fear-conditioned + DCS well-adapted, qPCR – quantitative PCR, ANOVA – analysis of variance. *MT2A p-value showed a trend towards a significant difference in gene expression between FDW and FSM

4.3 DNA Methylation Analysis

The subset of genes for which SYBR Green real-time qPCR was used to investigate differential expression in the LDH (Table 4.10, $p < 0.05$) was analysed using EMBL EMBOSS Cpgplot (http://www.ebi.ac.uk/Tools/seqstats/emboss_cpgplot/) to determine which of these genes contained CpG islands. We selected these genes based on the following (1) for two of the genes (*TRH* and *MT2A*) qPCR detected differential expression (*MT2A* showed a trend towards significanctly lower expression in the FDW vs. FSM group), (2) these genes have functions that could contribute to PTSD disease pathology (*NPY* has especially been implicated in anxiety disorders) and (3) if DNA methylation mediated the differential expression of these genes, results from the RNAseq, real-time qPCR and CpG island methylation would form a strong unit.

CpG islands were identified in *MT2A*, *TRH* and *NPY*. The DNA extracted from LDH as well as commercially available DNA standards (including 100%, 75%, 50%, 25%, 10%, 5% and 0% methylated DNA) from rat pre-mixed methylation controls, were subjected to bisulfite conversion. This was followed by real-time qPCR amplification and HRM analyses to determine the percentage methylation at each CpG island for each sample.

There were no significant differences in DNA methylation levels between the FDW and FSM groups in the screened CpG islands. For the *MT2A* CpG island the HRM melting profile of one of the FDW samples corresponded to that of the 5% methylated standard; the profiles of all the other samples however, corresponded to the 0% methylated standard and were thus unmethylated (Fig. 4.13 A - C). HRM melting profiles of *NPY* and *TRH* CpG islands all corresponded to the 0% methylation standard and were also unmethylated (Fig. 4.13 D - G). In conclusion, there were no significant differences in methylation levels between the FDW and FSM groups in the investigated *MT2A*, *NPY* and *TRH* CpG islands and therefore methylation of these CpG islands did not appear to mediate the downregulation of these genes in the FDW group.

MT2A CpG island mehtylation HRM melting profiles

A. HRM melting profile for DNA methylation standards for the *MT2A* CpG island
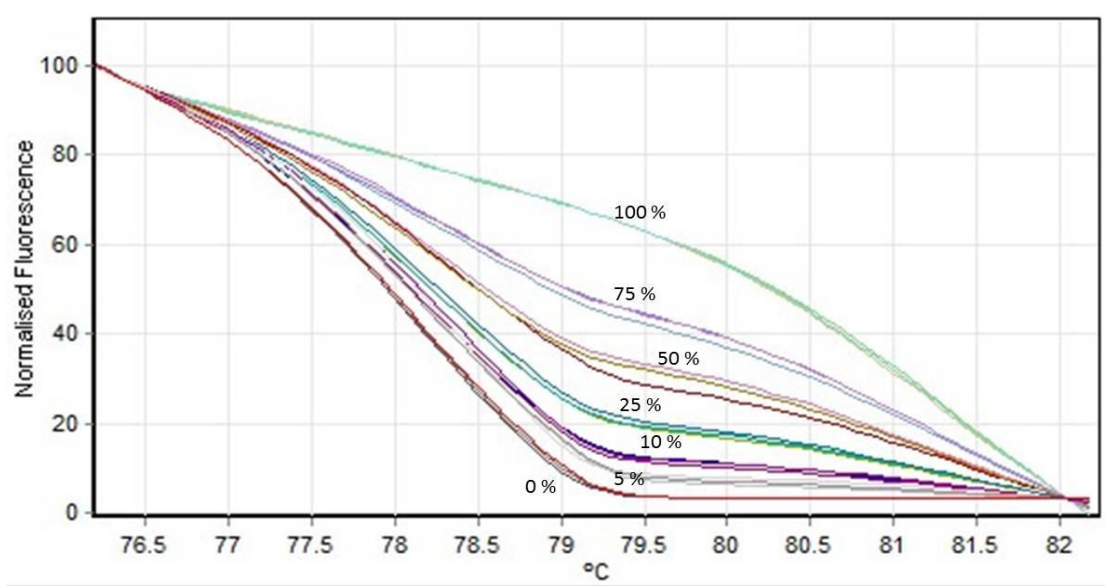
B. HRM melting profiles for FSM and FDW samples for the *MT2A* CpG island, showing 0% , 5% and 10% methylated standards. The FSM and FDW samples correspond to the 0% methylated standard

\

114
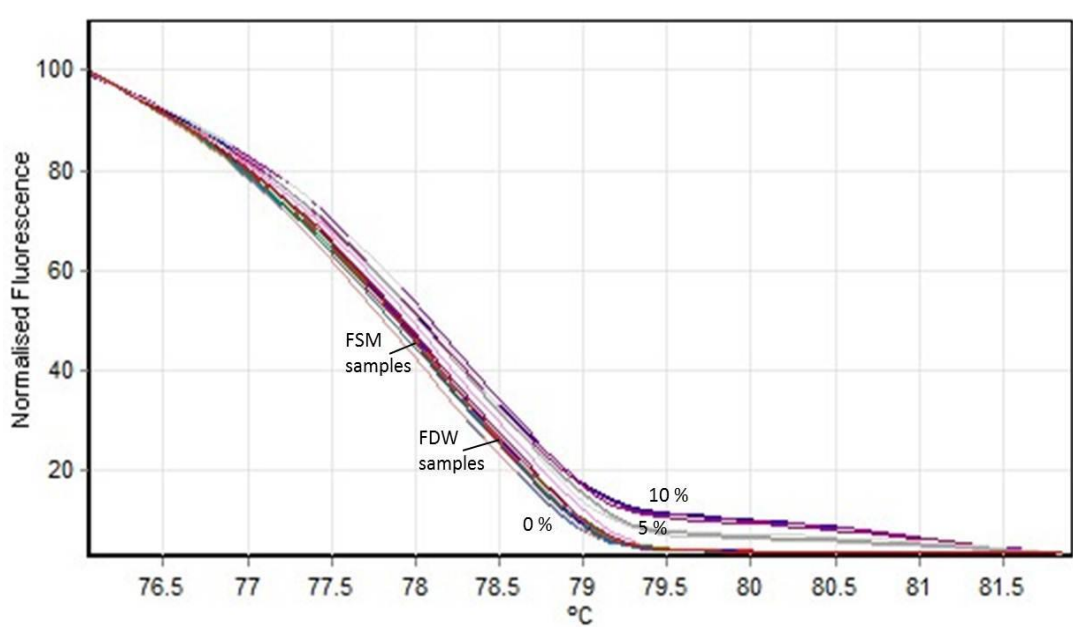
C. HRM melting profiles for one FDW sample for the *MT2A* CpG island, showing 0% , 5% and 10% methylated standards. The FDW sample correspond to the 5% methylated standard
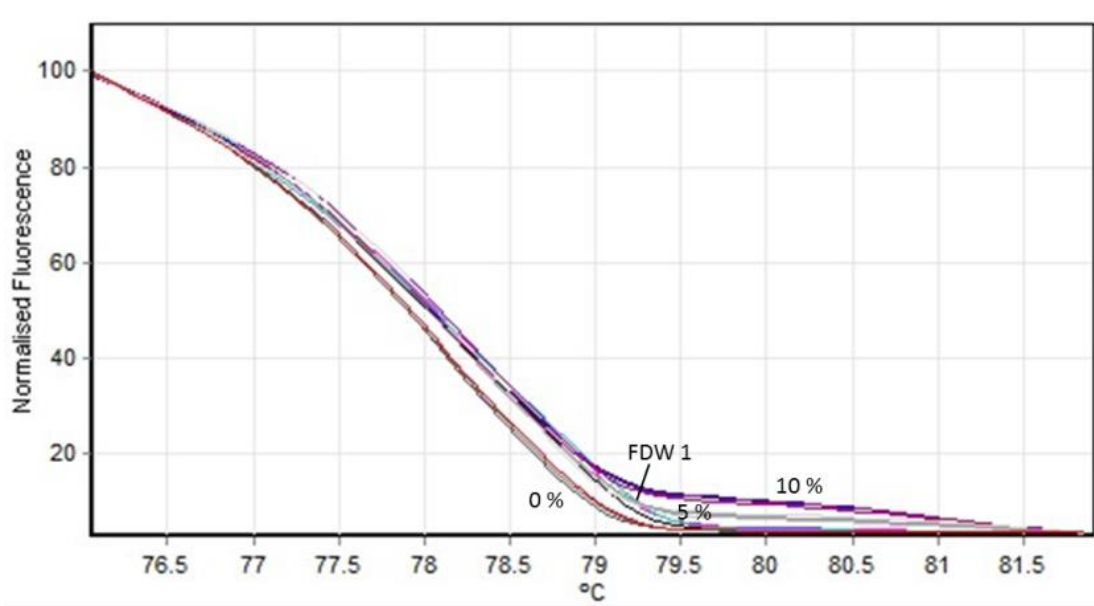
| *NPY* | | |

D. HRM melting profile for DNA methylation standards for the *NPY* CpG island
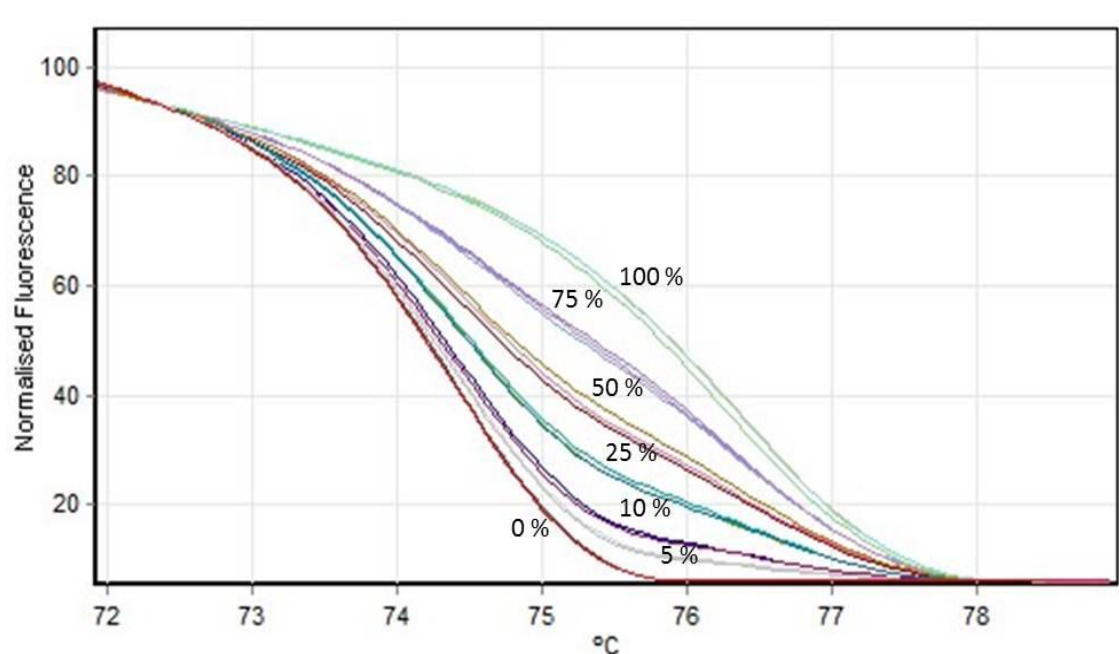
E. HRM melting profiles for FSM and FDW samples for the *NPY* CpG island, showing 0% , 5% and 10% methylated standards. The FSM and FDW samples correspond to the 0% methylated standard
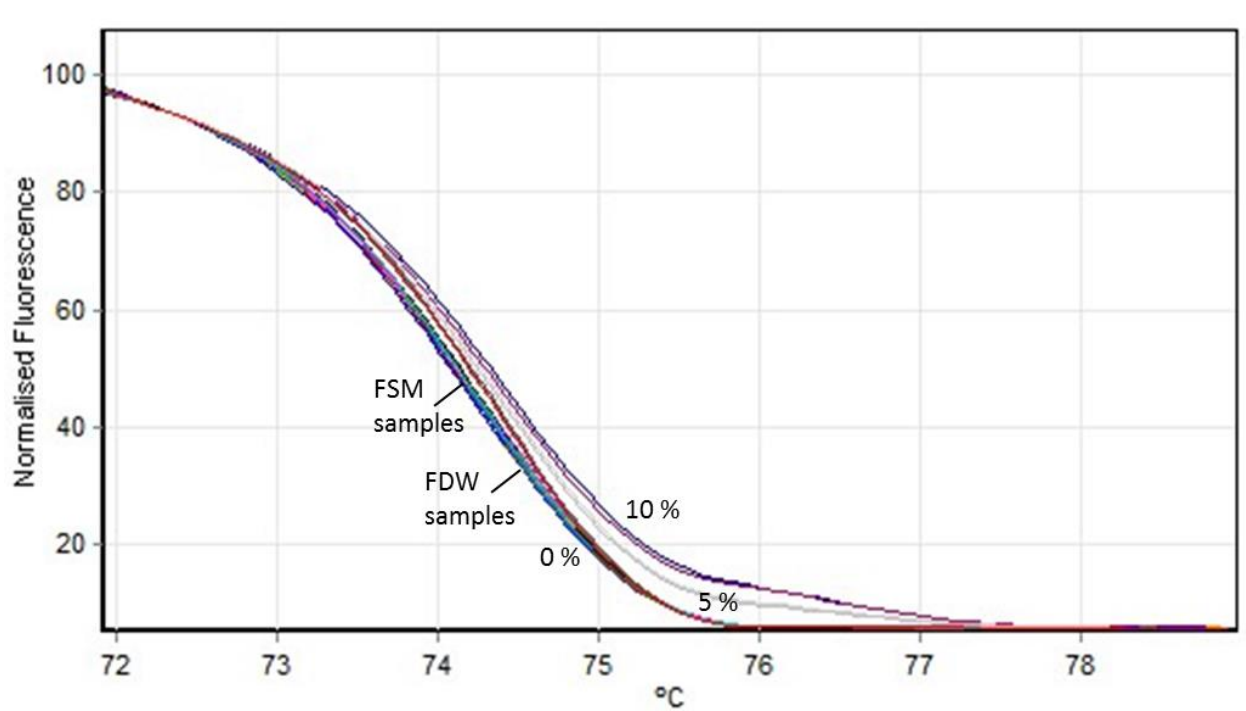
TRH

F. HRM melting profile for DNA methylation standards for the *TRH* CpG island
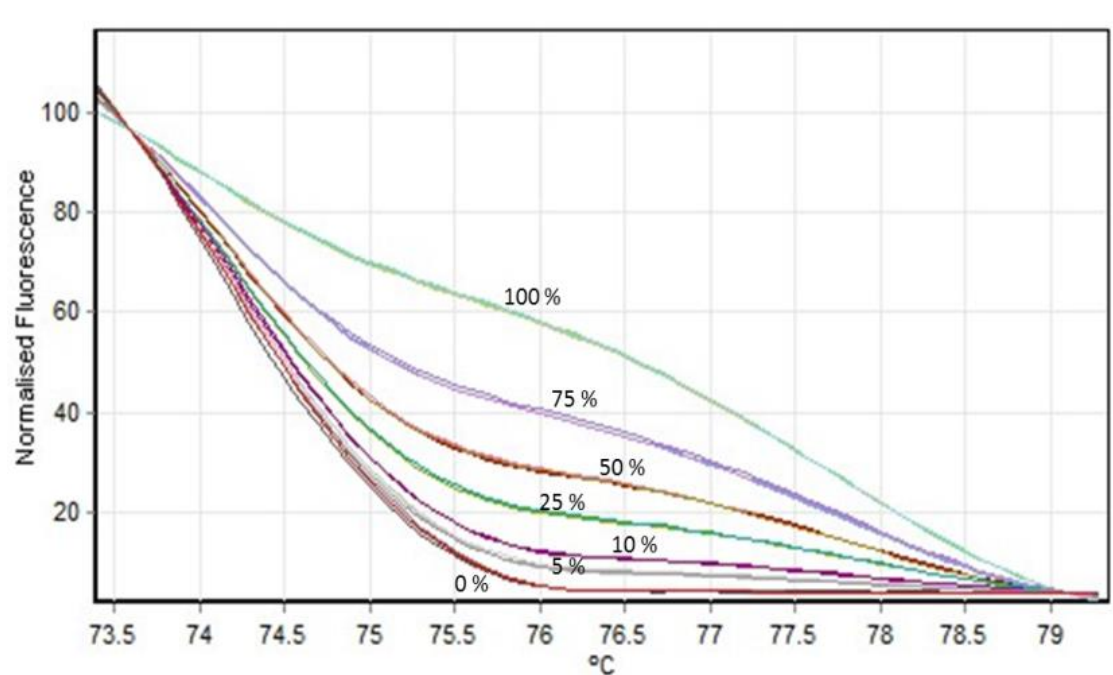
G. HRM melting profiles for FSM and FDW samples for the *TRH* CpG island, showing 0% , 5% and 10% methylated standards. The FSM and FDW samples correspond to the 0% methylated standard
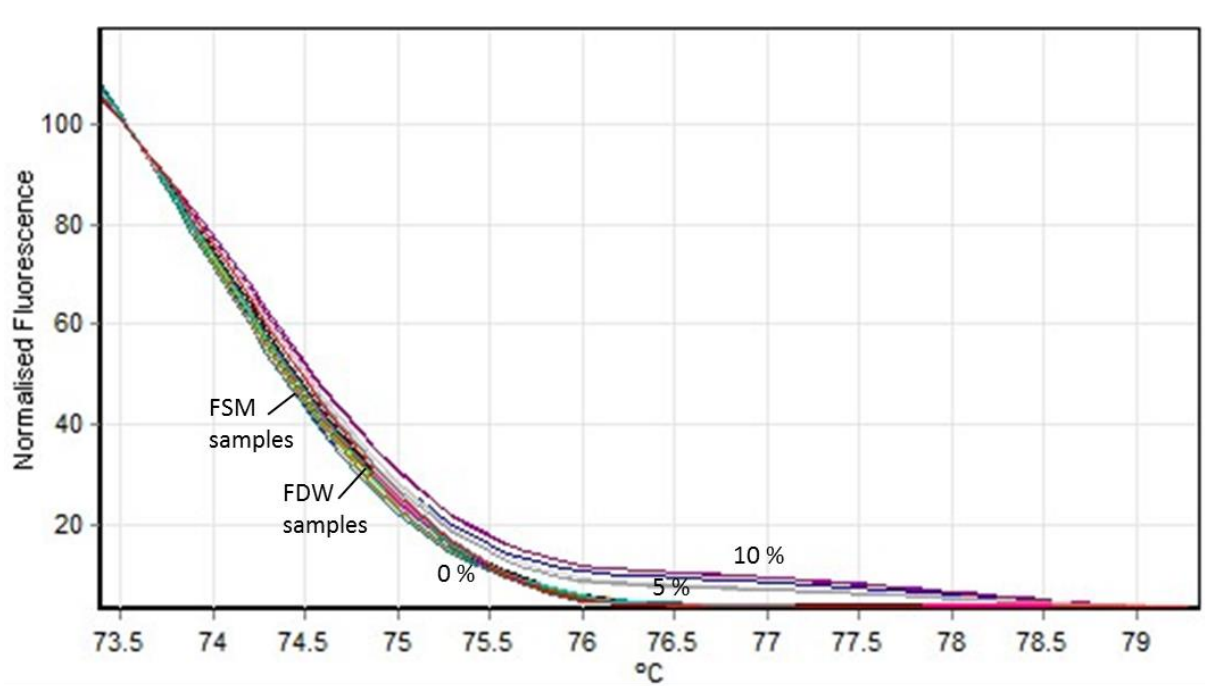
Figure 4. 13: HRM CpG island methylation analysis for MT2A, NPY and TRH. Figures show normalised HRM melt profiles for DNA methylation standards (ranging from 100% methylated to 0% methylated DNA) and the FDW and FSM samples for each gene. The x- axis shows the melting temperature range in °C and the y – axis shows normalised fluorescence values. HRM – high resolution melt, FSM – fear-conditioned + saline maladapted, FDW – fear-conditioned + DCS well-adapted, MT2A - metallothionein 2A, NPY - neuropeptide Y, TRH - thyrotropin releasing hormone

4.4 MicroRNA analysis

4.4.1   MicroRNA sequencing data analysis

The sequencing data from the three flow cells sequenced on the MiSeq® platforms yielded between 654 000 – 4 998 000 reads per sample, with an average of 1.9 million reads per sample. The quality of the sequencing data was high as illustrated by the high phred scores (mostly ≥ 30) (Fig. 4.14). Table 4.11 shows the number of reads generated for each sample as well as the number of reads which could be mapped to the *Rattus novergicus* rn4 rat reference genome. Data analysis of the miRNA sequencing data, using GFOLD (generalized fold change) count facility (Feng et al., 2012) (which only uses data from mapped reads), revealed 23 statistically significant differentially expressed miRNAs between the FDW and FSM groups

117

(Table 4.12). Three of these miRNAs were overexpressed and 20 were underexpressed in the FDW group compared to the FSM group.

Table 4.11: Total amounts of miRNA sequencing reads and number of reads mapped to the reference genome (*Rattus novergicus* rn4) for each sample

Sample	Total amount of sequencing reads	Numer and % of reads mapped to the reference genome	
FDW 1	7 249 534	79 721	(1.10%)
FDW 2	23 321 721	11 129 939	(47.72%)
FDW 3	6 227 221	2 041 530	(32.78%)
FDW 4	7 803 393	4 275 386	(54.79%)
FDW 5	3 737 149	1 473 210	(39.42%)
FDW 6	3 410 516	1 843 431	(54.05%)
FSM 1	4 016 154	198 989	(4.95%)
FSM 2	9 929 794	575 982	(5.80%)
FSM 3	15 840 090	223 005	(1.41%)
FSM 4	3 465 502	241 319	(6.96%)
FSM 5	9 356 673	1 466 464	(15.67%)
FSM 6	3 905 285	2 103 946	(53.87%)

FDW – fear-conditioned + D-cycloserine well-adapted, FSM – fear-conditioned + saline maladapted
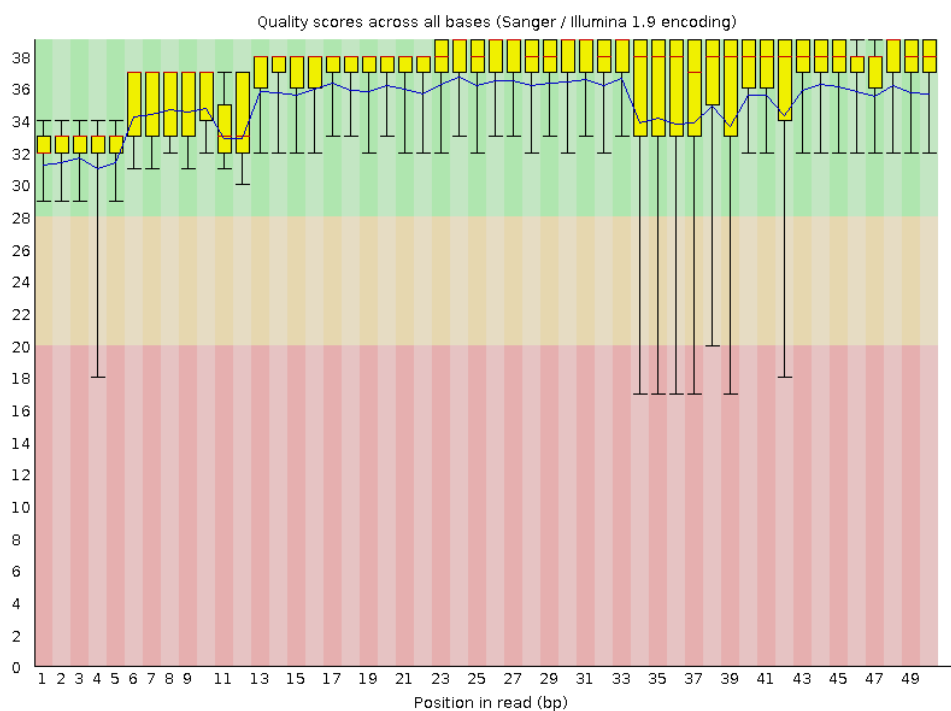
Figure 4.14: Distribution of phred (Q) score in reads. The x-axis indicates the bp position in the 50 bp read and the y-axis indicates the phred score at each position. The phred scores are usually lower at the start and end points and should be trimmed if the per base quality score is below 20

118

Table 4.12: Statistically significant differentially expressed miRNAs between the FDW and FSM groups as identified by GFOLD (generalized fold change) count facility (Feng et al., 2012). Positive fold change values indicate miRNAs that were upregulated in the FDW vs. FSM and negative values indicate downregulated miRNAs in FDW vs. FSM

Gene Symbol	miRNA	Fold Change	Associations of interest to the study
MI0000932	rno-mir-187-3p	1.27	↑ following ECT treatment in Ms rat model (O'Connor et al., 2013)
MI0000872	rno-mir-31a-5p	1.1	↑ in NSCs vs. MN (Wei et al., 2010) ↓following differentiation of adipose-derived stem cells (Wei et al., 2010) ↑ in NSC vs. MN (Wei et al., 2010)
MI0015412	rno-mir-31b	1.08	
MI0015476	rno-mir-3120	-1.50	Mirror miRNA located in neuronal cell bodies (Scott et al., 2012)
MI0021852	rno-mir-6328	-1.47	
MI0006168	rno-mir-488-3p	-0.94	Hsa-mir-488 tagged SNPs associated with different panic disorder phenotypes (Muiños-Gimeno et al., 2011)
MI0012585	rno-mir-490-3p	-1.11	↑ in liver and serum of alcoholic steatohepatitis (Chen et al., 2013) ↑ in insulin resistant adipocytes of mice (Ling et al., 2009)
MI0012590	rno-mir-362-5p	-1.59	
MI0012606	rno-mir-665	-1.55	
MI0000874	rno-mir-33-5p	-1.41	↑ following ECT treatment in Ms rat model (O'Connor et al., 2013)
MI0000847	rno-mir-19b-1-3p	-1.38	
MI0000963	rno-mir-223-3p	-1.71	
MI0000917	rno-mir-144-3p	-1.55	↑ hippocampal expression following chronic lithium and sodium valproate treatment in rats (Zhou et al., 2009) ↑ following KET treatment in Ms rat model (O'Connor et al., 2013) ↓ following FLU treatment in Ms rat model (O'Connor et al., 2013)
MI0000922	rno-mir-153-3p	-1.24	
MI0000842	rno-miR-10b-5p	-1.40	↓ following KET treatment in Ms rat model (O'Connor et al., 2013)
MI0000850	rno-miR-21-5p	-1.29	Glial cell differentiation (Chan et al., 2005)
MI0000620	rno-mir-339-3p	-1.48	Hso-mir-339 tagged SNPs associated with panic disorder (Muiños-Gimeno et al., 2011) 5p ↓ following KET treatment in Ms rat model (O'Connor et al., 2013) 3p ↑ following ECT treatment in Ms rat model (O'Connor et al., 2013) 5p ↓ following ECT treatment in Ms rat model (O'Connor et al., 2013)
MI0000936	rno-mir-193-3p	-1.77	↓ following ECT treatment in Ms rat model (O'Connor et al., 2013)

MI0015405	rno-mir-3549	-1.78	
MI0000915	rno-mir-142-5p	-1.46	
MI0000959	rno-miR-219-1-5p	-1.63	3p ↓ following KET treatment in Ms rat model (O'Connor et al., 2013) 5p↑ following KET treatment in Ms rat model (O'Connor et al., 2013) 3p ↓ following ECT treatment in MS rat model (O'Connor et al., 2013) 5p ↑ following ECT treatment in Ms rat model (O'Connor et al., 2013)
MI0001731	rno-mir-451-5p	-1.90	Expression of mmu-miR-451 correlated with behavioural measures for exploration on the elevated plus-maze task and learning and memory measures (Parsons et al., 2008) ↓ following FLU treatment in Ms rat model (O'Connor et al., 2013) ↓ following KET treatment in Ms rat model (O'Connor et al., 2013) ↓ following ECT treatment in Ms rat model (O'Connor et al., 2013)
MI0006121	rno-mir-879-5p	-1.70	

With regards to miRNA nomenclature, 3p and 5p indicates that the mature miRNA originated either from the 3' or the 5' arm. Hsa – *Homo sapiens*, rno - *Rattus norvegicus*, mmu – *Mus musculus*, SNP – single nucleotide polymorphism, NSC – neuronal stem cells, MSC – mature stem cells, MN – motor neurons, ECT - electroconvulsive shock therapy, Ms - maternal separation, FLU – fluoxetine, KET – ketamine, mir – miRNA, ↓ - downregulated, ↑ - upregulated

4.4.2   MicroRNA target enrichment analysis

Ingenuity pathway analysis (IPA) software program was used to perform miRNA target enrichment analysis, facilitating the identification of common networks shared between the differentially expressed miRNAs, based on the functions of their mRNA targets (Table 4.13). Furthermore, miRNA expression data was integrated with RNAseq expression profiles of biologically relevant differentially expressed genes (of the current study) to identify possible miRNA targets within this gene set (Table 4.14). IPA utilizes miRecords (http://mirecords.umn.edu/miRecords/), TargetScan human (http://www.targetscan.org/) and TarBase (http://www.microrna.gr/tarbase) to predict mRNA targets of the differentially expressed miRNAs. Additional manual searches were performed on databases that included *Rattus norvegicus* in the species list (MicroCosm, microrna.org and DIANA lab). Two miRNAs, rno-mir-187-3p and rno-mir-31a-5p, were significantly upregulated and were predicted to target several genes that were downregulated in the biologically relevant differentially expressed gene set (Table 4.14, Fig. 4.15).

Table 4.13: Common functions shared between differentially expressed miRNAs, based on the functions of their mRNA targets as predicted by Ingenuity Pathway Analysis (IPA).

Top Functions	Number of miRNAs	MiRNAs in Network
Reproductive system disease, Cellular movement, Hair and skin development and function	11	mir-31a, mir-193, miR-144-3p, miR-153, miR-187-3p, miR-193a-3p, miR-31a-5p, miR-31b, miR-3549, miR-490-3p, miR- 665
Cancer, Tumor morphology, Cardiovascular system development and function	9	mir-214, miR-10b-5p, miR-21-5p, miR-223-3p, miR-3120, miR-33-5p, miR-362-5p, miR-451, miR-488-3p, miR-879-5p

Table 4.14: Differentially expressed miRNAs, as identified by GFOLD, and their predicted mRNA targets, within the 42 biologically relevant differentially expressed gene set, as predicted by different software programs. Expression pairing show the direction of miRNA:mRNA expression regulation. Negative log-2 fold values denote down-regulated and positive values denote up-regulated miRNAs in the FDW group compared to the FSM group

miRNA ID	miRNA symbol	Log 2 fold	Prediction program	Confidence	Expression pairing (miRNA:MRNA)	Predicted mRNA target
rno-mir-187	rno-mir-187-3p	1.273	TargetScan human	Moderate (predicted)	↑↓	A2M
rno-mir-187	rno-mir-187-3p	1.273	TargetScan human	High (predicted)	↑↓	C1S
rno-mir-187	rno-mir-187-3p	1.273	TargetScan human	Moderate (predicted)	↑↓	S100A4
rno-mir-187	rno-mir-187-3p	1.273	MicroCosm	Moderate (predicted)	↑↓	CD4
rno-mir-187	rno-mir-187-3p	1.273	MicroCosm microRNA.org	Moderate (predicted)	↑↓	CD44
rno-mir-193	rno-mir-193-3p	-1.770	TargetScan human	Moderate (predicted)	↓↓	C1QC
rno-mir-193	rno-mir-193-3p	-1.770	MicroCosm microRNA.org	Moderate (predicted)	↓↓	S100A4
rno-mir-193	rno-mir-193-3p	-1.770	MicroCosm	Moderate (predicted)	↓↓	GRN
rno-miR-21	rno-miR-21-5p	-1.294	TargetScan human	Moderate (predicted)	↓↓	TSPO
rno-mir-19b-1	rno-mir-19b-1-3p	-0.206	microRNA.org	Moderate (predicted)	↓↓	CXCL13
rno-mir-19b-1	rno-mir-19b-1-3p	-0.206	microRNA.org	Moderate (predicted)	↓↓	RRM2
rno-mir-144	rno-mir-144-3p	-0.259	MicroCosm microRNA.org	Moderate (predicted)	↓↓	GPNMB
rno-mir-144	rno-mir-144-3p	-0.259	MicroCosm	Moderate (predicted)	↓↓	ANXA3
rno-mir-153	rno-mir-153-3p	-0.259	MicroCosm	Moderate (predicted)	↓↓	GPNMB

rno-mir-153	rno-mir-153-3p	-0.259	MicroCosm microRNA.org	Moderate (predicted)	↓↓	*C1S*
rno-miR-219-1	rno-miR-219-1-5p	-0.541	microRNA.org	Low (predicted)	↓↓	*IL1RN*
rno-miR-219-1	rno-miR-219-1-5p	-0.541	microRNA.org	Low (predicted)	↓↓	*CTSC*
rno-mir-223	rno-mir-223-3p	-1.715	TargetScan human	Moderate (predicted)	↓↓	*CD44*
rno-mir-223	rno-mir-223-3p	-1.715	TargetScan human	Moderate (predicted)	↓↓	*MSR1*
rno-mir-223	rno-mir-223-3p	-1.715	MiRecords DIANA lab MicroCosm microrna.org	Experimentally observed	↓↓	*VIM*
rno-mir-223	rno-mir-223-3p	-1.715	microrna.org	Moderate (predicted)	↓↓	*PTPRC*
rno-mir-223	rno-mir-223-3p	-1.715	microrna.org	Moderate (predicted)	↓↓	*CHRDL1*
rno-mir-31a	rno-mir-31a-5p	1.097	TargetScan human	Moderate (predicted)	↑↓	*IL1RN*
rno-mir-31a	rno-mir-31a-5p	1.097	TargetScan human	Moderate (predicted)	↑↓	*RAC2*
rno-mir-31a	rno-mir-31a-5p	1.097	MicroCosm	Moderate (predicted)	↑↓	*HMOX1*
rno-mir-3120	rno-mir-3120	-1.502	TargetScan human	High (predicted)	↓↓	*MSR1*
rno-mir-33	rno-mir-33-5p	-1.409	TargetScan human	Moderate (predicted)	↓↓	*MSR1*
rno-mir-33	rno-mir-33-5p	-1.409	TargetScan human	High (predicted)	↓↓	*SPP1*
rno-mir-33	rno-mir-33-5p	-1.409	MicroCosm	Moderate (predicted)	↓↓	*NPY*
rno-mir-33	rno-mir-33-5p	-1.409	MicroCosm	Moderate (predicted)	↓↓	*SAT*
rno-mir-339	rno-mir-339-3p	-1.475	MicroCosm	Moderate (predicted)	↓↓	*LYZ2*
rno-mir-339	rno-mir-339-3p	-1.475	MicroCosm	Moderate (predicted)	↓↓	*MMP9*
rno-mir-339	rno-mir-339-3p	-1.475	MicroCosm	Moderate (predicted)	↓↓	*RAC2*
rno-mir-362	rno-mir-362-5p	-1.586	TargetScan human	Moderate (predicted)	↓↓	*CTSC*
rno-mir-362	rno-mir-362-5p	-1.586	TargetScan human	Moderate (predicted)	↓↓	*LCP1*
rno-mir-451	rno-mir-451-5p	-0.750	MicroCosm	Moderate (predicted)	↓↓	*PTPRC*
rno-mir-488	rno-mir-488-3p	-0.945	TargetScan human	Moderate (predicted)	↓↓	*CTSC*
rno-mir-488	rno-mir-488-3p	-0.945	TargetScan human	High (predicted)	↓↓	*S100A3*
rno-mir-488	rno-mir-488-3p	-0.945	TargetScan human microRNA.org	High (predicted)	↓↓	*VIM*
rno-mir-488	rno-mir-488-3p	-0.945	microrna.org	Moderate (predicted)	↓↓	*RMR2*
rno-mir-490	rno-mir-490-3p	-1.114	TargetScan human	High (predicted)	↓↓	*CHRDL1*
rno-mir-490	rno-mir-490-3p	-1.114	TargetScan	Moderate (predicted)	↓↓	*GPNMB*

				human			
rno-mir-665	rno-mir-665	-1.549	TargetScan human	High (predicted)	↓↓	*CD4*
rno-mir-665	rno-mir-665	-1.549	TargetScan human	Moderate (predicted)	↓↓	*CP*
rno-mir-665	rno-mir-665	-1.549	TargetScan human	Moderate (predicted)	↓↓	*MSR1*
rno-mir-879	rno-mir-879-5p	-1.701	TargetScan human	High (predicted)	↓↓	*CXCL13*
rno-mir-879	rno-mir-879-5p	-1.701	TargetScan human	Moderate (predicted)	↓↓	*LCP1*
rno-mir-879	rno-mir-879-5p	-1.701	MicroCosm	Moderate (predicted)	↓↓	*CLEC7A*

FDW – fear-conditioned + DCS well-adapted, GFOLD - generalized fold change count facility, rno - *Rattus norvegicus*, mir – miRNA, ↓ shows downregulation, ↑ shows upregulation, Refer to Table 4.4 for full mRNA target gene names
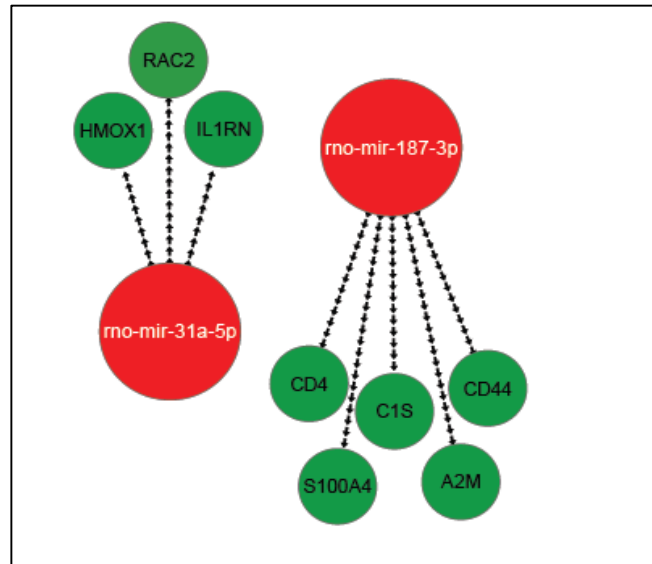
Figure 4.15: Integrative miRNA target enrichment. Integrative target enrichment diagram depicting the upregulated miRNAs in red circles and the downregulated genes (from the RNAseq data) predicted to be targeted by the upregulated miRNAs, in green circles. *HMOX* - heme oxygenase (decycling) 1, *RAC2* - ras-related C3 botulinum toxin substrate 2, *IL1RN* - interleukin 1 receptor antagonist, *CD4* - T cell surface glycoprotein CD4 molecule, *S100A4* - S100 calcium binding protein A4, *C1S* - complement component 1, s subcomponent, *A2M* - Alpha-2-macroglobulin, mir – miRNA, rno - Rattus norvegicus, mir - miRNA

4.4.3 SYBR Green real-time qPCR expression analysis for rno-miRNA-31a-5p in LDH and blood

Rno-miRNA-31a-5p was one of the significantly upregulated miRNAs identified in the miRNA sequencing analyses and was selected to be used for SYBR Green real-time qPCR expression analysis. This particular miRNA was selected for the following reasons: (1) its potential association with anxiety related traits (Parsons et al., 2012), (2) it is predicted to target *IL1RN*, which was four-fold downregulated in the FDW vs. FSM group and (3) its target gene, *IL1RN*, is also predicted to be involved in learning or memory processes (Palin et al., 2004). The aim was to determine whether SYBR Green real-time qPCR is sensitive enough to detect differential expression of this miRNA in the LDH and in blood samples between FDW and FSM animals. The small nuclear RNA (snRNA) component of U6 small nuclear ribonucleoprotein (snRNP) was used for normalisation. SYBR Green real-time qPCR expression was not able to detect a significant difference in the expression level of rno-miRNA-31a-5p between FDW and FSM animals in either the LDH cDNA ($p = 0.35$) or blood cDNA ($p = 0.65$) samples (ANOVA F-test).

4.4.4 Functional analysis of miRNA-target interaction

In order to determine whether rno-miR-31a-5p directly targeted *IL1RN*, as predicted by the software programs, a luciferase reporter assay was performed, using the GLuc-ON^{TM} Promoter Reporter Clones assay kit. Results from the mixed model repeated measures ANOVA indicated that co-transfection of a promoter clone containing the 3' UTR sequence of *IL1RN* and the rno-miR-31a-5p precursor clone, resulted in significantly lower luciferase activity compared to co-transfection of the miRNA precursor clone and a scrambled 3' UTR control clone ($p < 0.05$) (Fig. 4.15). This suggested that rno-miR-31a-5p did bind to the mRNA target region of *IL1RN*. However, when the luciferase signal was compared to that of the other negative controls, namely the scrambled miRNA precursor clone + *IL1RN* promoter reporter clone and the *IL1RN* promoter reporter clone without any miRNA precursor, no statistically significant differences were detected in the luciferase signals.
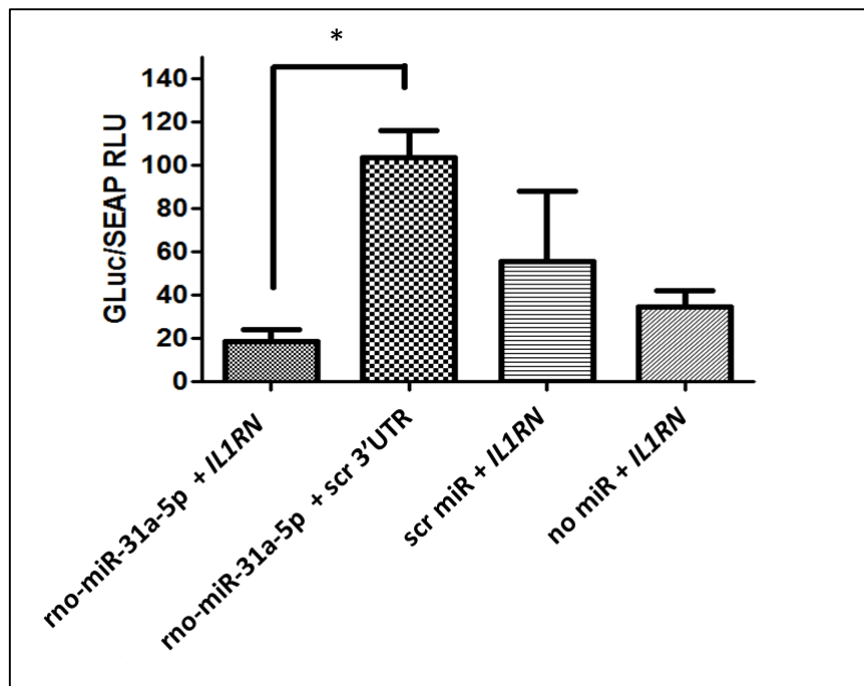
Figure 4.16: Functional luciferase analysis of miRNA-target interaction. Relative light units (RLU) of GLuc signal normalised to SEAP signal for each of the four different co-transfections into HEK 293 cells. HEK 293 cells co-transfected with the rno-miR-31a-5p precursor clone and *IL1RN* promoter reporter clone, showed significantly lower luciferase activity (18.16 ± 5.7) compared to cells co-transfected with the rno-miR-31a-5p precursor clone and a scrambled 3' UTR control clone (103.32 ± 12.655) (* $p < 0.05$) (mixed model repeated measures ANOVA). The data shown are the means ± SEM (standard error of the mean) of two independent experiments performed in duplicate. GLuc - Gaussia luciferase, SEAP - secreted Alkaline Phosphatase, RLU – relative light units, HEK 293 cells - Human Embryonic Kidney 293 cells, rno-miR31a-5p - rno-miR-31a-5p precursor clone, *IL1RN* - Interleukin 1 receptor antagonist promoter reporter clone, scr 3' UTR – scrambled three prime untranslated region negative control reporter clone, scr miR – scrambled miRNA negative control precursor clone.

5. Discussion

The current study investigated the molecular mechanisms underlying DCS-facilitated fear extinction in a PTSD animal model by investigating gene expression and epigenetic profiles in the LDH of male Sprague Dawley rats. The key findings of the study were firstly that co-administration of DCS and behavioural fear extinction downregulates genes that facilitate neuroinflammation, namely immune system genes, proinflammatory genes and genes involved in oxidative stress. Secondly, co-administration of DCS and behavioural fear extinction downregulated genes that are associated with processes in anxiety- and stress-related disorders, such as PTSD, as well as genes that are associated with disorders that often occur comorbidly with PTSD. In addition, DCS was found to downregulate genes that are involved in fear and anxiety, such as *TRH* (Wittmann et al., 2009; Lisowski et al., 2013), *CYBB* (Nair et al., 2011; Rammal et al., 2008; Hovatta et al., 2005; Masood et al., 2008; Schiavone et al., 2009), and genes transcribing S100 proteins (Ackermann et al., 2006). Moreover, DCS downregulated genes implicated in learning and memory, such as *IL1RN* (Oprica et al., 2005; Spulber et al. 2009) and *TRH* (Aguilar-Valles et al., 2007).

Epigenetic analyses indicated that CpG island DNA methylation of the investigated genes, did not mediate differential expression of those genes. Co-administration of DCS and behavioural fear extinction did, however, result in differential miRNA expression, and functional analysis suggested that the differential gene expression induced by DCS may have been mediated through differential expression of miRNAs.

The following paragraphs will briefly discuss the animal behavioural data. At this juncture, it should be noted that the animal behavioural data was not the focus of the current study, and was conducted by a fellow student. This is followed by a discussion of the GO enrichment analyses results and a more focussed discussion on a subset of the 42 biologically relevant differentially expressed genes, including those genes exhibiting the largest fold changes, genes for which differential expression was replicated using real-time qPCR as well as genes that have been described in literature to be involved in learning, memory and neuroinflammation. The chapter concludes with a discussion of the epigenetic results, including DNA methylation analysis, miRNA expression analysis as well as miRNA and gene expression correlation and functional miRNA analysis.

5.1 Central and peripheral effectors of the stress system

In order to adequately interpret molecular results from studies on stress-related disorders, it is imperative to understand the stress process and the downstream effects that stress elicits on an organism as a whole. The major CNS effectors of the stress system include hormones involved in the HPA axis (corticotropin-releasing hormone, arginine and vasopressin) and the pro-opiomelanocortin-derived peptides (α-melanocyte stimulating hormone, β-endorphin and norepinephrine which is produced in the locus coeruleus and autonomic norepinephrine centres in the brainstem) (Chrousos and Gold 1992; Charmandari et al., 2005). The main peripheral effectors are glucocorticoids, regulated by the HPA axis, and the catecholamines (epinephrine and norepinephrine), regulated by the systemic and adrenomedullary sympathetic nervous

system. These effectors target several systems, including the executive, cognitive, reward, fear and growth systems, and reproductive and thyroid hormone axes (Chrousos and Gold 1992; Chrousos, 1998), the circadian centres of the brain (Chrousos 2007; Vgontzas et al., 2003, 2004, 2007), gastrointestinal (Chrousos 2007; Taché and Bonas 2007), cardiorespiratory (Blanchard et al., 1990; Fredrikson and Matthews 1990; Georgiades and Fredrikson 2000; Buckley et al., 2001), metabolic (Goodwin et al., 2005; Lauterbach et al., 2005; Trief et al., 2006; Qureshi et al., 2009; Boyko et al., 2010; Pietrzak et al., 2012; Lukaschek et al., 2013; Dedert et al., 2010), and immune systems (Chrousos 1995; Chrousos 2000; Karalis et al., 1991; Elenkov et al., 2008). Dysregulation of the basal activity of this stress system could therefore result in a multitude of psychiatric, behavioural as well as somatic pathological conditions (Fig. 5.1). Throughout the discussion, the reader will be referred to Figure 5.1, which illustrates how stress and other factors, such as genetic variation, influences the stress system and different target tissues, which could ultimately lead to the development of various pathological conditions.
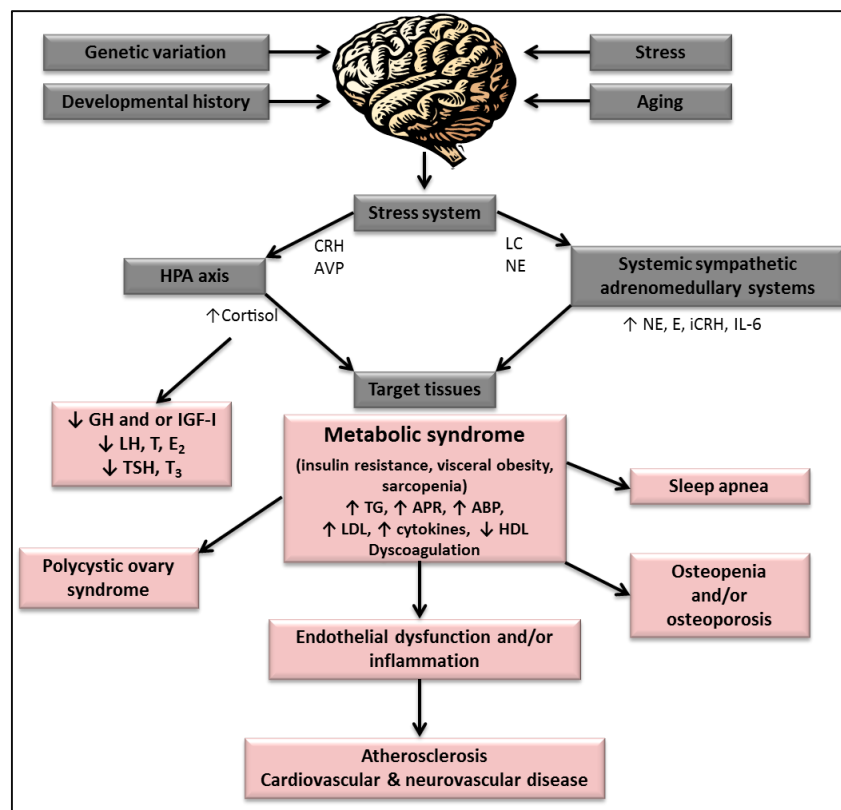
Figure 5.1: Chronic stress interacts with multiple environmental and genetic factors which subsequently influence the levels of various hormones and neurotransmitters. Altered profiles of hormones and neurotransmitters may ultimately contribute to the development of various diseases, such as metabolic syndrome, sleep disturbances, osteoporosis, inflammatory diseases, cardiovascular and neurovascular diseases and problems with the reproductive system. ABP – arterial blood pressure, ACTH - adrenocorticotropin hormone, APR – acute-phase reactants, AVP - arginine vasopressin, CRH - corticotropin-releasing hormone, iCRH – immune CRH, E – epinephrine (adrenalin), E2 – estradiol, GH – growth hormone, HPA - hypothalamic–pituitary–adrenal, IGF-I – insulin-like growth factor I, IL-6 – interleukin 6, LC – locus coeruleus, LH – luteinizing hormone, NE – norepinephrine, T – testosterone, TG - triglycerides (adapted from Chrousos, 2009)

5.2 PTSD animal model

The L/D avoidance test was compared to the other behavioural tests performed, namely the FST (forced swim test) and the OF tests, and was found to be the most sensitive behavioural test to detect the effects of fear conditioning and fear extinction. The L/D avoidance test showed statistically significant differences between the FS group and the CS group (fear conditioning), where the FS animals displayed more fearful behaviour than the CS animals. Furthermore, in the L/D avoidance test there was a statistically significant difference between the FS and FD animals (fear extinction). The other behavioural tests (FS test and the OF tests) either did not show statistically significant differences between all the aforementioned group comparisons or the differences were less significant compared to the L/D avoidance tests.

The results from the L/D avoidance test were thus subsequently used as the behavioural test on which to base the selection of well-adapted (WA) and maladapted (MA) subgroups within the fear-conditioned + saline (FS) and fear-conditioned + DCS (FD) groups. Statistical analyses of the L/D avoidance test behavioural data showed that there was a statistically significant difference between the FS group and all the other groups, with the FS group spending significantly more time in the dark compartment (Fig. 4.1A, Table 4.1, Section 4.1), indicative of anxiogenic or stress-related behaviour (Bourin and Hascoët, 2003). These results suggest that the modified animal model had face validity; where the model reproduced symptoms associated with the human syndrome (such as avoidance). Furthermore, based on the results from the L/D avoidance test, the fear conditioning paradigm used in the present study was successful in eliciting a significant fearful response in the fear-conditioned animals compared to animals that were not fear-conditioned. This indicated construct validity of the modified animal model as the L/D avoidance test measured what it was intended to measure, namely anxiety and stress-related behaviour. There was also a statistically significant difference detected between the control + saline (CS) group compared to all the other groups (Fig. 4.1A, Table 4.1, Section 4.1), where the CS group spent significantly more time in dark compartment. The significant difference between the CS and the CD was an unexpected result, as, in human studies, DCS is known to only elicit its anxiolytic effects when co-administered with behavioural fear extinction/CBT (Smits et al., 2013; de Kleine et al., 2012; Richardson et al., 2004). One explanation could be that outliers may have caused this effect, since after sub-group selection for the CD and CS groups, where 12 animals with behavioural values closest to the group mean were selected, there were no statistically significant group differences.

There were no statistically significant differences in the time spent in the dark compartment between the CD and FD groups (Fig. 4.1A, Table 4.1, Section 4.1). This result might suggest that the fear extinction induced by the co-administration of DCS and behavioural fear extinction was effective to such an extent that the animals in this group had behavioural parameters similar to those of the control group.

Furthermore, statistical analyses of the behavioural data of the WA and MA groups confirmed that there was indeed a statistical significant difference between MA and WA animals (Fig. 4.1 C, Table 4.2, Section 4.1);

WA animals spent significantly less time in the dark compartment. Furthermore, FDW animals spent significantly less time in the dark compartment compared to the FSM animals, which served as confirmation that the behavioural test used to group the animals into subgroups (the L/D avoidance test), was indeed suitable and that the phenotypes of these two subgroups can be utilized for correlations with downstream genetic and epigenetic results pertaining to fear conditioning and extinction. Another statistically significant difference was observed between the FSW and FDW animals (Fig. 4.1 D, Table 4.2, Section 4.1), where the FDW animals exhibited more anxiolytic behaviour compared to the FSW animals, indicating that co-administration of DCS and fear extinction was more effective in extinguishing fear than fear extinction alone.

5.3 Differential gene expression analysis

Next generation RNA sequencing data was analysed using bioinformatics tools (*TopHat*, *Bowtie*, *Cuffdiff* and *BORG* analysis tool) and the following between-group gene expression profiles were compared (Table 5.1):

Table 5.1: Different treatment groups that were used in the between-group gene expression profile comparisons

Groups	Processes associated with genes differentially expressed between groups
FSM vs. CS	fear conditioning in fear-conditioned compared to control animals
FSW vs. FSM	fear conditioning that could pertain to resilience or susceptibility to stress
FDW vs. FDM	efficacy of the drug in fear conditioning or resistance to the drug
FDW vs. FSM	fear extinction induced by DCS
FDW vs. FSW	fear extinction induced by DCS as opposed to the "natural" process of fear extinction
CD vs. CS	effects of DCS in the absence of fear conditioning and behavioural fear extinction

FSM - fear-conditioned + saline maladapted, CS - control + saline, FSW - fear-conditioned + saline well-adapted, FSM - fear-conditioned + saline maladapted, FDW - fear-conditioned + DCS well-adapted, FDM - fear-conditioned + D-cycloserine maladapted

Bio-ontological relationship graph (*BORG*) analysis was used to identify biologically relevant genes that could be associated with processes relating to fear conditioning, fear extinction, anxiety, memory and stress (i.e. any phenotypes associated with anxiety or PTSD). The main comparison group in the current study was FDW vs. FSM. From the total of 93 genes that were downregulated in the FDW group compared to the FSM group, 42 genes were identified as biologically relevant for the purposes of the present study. Only one gene, *CYR61*, was upregulated in the FDW group compared to the FSM group, however the *BORG* analysis did not detect this gene as biologically relevant. The general functions of the protein transcribed by this gene (provided by RefSeq 2011) is to promote the adhesion of endothelial cells and it also plays a role in cell proliferation, differentiation, angiogenesis, apoptosis and extracellular matrix formation (http://www.ncbi.nlm.nih.gov/gene?cmd=Retrieve&dopt=full_report&list_uids=3491). The current study hypothesised that some of these biologically relevant, differentially expressed genes, are possibly involved in

the fear extinction process, as there were no significantly differentially expressed genes between the CS and CD animals, suggesting that DCS administration in the absence of fear conditioning and behavioural fear extinction, did not induce differential gene expression.

In the FSW vs. FSM comparison (which would identify genes possibly involved in the "natural" process of fear extinction), three of the genes that were overexpressed in the FSW group compared to the FSM group, were found to be downregulated in the FDW group compared to the FSM. Initially, this result appeared to be counterintuitive, as both of these groups are well-adapted but in the FSW group these three genes are upregulated whereas in the FDW these same three genes are downregulated relative to the FSM group. However, when comparing the FDW to the FSW group, the same three genes, together with 32 other genes, were found to be downregulated in the FDW group compared to the FSW group (Table II.1, Appendix II). Genes that are differentially expressed between these groups (FDW vs. FSW) point to genes associated with DCS induced fear extinction pathway compared to the "natural" fear extinction pathway. It is therefore hypothesised that DCS-induced fear extinction possibly incorporates different genes and/or pathways compared to the "natural" processes of fear extinction. Furthermore, with reference to the L/D avoidance test results (Fig. 4.1 and Table 4.2, Section 4.1), where the FDW animals spent significantly less time in the dark compartment compared to the FSW animals, it is hypothesised that DCS administration in conjunction with the behavioural fear extinction (in the FDW group) was more effective in extinguishing fear compared to the behavioural fear extinction alone (in the FSW group).

One gene, *CXCL13*, was found to be biologically relevant and significantly downregulated in the FSM group compared to the CS groups. This was an unexpected result, as this gene was also found to be downregulated in the FDW vs. FSM groups, the FDW vs. FSW groups, the FDW vs. CS groups and the FSM vs. FSW groups. The fact that we see this gene differentially expressed between FDW vs. FSM as well as FDW vs. FSW suggests that this gene may be associated with a DCS-induced fear extinction pathway that is distinct from the "natural" fear extinction pathway.

Biological interpretation of large sets of differentially expressed genes can be improved by grouping genes together based on their functional similarity (Cline et al., 2007). Therefore, in the current study, GO enrichment analyses were performed to gain insight into the biological processes, diseases, molecular functions and pathways that these genes were associated with or involved in. Biologically relevant genes that were differentially expressed in FDW compared to FSM rats will be discussed in the following way: firstly the GO data will be discussed with regards to biological processes, diseases, molecular functions and pathways, after which the focus will be narrowed down to specific genes.

5.3.1 DCS downregulates immune system genes and proinflammatory molecules that facilitate neuroinflammation

The most enriched biological process in our dataset was immune response (p = 9.21E-21), with 24 of the biologically relevant differentially expressed genes associated with this process (Table 4.5, Figs. 4.3 – 4.5). Included in this set of genes were genes that transcribe complement components (*C6*, *C1QB*, *C1S*, *C1QA*), glycoproteins (*CD4*, *CD44*), cytokines (*IL1RN*), lipopolysaccharide-binding protein (*LBP*), alpha-2-macroglobulin (*A2M*), and cytochromes (*CYBB*). In addition, seven of the differentially expressed genes were also associated with the process of immune system development. The importance of the interrelationship between the CNS and immune systems has become more apparent in recent years and is best illustrated in the context of the innate immune system. The innate immune system is genetically programmed to respond to specific pathogen-derived or other danger signals. It sets a signal transduction cascade in motion which results in the release of proinflammatory cytokines and chemokines that neutralize pathogens and initiate tissue repair (Jones and Thomsen 2013). Research has shown that immune functioning is affected in individuals suffering from PTSD (Watson et al., 1993; Arranz et al., 2007). This has been attributed to the continuous state of sympathetic hyperarousal which is typical of the disorder (Wong et al., 2000; Geracioti et al., 2001; Paulus and Stein, 2006). Previously published gene expression analyses have also detected dysregulated expression of immune-related genes (Zieker et al., 2007; Yehuda et al., 2009; Glatt et al., 2013; Neylan et al., 2011).

Appropriate immune responses are vital for survival; however, dysregulated inflammatory and oxidative stress responses may result in cellular injury and even cell death (Lee et al., 2001; Marques et al., 2003; Andersen, 2004; Hovatta et al., 2010). Subsequently, cellular injury produces excess toxic substances which promote further inflammatory cascades and additional cellular toxicity (Licinio and Wong, 1997, 2003). CNS inflammation does not only originate in response to bacterial or viral infection, but could also develop as a consequence of endogenous neurotoxin production or variations in the protein oxidation and reduction balance, such as those occurring during the aging process, and neurodegenerative and autoimmune disorders (Glass et al., 2010; Bhat and Steinman, 2009). Furthermore, four of the biologically relevant differentially expressed genes in the present study were associated with the reactive oxygen species metabolic process biological process GO term (Table 4.5, Section 4.2.3.1). Increased oxidative stress could result in further increased inflammatory responses.

Recent research has indicated that the immune system has functions within the CNS beyond that of inflammation and neuroprotection, providing evidence for the involvement of immune system mediators in central behavioural functions including adult neurogenesis and processes underlying synaptic plasticity (such as learning and memory) (Khairova et al., 2009; McAfoose and Baune, 2009; Ransohoff, 2009; Yirmiya and Goshen, 2011). The involvement of the immune system in the response to psychological stress, apart from infectious or physical injury, has initiated a paradigm shift with regards to the understanding of the interrelations of immune system, brain and behaviour (Kioussis and Pachnis, 2009; McAfoose and Baune, 2009; Besedovsky and Del Rey, 2011; Molina-Holgado and Molina-Holgado, 2010; Schwartz and Shechter,

2010). According to this emerging model, the immune and nervous systems evolved together and they share numerous cellular, molecular and genetic mechanisms (such as gene regulation, cellular communication, signalling and supracellular organization). The model also states that the cascades that are induced (under normal conditions or in response to physical, psychological or infectious challenges) are tightly regulated, and aid in the maintenance of sufficient metabolic, adaptational and defensive functions. Lastly, the model states that the brain is supported by the immune system; where sufficient immune functioning is essential for learning and memory under basal conditions, and supports optimal stress-coping responses (Meffert and Baltimore, 2005; Kioussis and Pachnis, 2009; Besedovsky and Del Rey, 2011; Molina-Holgado and Molina-Holgado, 2010; Schwartz and Shechter, 2010; Su et al., 2010; Yirmiya and Goshen, 2011).

There is a growing body of literature that describes how acute and chronic stress contribute to the activation of the innate immune system, causing peripheral inflammation, which has been found to be associated with early morbidity and mortality (Elenkov et al., 2005; Fransson et al., 2010; Wassel et al., 2010; Gianaros and Manuck, 2010; Seeman et al., 2010). PTSD is one of the disorders that has been found to be associated with higher CNS reactivity, increased inflammation and a deteriorating state of health (Baker et al., 2003, 2012). Pre-clinical research has shed light on the functions of immune cells (such as astrocytes and microglia), proinflammatory cytokines (including IL-6 and IL-1), and tumor necrosis factor alpha (TNF-α) in vital brain functions such as learning and memory. These factors interact with neuronal glutamate and GABA signalling to control long-term potentiation (LTP) and facilitate neurogenesis. Therefore, the immune system could contribute to the resolution or exacerbation of fear memories following exposure to a trauma (Tambuyzer et al., 2009; Verkhratsky, 2010; Yirmiya and Goshen, 2011). Furthermore, pre-clinical research provides evidence to suggest that, in the context of stress, components of the acquired immune system, such as protective T cells, can be transported to the CSF blood brain barrier (BBB) interface by means of molecules such as chemokines and glucocorticoids (Lewitus et al., 2008; Ransohoff, 2009). These protective T cells are hypothesized to have a neuroprotective function during conditions of infection, injury or emotional or psychological stress (Miller, 2009; Schwartz and Shechter, 2010).

The results from the current study correlate with previous research that has found PTSD to be associated with dysregulated expression profiles of immune-related genes (Zieker et al., 2007; Yehuda et al., 2009; Glatt et al., 2013; Neylan et al., 2011). Additionally, in light of the functions of immune cells in learning and memory, it can be postulated that co-administration of DCS and behavioural fear extinction induces the downregulation of these immune-related and proinflammatory genes, and therefore attenuates neuroinflammation and learning and memory deficits, which contributes to the facilitation of fear extinction.

In addition to several complement components being associated with the immune response process, results from GO analysis indicated that six genes were also associated with the complement activation process. The complement components that were downregulated in the FDW group compared to the FSM group were mostly from the classical complement pathway (*C1QA*, *C1QB*, *C1QC* and *C1S*). The other complement component, *C6* (part of the alternative complement pathway), was also part of the list of genes associated

with the process of cell death. The complement system forms an essential part of the innate immune system. It provides defence against pathogens and initiates inflammatory responses (Frank and Fries 1991; Finehout et al., 2005; Coleman et al., 1989). Complement components are proinflammatory mediators and if the induced inflammatory response is dysregulated, it could result in tissue damage. The complement system has therefore been implicated in the pathology of several neurodegenerative disorders such as Parkinson's disease (PD) (Yamada et al., 1992; McGeer and McGeer 2004), Alzheimer's disease (AD) (May et al., 1989, 1990; Gasque et al., 2000; Tuppo and Arias 2005), multiple sclerosis (MS) (Kornek et al., 2003; Sellebjerg et al., 1998) and Pick's disease (Morgan and Gasque 1996, 1997). Expression levels of the classical complement cascade have also been found to be significantly higher in the hippocampus and temporal cortex of AD cases compared to controls (Shen et al., 1997), suggesting that neuroinflammation in these brain regions could contribute to the pathology of this neurodegenerative disorder.

More recent literature indicates that the complement system can also remodel and repair brain tissue by removing toxic protein deposits and facilitating phagocytosis of necrosed or apoptotic neurons via the microglia (Gasque et al., 2000). The complement system can thus elicit potentially threatening responses in the CNS, resulting in neuroinflammation, but can also induce positive effects in terms of repair and maintenance of brain tissue (Gasque et al., 2000). During the early stages of these brain disorders, the complement system may have a beneficial function (Osaka et al., 1999; Mukherjee et al., 2001, 2008), whereas in chronic CNS disorders, inflammation induced by these complement components is detrimental to neurons, as neuronal cells are not able to regulate non-specific complement component actions (Singhrao et al., 2000). In order to determine if upregulation of the complement system was associated with increased levels of anxiety, Kulkarni et al. (2011) administered an *in-vitro* inhibitor of complement activation to APPswePS1δE9 mice (transgenic mice with increased levels of anxiety, usually used to model neurological disorders, specifically Alzheimer's disease). Administration of this inhibitor of the complement system was shown to attenuate anxiety in this model of AD; inhibition of the complement system thus had an anxiolytic effect in these animals (Kulkarni et al., 2011). *In the context of DCS-induced fear extinction in the current study, one of the proposed processes whereby DCS facilitated fear extinction is by downregulating genes that transcribe complement components, thus reducing neuroinflammation and subsequent neuronal damage and ultimately eliciting an anxiolytic effect.*

5.3.2 DCS downregulates genes associated with behavioural processes implicated in stress-related disorders

Another enriched biological process of interest is behaviour, defined by the database as "the internally coordinated responses (actions or inactions) of whole living organisms (individuals or groups) to internal or external stimuli". Seven genes were associated with this process (p = 6.29E-03) (Table 4.5, Figs. 4.3 – 4.5, Section 4.2.3.1). Although the behavioural functions of *CD74*, *CXCL13* and *LBP* were associated with cellular behaviour, including neutrophil and T cell chemotaxis, the other genes enriched in this process were associated with behavioural responses such as memory, adult feeding, adult locomotory behaviour, eating

behaviour, circadian cycle and behavioural response to pain. It has been well documented that memory processes are impaired in PTSD (DSM-V, APA 2012; Elzinga and Bremner 2002; Layton and Krikorian 2002). Moreover, research has shown that NMDAR-dependent plasticity is crucial for spatial learning and memory (Morris 1981; Morris et al., 1982). *In the current study, DCS administration may have facilitated NMDAR-dependent plasticity, as DCS is a partial NMDAR1 agonist at the glycine site, and indirectly increases glutamatergic activity which subsequently facilitates neuroplasticity, and therefore also learning and memory. Furthermore, co-administration of DCS and behavioural fear extinction downregulates genes that have been shown to interfere with memory consolidation, such as IL1RN, (Spulber et al. 2009) and in this regard DCS may contribute to improved learning and memory, and ultimately to fear extinction. With regards to feeding and eating behaviours, the associated genes could help to explain the comorbidity between eating disorders (EDs) and anxiety disorders (Bulik et al., 2000; Brewerton et al., 1995; Swinbourne and Touyz 2007) and eating disorders and PTSD (Brewerton 1999, 2007).* In addition, several studies have also shown an association between trauma and EDs (Perkins and Luster 1999; Ackard and Neumark-Sztainer 2002; Fonseca et al., 2002; Wonderlich et al., 2000).

Circadian cycle was also amongst the GO enriched behavioural responses affected by DCS administration. Sleep disturbances are common amongst PTSD patients (Lavie et al., 2001); sleep-related symptoms of PTSD are included in the DSM-5 (APA, 2013) as diagnostic criteria where the traumatic events are often re-experienced in the form of nightmares and the initiation and maintenance of sleep is problematic (which is part of the hyperarousal symptom cluster of the disorder). Although the current study did not include an assessment of disturbances in circadian rhythms, it can be postulated that DCS induces gene expression changes that are associated with alterations in circadian rhythms, and as such may alleviate some of the sleep disturbances associated with PTSD. Further analysis is however required to establish this proposed effect of DCS.

With regards to the process of behavioural response to pain, DCS has previously been shown to reduce neuropathic pain (caused by nerve injury, characterized by spontaneous pain and exaggerated responses to painful (hyperalgesia) and non-painful stimuli (allodynia)) in rats with spared nerve injury (SNI) (Millecamps et al., 2007). Daily oral administration of DCS was found to reduce sensitivity of the injured limb in a dose-dependent manner. Direct infusion of DCS into the mPFC or the amygdala resulted in the acute reduction of sensitivity to painful stimuli in SNI rats. This effect was mimicked by NMDA and glycine and blocked by HA−966 (a glycine-recognition site antagonist). This led the authors to conclude that limbic NMDA-mediated circuitry facilitates a long-term decrease in neuropathic pain behaviour by enhancing the extinction of pain-related memories, thereby facilitating antinociceptive properties for neuropathic pain through manipulation of prefrontal neuronal properties (Millecamps et al., 2007). In this context, the downregulation of genes in the FDW group compared to the FSM group that are associated with behavioural response to pain, concurs with previous findings of NMDA-mediated decreases in neuropathic pain (Boyce et al., 1999; Chizh et al., 2001; Parsons et al., 2001; Millecamps et al., 2007; Kayser et al., 2011), it is

furthermore proposed that DCS might possibly facilitate the extinction of fear memories and pain-related memories in a similar fashion.

Research suggests that, amongst other things, PTSD is characterised by cytokine dysregulation, with decreased anti-inflammatory responses and increased proinflammatory responses, resulting in a state of heightened inflammation. This may result in comorbid immune related symptoms such as fatigue, malaise and altered patterns in sleep and appetite (Silverman et al., 2005; Dunn et al., 2006; Sternberg 2006). *Results from the current study indicate that co-administration of DCS and behavioural fear extinction decrease expression of certain proinflammatory genes and genes involved in inflammatory responses (such as certain complement component genes,* SPP1,MMPs, S100 *genes etc.). Furthermore, GO enrichment showed that differentially expressed genes between these two treatment groups were associated with immune-related processes and diseases as well as behavioural responses relating to sleep patterns and appetite. It is thus possible that one of the main molecular mechanisms whereby DCS facilitated fear extinction in this study was by regulating genes involved in the immune system.*

5.3.3 DCS downregulates genes that are associated with disorders that co-occur with PTSD

Gene ontology enrichment for diseases associated with the genes that were differentially expressed in response to co-administration of DCS and behavioural fear extinction, not only indicates diseases associated with the pathology and processes in question in the present study (fear extinction, anxiety, memory, learning, neurocognition etc.), but also identifies genes that could help to explain the comorbidity between PTSD and other disorders. The most significantly enriched GO disease term was immune system diseases (p = 4.02E-14), with 17 associated genes (Table 4.6, Figs. 4.6, 4.7, Section 4.2.3.2). Once again, numerous genes that transcribe complement components were associated with this particular disease term, as well as glycoproteins (*CD4*), cytokines (*IL1RN*), cytochromes (*CYBB*) and metallopeptidase (*MMP9*). Together with results from previous GO enrichment analyses (relating to enriched biological process terms, where immune response was the most enriched biological process), this association with immune system diseases underscores one of the main effects elicited by DCS, namely its immunological effect, whereby it downregulates immune-related genes and genes that transcribe proinflammatory molecules that mediate chronic inflammation, neuroinflammation, disease progression and cellular destruction.

The second most significant GO disease term was cardiovascular diseases (p = 1.02E-13), with 18 of the biologically relevant differentially expressed genes associated with this GO term. As illustrated in Fig. 5.1, Section 5.1, stress and other factors, such as genetic variation, can influence the stress system and different target tissues, which could ultimately lead to the development of various pathological conditions, including the cardiorespiratory systems (Blanchard et al., 1990; Fredrikson and Matthews 1990; Georgiades and Fredrikson 2000; Buckley et al., 2001). Research has found that PTSD patients are at increased risk for cardiovascular disease (Boscarino, 2008; Kessler, 2000; Schnurr et al., 2003; Kang et al., 2006; Boscarino et al., 2006; Kubzansky et al., 2009; Kubzansky et al., 2007). Furthermore, a recent study found that PTSD was

associated with ischemic changes on exercise treadmill tests, a result that was independent of traditional cardiac risk factors, C-reactive protein, and several health behaviors and psychosocial risk factors. The association remained significant even after patients with prior cardiovascular disease were excluded. Turner et al. (2013) suggested that additional mechanisms that could link PTSD and ischemia should be explored further. The authors mentioned that inflammation could be one of the factors that are involved as it has been shown to play a key role in the pathogenesis of cardiovascular disease. In addition, studies have shown that PTSD patients have elevated levels of multiple inflammatory biomarkers, as well as greater induction of inflammation in response to acute stress (Gill et al., 2009). Results from the present study correlate well with these findings, where several biologically relevant differentially expressed genes were found to be associated with GO terms such as immune response, immune system development, and immune system diseases.

The third most significant GO disease term associated with 17 of the biologically relevant differentially expressed genes, was digestive system diseases (p = 9.06E-11). In addition there were also 11 genes associated with the metabolic diseases GO term (p = 4.00E-05) (CTD defined metabolic diseases as diseases caused by an abnormal metabolic process, which could be congenital due to inherited enzyme abnormality (metabolism or inborn errors) or acquired due to disease of an endocrine organ or failure of a metabolically important organ such as the liver (according to STEDMAN, 26th edition) (Haqq et al., 2005)) and seven with diabetes mellitus (p = 1.63E-05), with some overlap noted between these three GO terms (Table 4.6, Figs. 4.6, 4.7).

The results from the present study correlate well with what has been described in literature. PTSD has been found to be significantly associated with a risk of diabetes (Goodwin et al., 2005; Lauterbach et al., 2005; Trief et al., 2006; Qureshi et al., 2009; Boyko et al., 2010; Pietrzak et al., 2012; Lukaschek et al., 2013) and hyperlipidemia (Solter et al., 2002; Karlovic et al., 2004; Maia et al., 2008; Dedert et al., 2010). Not only is PTSD associated with an increased risk for diabetes, but there is a strong association between diabetes and severe psychological distress (Egede et al., 2012) and experience of traumatic events (Mooy et al., 2000; Kumari et al., 2004). The stress response system includes the central and peripheral nervous systems, the immunological system and the endocrine system; it is thus possible that all these systems could concomitantly contribute to the progression from traumatic psychological stress to type 2 diabetes (Pickup et al., 2004; Black, 2006; Baker et al., 2012; O'Donovan et al., 2012; Lukaschek et al., 2013). However, it should also be noted that due to the cross-sectional nature of some of these studies, researchers were not always able to discern whether diabetes preceded the onset of PTSD or only developed later. A meta-analysis however found that depression was associated with a 60% increased risk of developing type 2 diabetes, whereas type 2 diabetes was only associated with a modest increased risk of depression (Mezuk et al., 2008).

The functional role of insulin in the brain's structural responses to stressors should not be overlooked. Insulin deficiencies result in a decrease in the number of dentate gyrus neurons which leads to increased dendrite remodelling of CA3 neurons (in the hippocampus) (Lisowski et al., 2013). This effect was found to be further accelerated following repeated restraint stress in two mice strains, bred for high and low swim stress-

induced analgesia (Lisowski et al., 2013). Furthermore, the combination of stress and hyperglycemia leads to increased oxidative stress in the brain, which could ultimately contribute to impaired neural function in conditions of chronic stress and diabetes (Lisowski et al., 2013). Additionally, negative health behaviours, such as substance abuse, smoking and eating disorders, as well as heightened levels of inflammation are commonly associated with PTSD and these factors may converge in their contribution to an increased risk for developing diabetes in humans (Rod et al., 2009).

Other diseases that were enriched in our set of differentially expressed genes were nervous system diseases (p = 2.28E-06) with 16 genes associated with this GO term. According to CTD nervous system diseases includes diseases of the central and peripheral nervous system, including disorders of the brain, spinal cord, cranial nerves, peripheral nerves, nerve roots, autonomic nervous system, neuromuscular junction, and muscle. Twenty of the differentially expressed biologically relevant genes were associated with nervous system diseases, with most of the genes being associated with central nervous system diseases and a few with peripheral nervous system diseases (Table 4.6, Figs. 4.7, Section 4.2.3.2). Central nervous system diseases include diseases of any component of the brain (including the cerebral hemispheres, diencephalon, brain stem, and cerebellum) or the spinal cord. Peripheral nervous system diseases include diseases of the peripheral nerves external to the brain and spinal cord, which includes diseases of the nerve roots, ganglia, plexi, autonomic nerves, sensory nerves, and motor nerves.

The CTD was mined for more specific disease terms (by searching for each gene's specifically associated diseases). Some of the main nervous system diseases that were associated with the gene set (that was enriched for the nervous system diseases GO term) were memory disorders, learning disorders, pain, neurotoxicity syndromes, Alzheimer's disease, Parkinson's disease and dementia. Some of these diseases were also enriched in the disease GO search, such as pain, mental disorders (including Alzheimer's disease, learning disorders, cognition disorders, autistic disorder, depressive disorders, anxiety disorders, narcolepsy, amnesia) and neurodegenerative diseases (including Alzheimer's disease, Parkinson's disease, etc.). Genes associated with these disorders were downregulated in the FDW animals compared to the FSM animals. *It is therefore hypothesised that in the current study DCS facilitated the downregulation of genes involved in learning and memory disorders, which may have subsequently assisted the fear extinction process.*

In PTSD, memory failure (specifically relating to memories of the traumatic event) is characterized as a symptom of avoidance (Horowitz, 1986; Horowitz & Reidbord, 1992; Larson et al., 2013). These dissociative memory problems are not defined to be neurological in nature by the diagnostic guidelines, and objective neuropsychological findings are not required in order to meet this criterion. Instead, diagnosis mostly relies on clinical observation and subjective complaint (Larson et al., 2013). However, research has shown that PTSD does in actual fact elicit neurological sequelae, which can be observed on brain imaging scans. PTSD can furthermore result in subsequent memory impairment, which can be confirmed by psychometric assessments (Larson et al., 2013). A decrease in hippocampal volume (Emdad et al., 2006; Woodward et al., 2009) and hippocampal connectivity (Daniels et al., 2010) and impaired performance on

memory testing (Daniels et al., 2010; Emdad et al., 2006; Woodward et al., 2009; Larson et al., 2013) has been observed in individuals with PTSD. *In the current study, differential expression of genes associated with learning and memory disorders are in accordance with previous findings regarding PTSD, as well as the understanding of the psychopathology of the disorder, since PTSD affects processes of learning and memory (Milad et al., 2009; LeDoux et al., 1988; Goldstein et al., 1987; Sutker et al., 1990, 1991). DCS has been shown to facilitate fear extinction (Ledgerwood et al., 2003; 2005; Walker et al., 2002; Yang and Lu, 2005), a process that also involves learning and memory processes (Berman and Dudai 2001; Bouton 2002; Myers and Davis 2002). Therefore, this study illustrates that co-administration of DCS and behavioural fear extinction facilitated fear extinction by regulating genes involved in learning and memory processes.*

Results from the current study found that DCS downregulated genes previously associated with Alzheimer's disease. This corresponds to what has previously been described in literature, where a direct relationship was found between experiential trauma in Alzheimer's disease and the rate of decline in Alzheimer's disease, as well as between previous life trauma and Alzheimer's disease onset (Burnes and Burnette 2013). Subsequently, it has been hypothesized that PTSD and correlates of PTSD could be mediators of Alzheimer's disease status, in a model recently described by Burnes and Burnette (2013). Moreover, research has shown some convergence of the neuroanatomical changes in individuals with trauma histories with cognitive and emotional disturbance and persons with Alzheimer's disease (Qureshi et al., 2011). This suggests that early trauma may mediate neuroanatomical alterations which increase susceptibility or vulnerability to Alzheimer's disease (Burnes and Burnette 2013). In the current study, administration of DCS in the fear-conditioned group facilitated the downregulation of genes that have previously been associated with Alzheimer's disease. Literature has also shown associations between dysregulated NMDAR trafficking and neuropsychiatric disorders, such as Alzheimer's disease (Lau et al., 2007), which is consistent with the critical role of glutamatergic neurotransmission during learning, memory formation and neuronal plasticity (Zhao et al., 2005; Nakazawa et al., 2006; Gardoni et al., 2009). In addition, Tsai et al. (1999) found that DCS administration (100 mg/day) significantly improved scores on the cognitive subscale of the Alzheimer's Disease Assessment Scale (improvement of 3.0 points). *The current study therefore supports the hypotheses that there are common mechanisms involved in the pathologies of PTSD and Alzheimer's disease; we furthermore propose that DCS functions by altering the expression of genes involved in these common mechanisms.*

Another hypothesis incorporates the important role of stress, brain inflammation and cerebrovascular alterations, all contributing in varying degrees to the cause and progression of various brain disorders. These disorders include, but are not limited to, affective and anxiety disorders, PTSD, traumatic brain disorder, schizophrenia, autism and numerous neurodegenerative diseases such as Parkinson's and Alzheimer's disease, multiple sclerosis, amyotrophic lateral sclerosis and HIV-associated dementia (Saetre et al., 2007; Dantzer et al., 2008, 2009; Miller et al., 2009; Glass et al., 2010; Tansey and Goldberg 2010; Bauer et al., 2010; Saavedra et al., 2011). With regards to Parkinson's disease, anxiety disturbances were found to be much more common in diseased patients compared to healthy or comparably disabled elderly controls, where

anxiety disturbances occurred in up to 40% of Parkinson's disease patients (Nuti et al., 2004; Stein et al., 1990; Pontone et al., 2009). This frequent co-occurrence of anxiety and Parkinson's disease has led researchers to believe that these disorders may share certain etiological processes (Stein et al., 1990; Siemers et al., 1993; Menza et al., 1993; Noble, 2000; Gravius et al., 2010; Naviaux, 2013). It has also been established that excessive, uncontrolled inflammation in the CNS plays a critical role in the pathophysiology of numerous psychiatric and neurodegenerative disorders, such as major depression, schizophrenia, PTSD, Parkinson's and Alzheimer's disease, HIV-associated dementia and traumatic brain injury (Fassbender et al., 2004; Hope et al, 2009; Miller et al, 2009; Rivest, 2009). *Results from the current study show that genes that have previously been associated with Parkinson's disease were downregulated in the FDW group compared to the FSM group, suggesting that there might be common pathogenic processes involved in these two disorders. Numerous proinflammatory genes were also downregulated in the FDW group, suggesting that DCS has the potential to attenuate these effects of neuroinflammation and other shared mechanisms that could contribute to disease progression.*

Research has shown that PTSD often co-occurs with chronic pain. Furthermore, PTSD severity was found to correlate with the severity of chronic pain (White and Fuastman 1989; Hickling et al., 1992; Chibnall et al., 1994; Beckham et al., 1997; Shipherd et al., 2007; Defrin et al., 2008). It has been suggested that PTSD patients display intense and extensive chronic pain, hyposensitivity to pain together with hyper-reactivity to suprathreshold harmful stimuli (Defrin et al., 2008). This could be due to the particular emotional interpretation and response to painful stimuli by PTSD subjects. These findings could also be attributed to altered sensory processing in PTSD patients (Defrin et al., 2008). Findings also suggest that in certain chronic pain patients (i.e., those classified as dysfunctional) the object of fear may be associated with prior traumatic and painful injury (Beck et al., 2001); these dysfunctional pain patients are also more vulnerable to developing PTSD (Asmundson et al., 2000). *The current study found not only that the differentially expressed gene set was enrichment for the biological process of behavioural response to pain, but also detected pain as a GO enriched disease term and as a nervous system disorder. In an earlier section of this chapter, NMDAR-mediated reduction of neuropathic pain was discussed in detail (Section 5.3.2). In light of that research and the research discussed above, it can be hypothesised that co-administration of DCS and behavioural fear extinction in the current study may have facilitated relief from pain, (including neuropathic pain that could have been experienced in the current model), through downregulating certain genes associated with pain and the response to pain. This suggests that DCS may have facilitated fear extinction by reducing pain and thereby diminishing the reminder of the prior, possibly painful (electric footshock) trauma.*

By downregulating genes that are both associated with PTSD pathologies and comorbid disorders of PTSD, DCS might be able to facilitate fear extinction whilst alleviating symptoms of these additional disorders. Identifying genes that are common between PTSD and these disorders can thus shed light on the molecular underpinnings of comorbidity or disease associations and could furthermore identify novel drug targets that facilitate better treatment of PTSD and these disorders.

5.3.3.1 DCS downregulates genes that have inferred associations with anxiety disorders and PTSD

In order to determine how many, and which, of the 42 biologically relevant differentially expressed genes have previously been implicated in PTSD and anxiety disorders, Venn diagrams were constructed using the myGeneVenn CTD tool (http://ctdbase.org/tools/myGeneVenn.go). These diagrams also showed that within the database there was a total of 6 816 genes with inferred (or indirect) associations with *both* PTSD and anxiety disorders. The database contained 12 506 genes that have only been found to be associated with anxiety disorders and 50 that have been found to be associated only with PTSD. The database also reported that 21 of the 42 differentially expressed genes have an inferred association with only anxiety disorders and 21 of the differentially expressed genes have inferred associations with both anxiety disorders and PTSD (Fig. 4.8, Table 4.7 Section 4.2.3.2). These results point to the possible efficacy of DCS for the treatment of anxiety disorders and stress-related disorders (Ressler et al., 2004; Hofmann et al., 2006; Kushner et al., 2007; Guastella et al., 2008; Wilhelm et al., 2008; Otto et al., 2010; Storch et al., 2010; Yamamoto et al., 2010; de Kleine et al., 2012).

5.3.3.2 Contributions of neuronal injury to neuropsychiatric disease

In light of the preceding discussions, it is important to discuss allostasis and its contribution to the development of disease. Allostasis is the process whereby physiologic systems adapt to meet the demands of external stressors. Allostatic load is the cost of repeated exposure to heightened or inconsistent neuroendocrine responses following exposure to stressful environmental challenges. Moreover, different types of stress can result in distinct patterns of CNS-neuroendocrine activation, whereby sustained stress yields neuroendocrine alterations that differ to those of acute stress (McEwen and Stellar 1993; Henninger 1995; McEwen, 2008). Figure 5.2 illustrates how genetic and environmental influences interact and impact on allostatic load. Allostatic load, together with chronic stress, trauma, inflammation and impaired cerebral circulation, could eventually result in neuronal dysfunction and injury which contributes to the development of neuropsychiatric disease, including PTSD. Results generated by the current study correlate well with the model, described by McEwen, (2008), which explains how neuronal injury leads to neuropsychiatric disease. In the present study, intrahippocampal DCS administration (together with behavioural fear extinction) had an anxiolytic effect on the animals, DCS downregulated proinflammatory genes and DCS resulted in differential expression of genes that are associated with affective, stress-related, cognitive and neurodegenerative diseases.
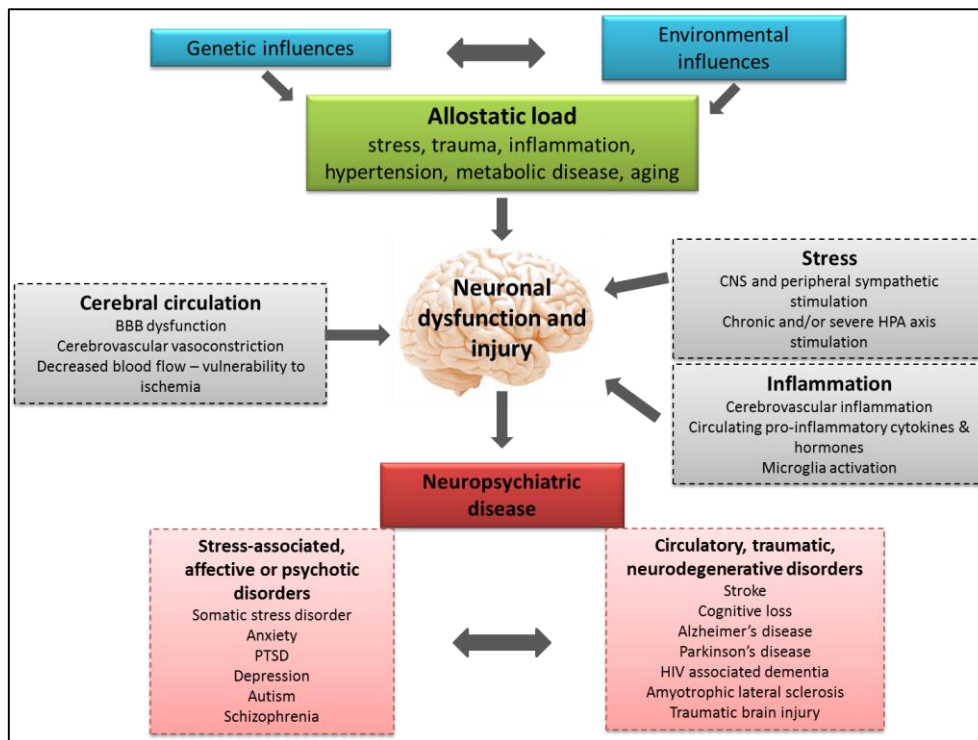
Figure 5.2: Diagram illustrating the mechanisms of neuronal injury leading to neuropsychiatric disease. Genetic and environmental factors interact with each other which could result in the failure of compensatory mechanisms to maintain homeostasis. This, in turn, contributes to increased allostatic load on the CNS which translates into pathological reactivity to stress, uncontrolled inflammation and alterations in blood flow, ultimately resulting in variable degrees of neuronal dysfunction and injury. Various combinations of these alterations and their CNS localization affect different regulatory systems. The initiation, development and combinations of particular affective, stress-related, psychotic, cognitive and neurodegenerative diseases of the brain, is dependent on individual vulnerability, the combination of pathological factors, the localization of the neuronal injury, and the regulatory mechanisms affected. BBB – brain blood barrier, CNS – central nervous system, HIV - human immunodeficiency virus, PTSD – posttraumatic stress disorder (Adapted from McEwen, 2008 and Saavedra et al., 2011).

5.3.3.3 Neuroinflammation and its effects on neurogenesis and memory

The discussion below provides some explanation of how inflammation facilitates neuronal dysfunction. The majority of the genes discussed in the following sections are involved in the immune system, oxidative stress response and inflammation. Dysregulated inflammatory and oxidative stress responses may result in cellular injury and even cell death (Lee et al., 2001; Marques et al., 2003; Andersen, 2004; Hovatta et al., 2010). In addition, cellular injury produces excess toxic substances which promote further inflammatory cascades and additional cellular toxicity (Licinio and Wong, 1997, 2003). CNS inflammation does not only originate in response to bacterial or viral infection, but could also develop as a consequence of endogenous neurotoxin production or variations in the protein oxidation and reduction balance, such as those occurring during the aging process, and neurodegenerative, and autoimmune disorders (Glass et al., 2010; Bhat and Steinman, 2009). Models describing the interrelations of the immune system, brain and behaviour have described that the brain is supported by the immune system; where sufficient immune functioning is essential for learning

and memory under basal conditions, and supports an optimal stress-coping response (Meffert and Baltimore, 2005; Kioussis and Pachnis, 2009; Besedovsky and Del Rey, 2011; Molina-Holgado and Molina-Holgado, 2010; Schwartz and Shechter, 2010; Su et al., 2010; Yirmiya and Goshen, 2011).

One of the proposed mechanisms whereby neuoinflammation mediates neuronal dysfunction is via the inflammatory mediators, such as the cytokines, interleukin 1beta (IL-1β), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNFα). All of these cytokines are released by macrophages, peripheral immune cells and microglia (in the CNS), during the early acute phase defence reaction against invading pathogens. These cytokines are robustly stimulated by molecules associated with pathogens, such as lipopolysaccharide (LPS) and viral nucleic acids that bind to toll-like receptors (TLRs) and activate the NFκβ pathway (Fig. 5.3). These cytokines also interact with neuronal glutamate GABA signalling to modulate LTP (Tambuyzer et al., 2009; Verkhratsky, 2010; Yirmiya and Goshen, 2011). Microglia play a vital role in the innate immune system in the brain and respond rapidly to stimuli such as infection or injury (Hanisch and Kettenmann 2007). Upon activation (induced by cell damage), microglia migrate to the site of infarct, where they clear damaged neurons or other cellular components. However, in the absence of injury, microglia provide surveillance of the environment and trophic support to healthy neurons (Kettenmann et al., 2011). However, stress-induced signalling molecules and glucocorticoids induce a proinflammatory response in microglia which may lead to aberrant activation in the absence of infection or injuries (Nair and Bonneau, 2006; Hanisch and Kettenmann 2007).
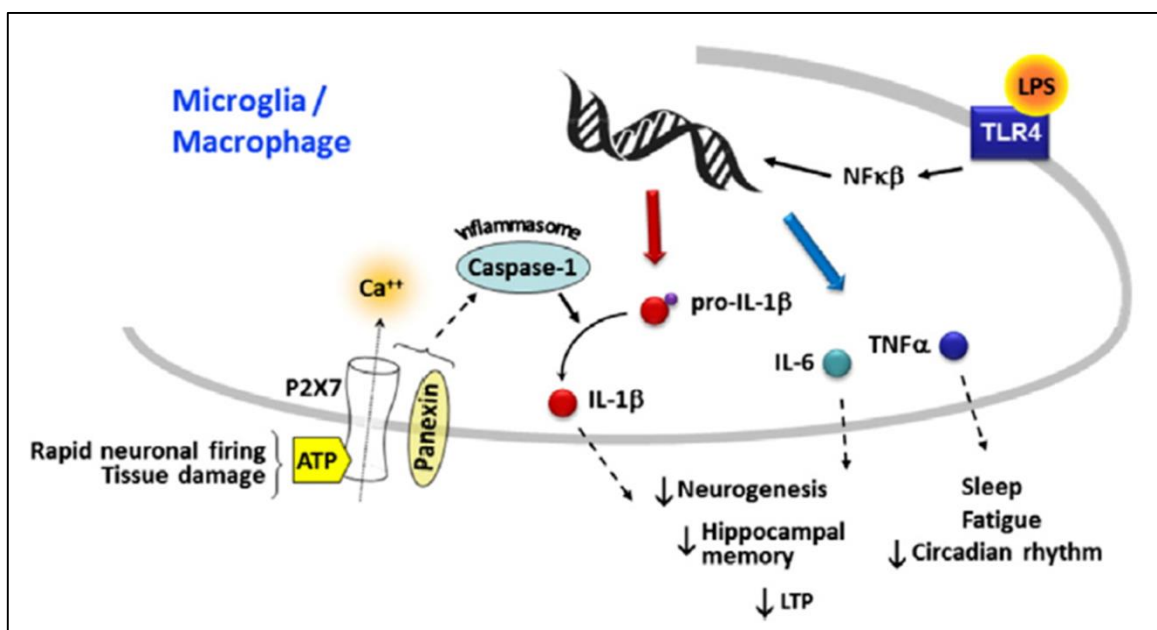
Figure 5.3: One of the proposed pathways whereby neuroinflammation mediates neuronal dysfunction. Pathways that regulate and secrete IL-1β and other neuroactive cytokines, and the effects of these cytokines on the nervous system. Regulation of IL-6, TNFα and IL-1β is primarily via the NFκβ pathway through transcriptional activation. Inactive pro-IL-1β (as well as pro-IL-18 — not shown) requires further processing by the inflammasome component caspase-1. Endogenous stimulants include extracellular ATP and bacterial LPS. There are many other endogenous and exogenous inflammatory mediators that cause acute inflammation via other TLR subtypes and additional components of the inflammasome (not shown). Studies have shown that stress-stimulated and exogenously administered IL-1β decrease memory in the conditioned fear and water maze paradigms (Buchanan et al., 2008; Goshen et al., 2007; Pugh et al., 1999) (Jones and Thomsen 2013).

Numerous studies have demonstrated the key role of IL-1β as a stress-sensitive neurohormone (Goshen and Yirmiya, 2009). Following a period of intense stress, IL-1β levels were found to be increased in hypothalamus and other brain areas (Nguyen et al., 2000). Stress-induced IL-1β release has been implicated in reduced cognitive performance, as both exogenously administered and stress-stimulated IL-1β resulted in reduced memory in the conditioned fear and water maze paradigms (Buchanan et al., 2008; Goshen et al., 2007; Pugh et al., 1999). Studies have furthermore shown that the anti-neurogenic action of stress is ablated in knockout mice lacking the receptor for IL-1β (IL-1RI) (Goshen et al., 2008; Koo and Duman, 2008). The anti-neurogenic action was also inhibited following the administration of IL1RN in wild-type animals (Goshen et al., 2008; Koo and Duman, 2008).

However, it is not only excess CNS IL-1 signalling that elicits negative effects on memory tasks, earlier studies have shown that deficits in CNS IL-1 signalling also has a negative effect on memory. During the maintenance phase of LTP, there is a strong and sustained increase in the levels of IL1β (Balschun et al., 2004; Schneider et al., 1998). Long-term potentiation at the synaptic level is believed to underlie memory consolidation (Bliss and Lømo, 1973; Teyler, 1987). Accordingly, researchers have found that elevated levels of IL1RN, which blocks the binding of IL-1β, impair learning (Oprica et al., 2005) and interferes with the memory consolidation process (especially in the hippocampus) (Spulber et al. 2009). These findings, together with earlier research showing that IL1 receptor blocking interferes with the BDNF-ERK1/2 pathway (which mediates the effects of BDNF on synaptic plasticity, nuclear signalling, and memory formation (Finkbeiner et al., 1997; Gottschalk et al., 1999; Blanquet 2000; Pizzorusso et al., 2000; Alonso et al., 2002a,b)), suggests that physiological levels of IL1 are essential in learning and long-term memory consolidation. Additionally, blocking of IL-1R-mediated signalling, through intracerebroventricular (ICV) administration of IL1RN, significantly impaired memory in the Morris water-maze and in the passive avoidance test in rats (Yirmiya et al., 2002). It was also proposed that IL1RN elicits a possible direct effect on hippocampal function, in view of findings that exogenous IL1RN exerts agonist activity in the hippocampus, independent of IL-1RI (Loscher et al., 2003). This study found that *in vitro* stimulation of synaptosomes with IL1RN mimicked the effects of IL-1β by decreasing glutamate release and increasing c-Jun N-terminal kinases (JNK) phosphorylation. These effects were maintained in mice with defective IL-1 type I receptor (IL-1RI). Furthermore, IL1RN mimicked the IL-1β inhibitory effect on long-term potentiation (LTP) in the hippocampus (Loscher et al., 2003).

It has been established that IL-1β is involved in the modulation of anxiety, exerting either anxiogenic (Connor et al., 1998; Montkowski et al., 1997), or anxiolytic (Montkowski et al., 1997) effects, depending on the dose (Oprica et al., 2005; Goshen et al., 2007). It was subsequently proposed that IL-1β has a modulatory effect on anxiety, where higher levels of IL-1β are anxiogenic and low levels of IL-1β or a deficit in available IL-1Rs, seem to have anxiolytic effects (Oprica et al., 2005; Goshen et al., 2007). Therefore, it is proposed that neuroinflammation, induced under stressful conditions, could mediate neuronal dysfunction through decreased neurogenesis and hippocampal memory, via dysregulation of the levels of IL-1β in the microglia.

It is however not only IL-1β that affects the neuronal functioning, but studies have also investigated the effects of IL-6. In a study by Raison et al. (2010) where 24 patients were treated with IFN-α (which induces inflammation), they found significantly increased CSF IL-6 levels. These levels were negatively correlated with concentrations of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in the CSF (Raison et al., 2009). The authors proposed that, based on pre-clinical research, increased CSF IL-6 may have elicited direct as well as indirect effects on serotonin and impaired growth of neuronal progenitor cells (Dunn, 2006; Monje et al., 2003; Song et al., 1999).

Another important factor that influences neuroinflammation is oxidative stress. During oxidative stress, an excess of oxidants damage or modify biological macromolecules such as lipids, proteins and DNA (Scapagnini et al., 2012; Ng et al., 2008; Salim et al., 2012; Anderson et al., 2013). This excess could be as a result of increased oxidant production, decreased oxidant elimination, defective antioxidant defences, or a combination of these factors (Scapagnini et al., 2012; Ng et al., 2008; Salim et al., 2012; Anderson et al., 2013). The brain is particularly vulnerable to oxidative stress due to high levels of peroxidizable polyunsaturated fatty acids, high oxygen utilization and limited anti-oxidation mechanisms (Scapagnini et al., 2012; Ng et al., 2008). It has been suggested that oxidative stress contributes to the aetiology of various psychiatric disorders, including anxiety disorders, depression, and alcohol use disorder (Hovatta et al., 2010). Oxidative stress could results in neuroinflammation through the activation of microglia, which increase oxidative stress through the production of proinflammatory cytokines and nitric oxide (NO) (Scapagnini et al., 2012; Ng et al., 2008; Salim et al., 2012; Anderson et al., 2013). In turn, these proinflammatory cytokines and high NO levels may promote further reactive oxygen species (ROS) formation, which ultimately results in the damage of membrane phospholipids and their membrane-bound monoamine neurotransmitter receptors (which depletes endogenous antioxidants). Increased ROS products could result in enhanced microglial activation and increase proinflammatory production via NF-κB stimulation (Salim et al., 2012). This, in turn, maintains oxidative injury (Salim et al., 2012), creating a potential pathological positive feedback loop in some psychiatric disorders (Scapagnini et al., 2012; Ng et al., 2008; Salim et al., 2012; Anderson et al., 2013).

In the Sections that follow, reference will be made to the effects of neuroinflammation on neurogenesis and hippocampal memory, and its possible contribution to neuronal dysfunction in the context of PTSD.

5.3.4   DCS downregulates genes that are associated with protein, receptor and ion binding molecular functions

Protein binding was the molecular function that was most enriched in the set of differentially expressed genes (p = 1.34E-12), with 34 genes associated with this function (Table 4.8, Figs. 4.9, 4.10 Section 4.2.3.3). The database defines this function as "Interacting selectively and non-covalently with any protein or protein complex (a complex of two or more proteins that may include other nonprotein molecules)". This definition is very broad, which could explain why so many genes were associated with it. Individual protein-binding terms associated with the differentially expressed genes included (but were not limited to) enzyme binding,

receptor binding, ion binding, calcium-dependent protein binding, tumor necrosis factor binding, MHC class-I protein binding, protein homodimerization activity, RAGE (receptor for advanced glycation end products) receptor binding, G-protein coupled receptor binding, protein kinase binding, chemokine activation as well as receptor agonist activity. Some of these individual terms were also detected to be enriched molecular functions within our gene set, such as receptor binding, ion binding, calcium ion binding and MHC protein binding.

Most of these molecular functions should be investigated and explained by looking at the NMDA receptor and its activation, since DCS is a partial NMDAR1 agonist. NMDAR activation is a relatively complex process, since binding of both glutamate and glycine is required to open the ion channel and allow calcium entry (Fig. 2.5 Section 2.4.1). The ion-channel is blocked by magnesium (voltage-dependently) and this block is removed by depolarization (McBain and Mayer 1994). Continued activation of the NMDA receptor promotes signalling to the nucleus (through calcium signalling pathways), which ultimately results in CREB phosphorylation, gene activation and long-term synaptic plasticity (which underlies learning and memory) (Schwarcz 2001). However, excess glutamate could lead to overstimulation of NMDA receptors, resulting in excess intracellular calcium, which has been shown to cause excitotoxicity, a process whereby nerve cells are damaged and die due to excessive stimulation by neurotransmitters such as glutamate. It is one of the process whereby neurons die in various CNS disorders (Schwarcz 2001). A fine balance of neurotransmitters, as well as calcium ion levels, is thus required for optimal neuronal function (Kemp and McKernan 2002). Therefore, the detection of differentially expressed genes with functions in ion binding, specifically calcium ion binding, suggests that DCS regulates the expression of genes that could help to maintain the homeostatic balance of calcium ions and prevent excitotoxicity, whilst ensuring optimal levels for sufficient neuronal functioning, learning and memory.

Receptor for Advanced Glycation Endproducts (RAGE) binding was another enriched protein binding molecular function, detected in the current study. RAGE is part of the immunoglobulin superfamily and is a transmembrane receptor (Neeper et al., 1992) that has the ability to bind advanced glycation endproducts (AGE), including mainly glycoproteins and glycans (Schmidt et al., 1995; Bierhaus et al., 1998). RAGE has also been implicated in inflammation through its binding to S100A12 (of the calgranulin family) (Hofmann et al., 1999; Marenholz et al., 2004) in mice macrophages (Schmidt et al., 2000), resulting in increased levels of IL-1β and TNF-α (factors involved in inflammation). Interestingly, one of the other protein binding molecular functions associated with the differentially expressed genes, was TNF binding. RAGE cross-linking of peptides and proteins results in irreversible tissue damage (Bierhaus et al., 2001). It also activates cell types such as macrophages, monocytes, vascular smooth muscle cells and cardiac fibroblasts, which consequently generate ROS. Interaction between RAGE and its ligands is believed to result in the activation of proinflammatory genes (Bierhaus et al., 2001). Research has shown that RAGE plays an important role in the process of ageing and age-related degenerative diseases (Park et al., 1998; Schmidt et al., 2000). In addition, elevated levels of RAGE ligands have been detected in diabetes and other chronic disorders, leading researchers to hypothesize that this receptor has a causative effect in various inflammatory diseases

such as diabetic complications, Alzheimer's disease and even some tumors (Park et al., 1998). *In the present study, DCS downregulated genes associated with RAGE receptor binding, which may have resulted in reduced levels of TNF. This suggests that this is one of the mechanisms whereby DCS attenuates neuroinflammation, tissue damage, and possibly also learning and memory deficits, which may have contributed to the facilitation of fear extinction.* This correlates with other findings in the present study, where DCS induced a reduction in the expression of immune system and proinflammatory genes as well as genes that have been associated with other inflammatory diseases, such as Alzheimer's disease and diabetes.

5.3.5 DCS downregulates genes that are associated with immune system-related and complement activation pathways

The immune system pathway was the most enriched biochemical pathway associated with the genes that were differentially expressed between FDW and FSM (p = 1.24E-14), with 18 biologically relevant genes associated with this pathway (Table 4.9, Figs. 4.11, 4.12, Section 4.2.3.4). The second most enriched pathway (associated with the biologically relevant differentially expressed genes) was complement and coagulation cascades (p = 1.37E-10). The results for the immune system and complement pathways correlate with the earlier results from the current study, where immune response and complement activation pathways were part of the enriched GO biological processes, as well as with the enriched GO disease terms, where immune system diseases were enriched. This emphasises the important effects that DCS elicits on immunological and complement processes and pathways and how they contribute to the facilitation of fear extinction.

Other enriched pathways associated with the biologically relevant, differentially expressed genes included the following pathways: leukocyte transendothelial migration, phagosome, Fc gamma receptor-mediated phagocytosis, signal transduction, systemic lupus erythematosus, hemostasis, antigen processing and presentation, cell adhesion molecules, T cell receptor signalling pathway and primary immunodeficiency. Most of these pathways are involved in or associated with the immune response or inflammation. During migration of leukocytes, the leukocytes bind to endothelial cell adhesion molecules (CAM) and subsequently migrate across the vascular endothelium. Migration of leukocytes from the blood into tissues is essential for immune surveillance and inflammation (Worthylake and Burridge, 2001). Phagocytosis is the process whereby phagocytes engulf relatively large particles (> 0.5 μm) and forms an internal phagosome. The process of phagocytosis is primarily involved in the uptake and degradation of infectious agents and senescent cells. Furthermore, phagocytosis participates in the immune response, inflammation, development and tissue remodelling. The innate immune response is initiated following phagocytosis of pathogens by macrophages; this in turn activates the adaptive response (Silverstein, 1995; Allen and Aderem 1996). Research has also shown that by presenting antigens derived from phagocytized apoptotic cells, dendritic cells are capable of stimulating class I–restricted CD8+ cytotoxic T lymphocytes (Albert et al., 1998), facilitating the presentation of self-antigens, leading to the breaking of tolerance and subsequent activation of autoimmune disease (Casciola-Rosen et al., 1994).

146

Furthermore, one of the other enriched pathways associated with the biologically relevant differentially expressed genes, was systemic lupus erythematosus (an autoimmune disease). It was therefore proposed that the phagosome pathway was activated due to dendritic cells that presented antigens from phagocytized apoptotic cells (cell death was one of the enriched biological processes which was probably the end-result from inflammation and proinflammatory responses in the LDH in response to fear conditioning); this may have facilitated the activation of autoimmune responses. *In the present study, DCS downregulated genes associated with the phagosomal pathway (including genes that transcribe proteins that initiate phagocytosis, such as CLEC7A and phagocytic receptors, such as MSR1 as well as a regulatory subunit of the phagocytic NADPH oxidase, RAC2) thereby attenuating increased inflammatory responses, subsequent neuroinflammation and associated memory end learning deficits, which may have facilitated fear extinction.*

5.3.6 DCS downregulates genes that have previously been implicated in learning, memory, fear and anxiety

This Section will focus on those genes that exhibited the largest fold changes between the FDW and FSM animals as well as genes that were previously found to be associated with fear, anxiety and PTSD. In addition, this section will focus on genes that exhibited differential expression with real time qPCR analyses (which included genes with large fold changes as well as genes previously implicated in anxiety disorders), thereby verifying the gene expression data from the RNAseq. Lastly, this Section also discusses a selection of biologically relevant differentially expressed genes that have previously been implicated in fear, anxiety, learning, memory and other neurocognitive diseases.

5.3.6.1 *SPP1*

The gene that had the biggest fold change between the FDW and FSM groups was secreted osteopontin or phosphoprotein 1 (*SPP1*), which was downregulated about six-fold in the FDW group (Table 4.4, Section 4.2.2, refer to Tables 4.5 – 4.9 for GO enrichment results under Section 4.2.3). This highly glycosylated multifunctional phosphoprotein functions as a free cytokine in body fluids (Gravallese 2003) and influences adhesion, migration, cell survival, and inflammation (Denhardt et al., 2001a, 2001b; Scatena et al., 2007). Furthermore, this gene was proposed to act as a novel regulator of myelination and remyelination (Selvaraju et al., 2004). Osteopontin has the ability to regulate pathways that are involved in the development of distinct effector T helper cells (T_H) cells, including T_H1 and T_H17 cells by interacting with cytokines and their receptors (Steinman 2008; Cantor and Shinohara 2009). Osteopontin is expressed in numerous tissues and cell types, including inflammatory cells and in the CNS (such as in primary sensory neurons in the mesencephalic Vth nucleus, the vestibular, auditory, trigeminal, and dorsal root ganglia (Loez et al., 1995; Ishikawa et al., 2000) as well as in the olfactory bulb, the cerebellum, and the brainstem (Shin et al., 1999)). Osteopontin has also been found to be present below the plasma membrane in association with the intracellular domain of CD44 (Zohar et al., 2000) (interestingly, the *CD44* gene was also downregulated in the FDW group). In addition to being upregulated in many peripheral tissues following injury such as

147

ischemia (Ellison et al., 1998; Wang et al., 1998; Lee et al., 1999; Choi et al., 2007) or spinal cord injury (Hashimoto et al., 2007), osteopontin has also been shown to be upregulated in various brain disorders, kainite-induced seizures (Kim et al., 2002), cryolesions (Shin et al., 2005), HIV-induced CNS dysfunction (Burdo et al., 2008) and experimental autoimmune encephalomyelitis (EAE) (Chabas et al., 2001). Osteopontin has been reported to be protective against ischemic brain injury (Noiri et al., 1999; Denhardt et al., 2001a, 2001b; Meller et al., 2005), however it was also shown to promote inflammation and exacerbate autoimmune diseases (Cantor, 1995; O'Regan et al., 2000a), such as EAE (Chabas et al., 2001) and multiple sclerosis (Steinman 2008). Furthermore, increased plasma levels of osteopontin have previously been associated with obesity-associated insulin resistance in mice, osteopontin-promoting inflammation and macrophage accumulation in adipose tissue (Nomiyama et al., 2007).

In a recent study, a unique CCR2^{+}CCR5^{+} T cell population was found to be enriched in the CSF of patients with exacerbated MS (Sato et al., 2012). The authors found that this T cell population was specifically involved in the autoimmune pathology of MS. Interestingly, they found that the CCR2^{+}CCR5^{+} T cells produced a large amount of osteopontin (at both the mRNA and the protein levels) and MMP9 (Sato et al., 2012) (which is discussed in detail in Section 5.3.6.7), which was previously found to be abundantly expressed in active MS lesions (Chabas et al., 2001). In addition to being an adhesion molecule, osteopontin also promotes the survival of activated T cells and production of proinflammatory cytokines by antigen presenting cells (Denhardt et al., 2001). Denhardt et al., proposed that osteopontin (produced by the CCR2^{+}CCR5^{+} T cells) would promote the survival of these T cells in the CNS, leading to further enrichment of T cell population in the CSF. The authors furthermore proposed that CCR2^{+}CCR5^{+} T cells capable of producing MMP9 and osteopontin, have a greater ability to invade the brain parenchyma (consisting of neurons and glial cells) (Sato et al., 2012).

In the current study it could be hypothesised that downregulation of SPP1, *which transcribes osteopontin, in the FDW group compared to the FSM group, may have attenuated neuroinflammation, by downregulating harmful T cell populations and the production of proinflammatory cytokines. This decrease in neuroinflammation in the FDW group could subsequently have contributed to neurogenesis and increased hippocampal memory (as discussed in 5.3.3.3, also refer to the model in Fig. 5.2) which possibly contributed to facilitate fear extinction.*

Additionally, if expression levels of *SPP1* in LDH are also reflected systemically, *SPP1* expression could be one of the genes mediating comorbid metabolic diseases such as diabetes. Metabolic diseases were one of the enriched diseases detected in this study, and *SPP1* was found to be associated with this disease.

5.3.6.2 *CXCL13*

Decreased levels of the chemokine (C-X-C motif) ligand 13 (*CXCL13*) were observed in the LDH of FDW compared to FSM animals. It was more than five-fold downregulated in the FDW animals and was the second most downregulated gene in the FDW group (Table 4.4, Section 4.2.3 and refer to Tables 4.5 – 4.9,

Section 4.2.3 for GO enrichment results). Differential expression of this gene was also detected with SYBR Green real-time qPCR analysis in the LDH of the rats. Differential expression of this gene was also detected in some of the other between-group comparisons (refer to Section 5.3 for a detailed discussion). CXCL13 is a potent lymphoid chemokine that is constitutively expressed in secondary lymphoid tissue (Cyster et al., 2000) and is one of the chemokines that plays a role in the migration of B cells within the follicular dendritic cell-rich area which and is critical for antigen scanning (Brandes et al., 2000; Pereira et al., 2010). B cells have an important function in the humoral immune response in neuroinflammation and perform the role of antigen-presenting cells for T cells (Pashenkov et al., 2003).

In chronic inflammatory CNS diseases, CXCL13 has been shown to play a role in the formation of ectopic lymphoid tissues within the CNS (Serafini et al., 2004; Magliozzi et al., 2004). Furthermore, elevated levels of CXCL13 were detected in the CSF of patients with MS, neuroborreliosis and other neurological inflammatory diseases (Aloisi et al., 2008; Krumbholz et al., 2006; Rupprecht et al., 2009; Brettschneider et al., 2010; Khademi et al., 2011). Levels of *CXCL13* mRNA were also found to be increased in actively demyelinating MS lesions (Krumbholz et al., 2006). A recent study implicates CXCL13 as the major determinant for B cell recruitment to the CNS in different neuroinflammatory diseases (including clinically isolated syndrome, MS, Lyme neuroborreliosis and patients with other inflammatory neurological diseases) (Kowarik et al., 2012). The authors furthermore state that elevated levels of CXCL13 in the CSF reflects a strong humoral immune response in the CNS, rather than being a marker specific for a particular disease entity (Kowarik et al., 2012).

It is thus proposed that, in the current study, that co-administration of DCS and behavioural fear extinction in the FDW animals resulted in the downregulation of this indicator of enhanced immune activation, CXCL13. *This indicated that DCS counteracted excessive immune system responses and subsequent neuroinflammation and possible neurodegeneration in the LDH. This effect, together with other effects elicited by DCS, possibly contributed to effective fear extinction.*

5.3.6.3 *CLEC7A*

The gene that transcribes the C-type lectin domain family 7, member A (*CLEC7A*), also known as Dectin-1, was more than five-fold downregulated in the FDW compared to the FSM animals (Table 4.4, Section 4.2.2 and refer to Tables 4.5 – 4.9 Section 4.2.3 for GO enrichment results). *CLEC7A* is a C-type lectin domain family member and microglia marker gene that recognizes glucans (Langmann et al., 2009) and has been found to be associated with inflammatory responses (Israelsson et al., 2009). It is a membrane-associated surface glycoprotein that is part of the pattern recognition receptors (PRRs). CLEC7A can stimulate the release of proinflammatory mediators and other cellular responses such as phagocytosis and respiratory burst (also termed oxidative burst, is the rapid release of reactive oxygen species, such as superoxide radical and hydrogen peroxide), in collaboration with TLR-2 (Brown et al., 2002; Gantner et al., 2003). This gene has been found to be upregulated in lysosomal storage disorder, a neurodegenerative disease that is characterised

by astrogliosis and neuroinflammation (Ballabio and Gieselmann 2009). A study by Shah et al. (2008) showed that CLEC7A is expressed on the surface of murine primary microglia, and engagement of the receptor with β-glucans results in increased tyrosine phosphorylation of spleen tyrosine kinase, which is a hallmark feature of the CLEC7A/Dectin-1 signalling pathway. Furthermore, they showed that CLEC7A mediates phagocytosis of β-glucan particles and facilitates subsequent intracellular production of ROS (Shah et al., 2008). However, it was found that β-glucan-mediated microglial activation did not result in significant production of cytokines or chemokines (as is typically found in macrophages and dendritic cells), thus, the interaction of microglial CLEC7A with glucan elicits a unique immunological response.

Expression of *CLEC7A* was also shown to be increased in the mouse neocortex in animal models of closed traumatic brain injury (Israelsson et al., 2009; Israelsson et al., 2010) and an Alzheimer's disease animal model (Israelsson et al., 2010), illustrating that a response elicited by CLEC7A is not limited to only responses to yeasts and fungal pathogens. Furthermore, blockade of CLEC7A significantly reduced the incidence and severity of arthritis in SKG mice (mice that are genetically prone to develop autoimmune arthritis) (Yoshitomi et al., 2005). There is growing evidence that the release and detection of alarmins, or endogenous ligands, by the innate immune system significantly contributes to the failure to resolve inflammation in chronic inflammatory diseases (Lotze Tracey 2005; Scheibner et al., 2006; Okamura et al., 2001; Ohashi et al., 2000; Shelton et al., 2008). Therefore the identification of innate immune receptors and their ligands is critical for intervening in chronic inflammatory responses. A recent study aimed to identify whether an endogenously generated inflammatory ligand exists that could trigger superoxide radicals (O_2^-) production through CLEC7A in atherosclerosis, a chronic inflammatory disease characterized by lipid accumulation in macrophage foam cells in the artery wall (Thiagarajan et al., 2013). Their results revealed that vimentin is an endogenous, activating ligand for Dectin-1 and that it induces NADPH oxidase (NOX) activity to produce O_2^- in a Dectin-1-dependent manner (in primary human monocytes) (Thiagarajan et al., 2013). In the present study, gene expression levels of vimentin, *CLEC7A* and *NOX2* (*CYBB*) were all downregulated in the FDW vs. FSM animals. It can therefore be hypothesised that decreased levels vimentin resulted in lower levels of *CLEC7A* and *NOX2*, subsequently leading to lower levels of O_2^-, which could decrease oxidative stress and inflammation.

Although the current study investigated gene expression patterns in the LDH, it could be hypothesised that CLEC7A may facilitate neuroinflammation in response to fear conditioning or exposure to stress, in a similar fashion as that described in atherosclerosis in human monocytes. Moreover, the current study detected a 1.8 fold downregulation of vimentin in the FDW compared to FSM animals as well as a 2.5 fold downregulation of *NOX2* (also known as *CYBB*) in the FDW compared to FSM animals.

In the current study, CLEC7A *was downregulated in the FDW group compared to the FSM group and it is hypothesised that downregulation of its alarmin, vimentin, resulted in reduced activation of CLEC7A. This could have, in turn, impeded NOX activity, ultimately resulting in decreased O_2^-. This may have prevented further proinflammatory responses in the LDH (since high levels of O_2^- could lead to toxic effects) which*

150

possibly contributed to neurogenesis and hippocampal memory (as discussed in 5.3.3.3 and illustrated in Fig. 5.3). DCS administration can enhance consolidation and retrieval of memory (Quartermain et al., 1994; Gabriele and Packard 2007) and in the present study DCS was co-administered with behavioural fear extinction. It is therefore proposed that co-administration of DCS and behavioural fear extinction may have facilitated consolidation and retrieval of extinction memories thereby facilitating the process of fear extinction. Furthermore, the hippocampus is one of the structures that play a role in consolidation of extinction (Cammarota et al., 2005); as the LDH was the site of injection, this may have further facilitated the fear extinction process.

5.3.6.4 *IL1RN*

The antagonist of the interleukin 1 receptor (*IL1RN*) was downregulated four-fold in the FDW compared to FSM group (Table 4.4, Section 4.2.2 and refer to Tables 4.5 – 4.9 Section 4.2.3 for GO enrichment results). Differential expression of this gene was also detected with SYBR Green real-time qPCR analysis in the LDH of the rats. Interleukin 1 (IL1) is one of the most important and predominant proinflammatory cytokines (Dinarello, 1998). The IL-1 system is composed of two known agonists, IL-1alpha (IL-1α) and IL-1beta (IL-1β), and their levels are regulated by the endogenous IL1RN. There is, however, a very narrow margin between clinical benefit and unacceptable toxicity when it comes to the levels of IL1 in humans (Dinarello, 1998). Both IL1 and IL1RN are released from glia and microglia and act on neuronal and glial receptors (Molina-Holgado et al. 2003; Tsakiri et al. 2008).

During the maintenance phase of LTP, there is a strong and sustained increase in the levels of IL1β (Balschun et al., 2004; Schneider et al., 1998). Long-term potentiation at the synaptic level is believed to underlie memory consolidation (Bliss and Lømo, 1973; Teyler, 1987). Accordingly, researchers have found that elevated levels of IL1RN, which blocks the binding of IL-1β, impair learning (Oprica et al., 2005) and interferes with the memory consolidation process (especially in the hippocampus) (Spulber et al. 2009). These findings, together with earlier research showing that IL1 receptor blockage interferes with the BDNF-ERK1/2 pathway (which mediates the effects of BDNF on synaptic plasticity, nuclear signalling, and memory formation (Finkbeiner et al., 1997; Gottschalk et al., 1999; Blanquet 2000; Pizzorusso et al., 2000; Alonso et al., 2002a,b)), suggests that physiologically appropriate levels of IL1 are essential in learning and long-term memory consolidation (refer to Section 5.3.3.3 and Fig. 5.3).

The current study therefore hypothesised that neuroinflammation, induced under stressful conditions such as fear conditioning could mediate neuronal dysfunction through decreased neurogenesis and hippocampal memory (pertaining to consolidation and retrieval of extinction memories), which may be induced by dysregulated levels of IL-1β in the microglia. *In the present study, lower levels of the* IL1RN *gene were detected in the in FDW group compared to the FSM group. Since overexpression of IL1RN impairs IL1 signalling in the brain, which could result in subsequent impairments in neurogenesis, learning and hippocampal memory (refer to Section 5.3.3.3 and Fig. 5.3) (especially hippocampal consolidation of*

extinction memories), downregulation of this gene is therefore hypothesised to attenuate these impaired neuronal processes and may possibly assist in the fear extinction process.

5.3.6.5 *FCER1G*

The gene that encodes the gamma chain of the IgE receptor CD23, *FCER1G*, was downregulated about 1.5 fold in the FDW group compared to the FSM group (Table 4.4, Section 4.2.2 and refer to Tables 4.5 – 4.9 Section 4.2.3 for GO enrichment results). This gene has also been found to be upregulated in the brains of neuromyelitis optica (NMO), Parkinson diseases (PD) and amyotrophic lateral sclerosis (ALS) patients compared to a universal control (Satoh et al., 2008). Following further bioinformatics analyses (to analyse molecular interaction on the curated knowledge database) Satoh et al. (2008) proposed that the up-regulated genes in NMO brain lesions were possibly transcriptionally regulated by NFkB and B-lymphocyte-induced maturation protein-1 (Satoh et al., 2008). NF-kB is an essential regulator of innate and adaptive immune responses, cell proliferation, and apoptosis (Li and Verma 2002), and it also regulates the expression of various proinflammatory target genes (Kumar et al., 2004). Furthermore, studies have found that the stress-induced upregulation of NF-κB complex in the hippocampus may contribute to the impaired physiological and behavioural processes in stress-related disorders (Cohen et al., 2011). In a study that investigated gene expression profiles of immune/inflammation-related genes in the aging brain, *FCER1G* was found to be significantly upregulated throughout the aging brain, including the hippocampus, and the authors hypothesised that the extensive innate immune activation they observed in their study may increase vulnerability to cognitive decline and neurodegeneration (Cribbs et al., 2012). This suggests that activation of immune system genes and proinflammatory genes, induced in response to exposure to stress, could contribute to cognitive decline and neurodegeneration.

A recent study, using a mouse model of chronic mild stress (CMS) found that *FCER1G* was downregulated in the prefrontal cortices (PFC) of WA (well-adapted) animals (the low swim stress-induced analgesia [LA] mice exhibiting low stress responses) (Lisowski et al., 2013). Furthermore, GO enrichment analysis by Lisowski et al. (2013) revealed that *FCER1G* was associated with the following GO terms (as indicated by the Expression Analysis Systemic Explorer program): signal transduction, response to stress, immune response and behavioural response to external stimulus, regulation of apoptosis or cell death as well as signal transducer activity, receptor activity or GPCR (Lisowski et al., 2013). This corresponds to enrichment analysis results from the present study, where this gene was associated with enriched GO terms such as immune system disorders, immune response, cell death, response to stimuli and signal transduction.

In the current study, FCER1G *downregulation may have facilitated fear extinction in the LDH in a similar fashion as has been observed in the PFC in the study by Lisowski et al. (2013). Furthermore, in light of its increased expression in autoimmune diseases (such as NMO, PD, and ALS), the current study hypothesises that downregulation of* FCER1G *(possibly facilitated by NF-kB) in the FDW compared to the FSM group, may have attenuated neuroinflammation in the LDH, which possibly mediated hippocampal memory*

(especially hippocampal consolidation of extinction memories) and neurogenesis (refer to Section 5.3.3.3 and Fig. 5.3). In this way administration of DCS in the FDW group may possibly have contributed to fear extinction.

5.3.6.6 TRH

Thyrotropin releasing hormone gene (*TRH*) was found to be 2.8 fold downregulated in the FDW group relative to the FSM group (Table 4.4, Section 4.2.2 and refer to Tables 4.5 – 4.9 Section 4.2.3 for GO enrichment results). Differential expression of this gene was also detected with SYBR Green real-time qPCR analysis in the LDH of the rats. TRH was the first hypothalamic hormone to be isolated (Nillni and Sevarino 1999). It is a neuropeptide and is the central regulator of the hypothalamic-pituitary-thyroid (HPT) axis (Boler et al., 1969). Produced in the paraventricular nucleus of hypothalamus, TRH stimulates the anterior pituitary to synthesise and secrete thyroid-stimulating hormone (TSH), which subsequently stimulates the synthesis and release of thyroid hormones (Dietrich et al., 2012). TRH is widely distributed in various brain regions (such as the limbic, hypothalamic, cortical and spinal regions) and exerts a range of effects as a neurotransmitter or neuromodulator (Nillni and Sevarino 1999). It is also present in numerous peripheral tissues and is therefore implicated in diverse physiological functions. The traditional role of TRH is to regulate metabolic homeostasis, which also extends to its neurobiological functions, namely the promotion of homeostasis in the CNS (Gary et al., 2003). TRH can elicit endogenous antidepressant effects, has neuroprotective as well as anti-epileptic properties and is one of several co-transmitters within glutamatergic neurons (Kubek et al., 1989; Low et al., 1989; Hrabovszky and Liposits 2008). Furthermore, TRH was shown to be involved in the pathophysiology of various neuropsychiatric disorders (Sattin, 1999).

Exogenous administration of TRH has been shown to elicit anxiolytic effects (Vogel et al., 1980 Horita, 1998; Prokai, 2002). Fear-conditioned rats have been shown to exhibit decreased acoustic startle and freezing responses following ICV TRH injection into the amygdala. However, it was shown that, using this paradigm, the HPT was not activated (Thompson and Rosen, 2000). To this end, TRH decreased acoustic startle and freezing responses, but had a limited effect on fear conditioning and active avoidance (Thompson and Rosen, 2000). The authors suggested that the results may be due to the effects of TRH on motor activity and arousal, independent of its effects on fear. Following these results, doubt was shed on the ability of TRH to truly reduce fear. It could therefore be deduced that endogenously produced TRH and exogenously administered TRH, utilise different mechanisms to elicit responses and a fine balance is required in order to maintain homeostasis. Furthermore, hippocampal TRH neurons have been shown to be involved in spatial learning and memory processes, whereas in the amygdala, TRH has an anxiolytic role (Guilar-Valles et al., 2007).

Cholinergic transmission is activated by TRH (Sabbatini et al., 1998) through the activation of cortical (Horita et al., 1986) and septohippocampal (Lamour et al.,1985) cholinergic neurons, thereby inducing release of acetylcholine (ACh) in the hippocampus (Hutson et al.,1990; Itoh et al., 1994). In the

hippocampus, TRH has the ability to affect glutamatergic and GABAergic neurotransmitter systems (Ishihara et al., 1992; Stocca and Nistri, 1995). Due to its ability to influence hormones and transmitters that form part of the HPA axis, it is hypothesised that dysregulation of TRH could contribute to dysregulation of the HPA axis.

Lisowski et al. (2013) found increased expression levels of this gene in MA animals (the high swim stress-induced analgesia [HA] mice, exhibiting increased stress responses) in a CMS animal model. Another study has shown that the ventral hippocampus, another major projection area of TRH neurons, is involved in anxiety-like behaviour; the presumed stimulatory actions of TRH axons in these areas seem to promote stress responses and increase anxiety (Wittmann et al., 2009).

In the current study we hypothesise that DCS-induced downregulation of TRH *in the FDW group might have reduced stress responses in the animals (similar to what was seen in the study by Lisowski et al. (2013)). Furthermore, in light of the fact that* TRH *regulates hormones and transmitters (especially in the hippocampus) involved in the functioning of the HPA axis, and the fact that PTSD is characterised by dysregulation of the HPA axis, it is hypothesised that increased* TRH *in response to stress might further contribute to the dysregulation of the HPA axis. Therefore, it is hypothesised that by downregulating* TRH *in the FDW animals, DCS may possibly have facilitated appropriate HPA axis regulation which may have enabled a more normal stress response in the FDW group*.

5.3.6.7 *MMP9*

Expression levels of the matrix metallopeptidase 9 gene (*MMP9*), also known as gelatinase B, were more than two-fold lower in the FDW compared to the FSM animals (Table 4.4, Section 4.2.2 and refer to Tables 4.5 – 4.9 Section 4.2.3 for GO enrichment results). Downregulated expression of this gene was also detected in the blood samples of FDW compared to FSM animals with SYBR Green real-time qPCR analysis. Matrix metalloproteinases are a family of secreted, cell-surface zinc- and calcium-dependent endopeptidases that catalyse the proteolysis of the extracellular matrix (ECM) and associated proteins (Dzwonek et al., 2004; Kaczmarek et al., 2002; Wright and Harding, 2004). Numerous studies have implicated MMPs in damage and repair in the brain under pathological conditions (Yong et al., 2005; Milward et al., 2007). In addition, MMP9 has also been shown to be involved in learning and memory processes (Nagy et al., 2006; Meighan et al., 2006, 2007; Wright et al., 2007). Inhibition of MMPs in the lateral ventricles (Meighan et al., 2006) or dorsal hippocampus (Wright et al., 2007), has been found to result in suppressed spatial learning. Also, the induction and stability of LTP is abolished in response to MMP9 blockade or broad MMP inhibitors (Meighan et al., 2007; Nagy et al., 2006). Impairment in contextual fear conditioning was observed in *MMP9* knockout mice (Nagy et al., 2006) and MMP9 and MMP3 have been shown to have a critical function in inhibitory avoidance learning (Nagy et al., 2007; Olson et al., 2008).

While the studies above emphasised the beneficial roles of MMPs in the development and normal physiology of the healthy CNS, results from the current study regarding *MMP9* expression appear to contradict the aforementioned results, since *MMP9* was found to be downregulated in the FDW animals. However, numerous studies utilise knockout animal models in order to determine the function of a particular gene. However, as was illustrated for numerous proteins or genes expressed in the brain, there is a very delicate balance between physiologically appropriate levels and levels that contribute to disease pathology. Under normal developmental and physiological conditions in the healthy CNS, specific MMP members are discretely expressed, however, the simultaneous upregulation of several MMP members at high levels have been observed during disease states; this synchronised upregulation tend to promote disease progression even further (Agrawal et al., 2008).

Increased levels of active MMP9 protein were observed in MS patients compared to controls (Leppert et al., 1998; Liuzzi et al., 2002; Fainardi et al., 2006) and elevated serum levels of MMP9 in MS patients correlated with the increased lesions as detected by magnetic resonance imaging (MRI) (Lee et al., 1999; Waubant et al., 1999). In addition, MMP9 serum levels have been found to rise progressively when patients convert from clinically isolated syndromes (a first demyelinating event, where MS diagnosis has not yet been confirmed) to the clinical diagnosis of MS (Correale et al., 2003). High expression levels of MMPs can furthermore mediate apoptosis of neural cells which results in tissue injury and subsequent neurodegeneration (Yong et al., 2001). Furthermore, MMPs have been implicated in the involvement in processes that promote neuroinflammation (Gijbels et al., 1993). A recent study found a unique CCR2^{+}CCR5^{+} T cell population to be enriched in the CSF of patients with exacerbated MS (Sato et al., 2012). The authors found that this T cell population was specifically involved in the autoimmune pathology of MS. In comparison to other T cell populations, the CCR2^{+}CCR5^{+} T cells have an exceptional ability to produce MMP9. Since MMP9 has the ability to disrupt the *glia limitans* (the outermost layer of neural tissue, it works in concert with other CNS components, such as BBB to regulate movement of small molecules and cells into the brain parenchyma), the authors speculated that they may have the potential to destroy the integrity of the BBB and trigger an inflammatory pathology cascade. An *in vitro* model of glia limitans has also shown that the MMP9–producing CCR2^{+}CCR5^{+} T cells were indeed superior to the other T cells in their ability to cross the *in vitro* model of glia limitans (Sato et al., 2012).

Increased neuroinflammation is one of the contributing factors to CNS disease pathologies (including PTSD) (McEwen, 2008 and Saavedra et al., 2011). In the present study, is has been postulated that DCS possibly attenuates excessive inflammatory responses in the LDH by downregulating genes involved in inflammatory and oxidative stress responses. Interestingly, MMP9 has been found to be induced by various proinflammatory cytokines: TNF-α and IL-1α significantly induced MMP9 synthesis and secretion in mesenchymal stem cells-derived osteoprogenitors (Ben David et al., 2008). Similar results were found in the brain, where IL-1β was found to be a key regulator of neuronal MMP9 in culture and it mediated the upregulation of MMP9 following CNS trauma in mice (Vecil et al., 2000).

Taken together, the aforementioned studies illustrate that there is a delicate balance of MMP levels, including that of MMP9, that must be preserved in the CNS to maintain homeostasis. In the current study the expression of *MMP9* was downregulated in the FDW group. *It is hypothesised that in the present study administration of DCS alleviated neuroinflammation by downregulating genes involved in inflammatory and oxidative stress responses. This possibly resulted in lower levels of inflammatory cytokines and since* MMP9 *is induced by cytokines, it is hypothesised that decreased levels of cytokines possibly resulted in lower levels of* MMP9 *in the FDW animals compared to the FSM animals. Furthermore, since high expression levels of MMPs can mediate apoptosis of neural cells, resulting in tissue injury and subsequent neurodegeneration (Yong et al., 2001), it is hypothesised that in the present study, DCS may possibly have prevented neuronal apoptosis and degeneration in the LDH by downregulating* MMP9*. This, together with the other effects mediated through the co-administration of DCS and behavioural fear extinction, may have facilitated the fear extinction process.*

The current study was also able to detect decreased levels of *MMP9* in the blood samples of the FDW animals compared to FSM animals, which is in accordance with MS studies that found these perturbations in serum (Correale et al., 2003). Sato et al. (2012) showed that MMP9 expressing T cell populations in the CSF compromised the integrity of the BBB and was able to cross it and enter the CNS. Disruption of the BBB can also enable T cells to invade into the parenchyma and cause further CNS inflammation (Engelhardt, 2010). It is therefore hypothesised that in the present study, exposure to stress induced a systemic inflammatory response whereby *MMP9* was upregulated (possibly through specific T cell populations) (enabling the detection of *MMP9* in the blood of the rats). It is further hypothesised that, in a similar fashion as described by Sato et al. (2012), increased MMP9 might have compromised the integrity of the BBB, enabling MMP9 to enter the CNS and induce further neuroinflammation and cell destruction. Expression levels of *MMP9* in the blood may therefore possibly be used as a screening tool to detect corresponding levels of *MMP9* in the brain. In addition, in the context of anxiety, *MMP9* levels may even be used to identify individuals susceptible to excessive neuroinflammatory responses and neurodegeneration and the possible development of anxiety disorders or PTSD, following exposure to a traumatic event.

5.3.6.8 *CYBB*

Expression levels of the cytochrome b-245, beta polypeptide gene, *CYBB* (also known as NADPH Oxidase 2 [NOX2]), was 2.5 fold downregulated in the FDW compared to the FSM group (Table 4.4, Section 4.2.2 and refer to Tables 4.5 – 4.9 Section 4.2.3 for GO enrichment results). Differential expression of this gene was also detected with SYBR Green real-time qPCR analysis in the LDH of the rats. CYBB is a key component of the innate host defence system (Leto and Geiszt 2006). One of its primary functions is O_2^- and ROS production in order to induce oxidative burst (explained in Section 5.3.6.3) in phagocytes, which enables digestion of engulfed bacteria (Datla and Griendling 2010; Wang et al., 2008; Tyagi et al., 2011; Brown and Griendling 2009; Lambeth et al., 2008).

However, CYBB is also widely distributed in non-phagocytic cells (Garrido et al., 2009; Sorce and Krause 2009). CYBB is distributed in somatodendritic and axonal profiles in the hippocampus, prefrontal cortex and nucleus tractus solitarius (NTS) (Wang et al., 2004). NTS dysregulation not only disrupts cardiorespiratory homeostasis, leading to hypertension, but it was also proposed to be involved in anxiety and mood disorders (such as depression, PTSD, panic disorder etc.) (Liu et al., 2012). Anxiety and mood disorders have been closely linked to CYBB-mediated oxidative stress, especially following social isolation (Nair et al., 2011; Rammal et al., 2008; Hovatta et al., 2005; Masood et al., 2008; Schiavone et al., 2009). Indeed, a growing body of literature supports the hypothesis that CYBB-derived ROS is involved in the pathophysiology of anxiety and bipolar disorders (Gard 2004; Sorce and Krause, 2009; Inaba et al., 2009; Andreazza et al., 2009; Tsaluchidu et al., 2008; Yumru et al., 2009; Steckert et al., 2010).

Furthermore, in an animal model of AD, mice that were CYBB-deficient did not develop oxidative stress, cerebrovascular dysfunction, or behavioural deficits associated with AD (Park et al., 2008). A lack of CYBB (mice lacking NOX2) was also associated with improved cognitive performance (as measured by the spatial-memory task). Genetic inactivation of CYBB counteracts oxidative stress and vascular dysfunction, therefore Park et al. (2008) hypothesised that cerebrovascular dysfunction, induced by CYBB-derived radicals, may have a role in the neuronal dysfunction underlying the cognitive impairments in Alzheimer's disease (Park et al., 2008).

The renin-angiotensin system (RAS) and its active peptide angiotensin II (AngII) are not only actively involved in hypertension but also play a major role in mood and anxiety disorders. Studies have revealed that CYBB-induced ROS production in the brain was mediated by the AngII peptide (Wang et al., 2004; Hirooka et al., 2011; Sorce and Krause 2009; Inaba et al., 2009; Zimmerman, 2011; Wang et al., 2008). Moreover, continuous activation of RAS in the brain impairs cognitive functions through CYBB-derived ROS (Inaba et al., 2009). Two subtypes of AngII receptors, namely, AT1R and AT2R, mediate the effects of AngII (Nguyen Dinh Cat et al., 2011). Furthermore, AT1Rs was found to be highly expressed in the HPA axis (Karamyan and Speth 2008) and to contribute to the majority of harmful effects induced by AngII (such as hypertension, heart failure and mood disorders (Phillips et al., 2008; Davisson et al., 2006)). Considerable evidence points to the involvement of AngII in anxiety disorders, which is mediated by the effects of AngII in the HPA and sympatho-adrenal axis (Shelton, 2007), as AngII in the brain was associated with higher HPA axis activity as well as enhanced responses to stress and anxiety (Pavel et al., 2008). In context of the current study, it is therefore hypothesised that decreased AngII may result in lower levels of CYBB-induced ROS, which could lead to lower levels of oxidative stress and inflammation. As continuous activation of RAS through CYBB-derived ROS impairs cognitive functions, a decrease could attenuate cognitive impairment.

In the current study, expression levels of *CYBB* were found to be downregulated in the FDW compared to the FSM animals. Stress has been shown to increase oxidative stress in the brain (Zieker et al., 2007), we therefore propose that in the current PTSD animal model, conditioned fear may have resulted in increased

157

oxidative stress in the fear conditioned animals. *Given the functional role of* CYBB *in immunity and ROS production, and that* CYBB-*induced ROS (possibly mediated by the AngII's effect on the HPA axis, as explained above) is proposed to be involved in the pathophysiology of anxiety disorders, it is proposed that, in the FDW group, the co-administration of DCS and behavioural fear extinction resulted in the downregulation of* CYBB *(which may have been mediated through AngII and AT1Rs), this possibly resulted in decreased levels of ROS, oxidative stress and further proinflammatory reactions (induced by ROS). This possible decrease in cytokines may have facilitated increased hippocampal memory and neurogenesis (through the mechanisms proposed in Section 5.3.3.3 and Fig. 5.3). Furthermore, since ROS may mediate neuronal cell death, it is hypothesised that downregulation of* CYBB *may have attenuated neuronal damage in the LDH (a brain region involved in fear) and may have contributed to fear extinction.*

5.3.6.9 *S100A3*, *S100A4* and *S100A9*

Three other genes that were downregulated in the FDW group belonged to the family of S100 calcium binding proteins. These genes were *S100A3*, *S100A4* and *S100A9*, which were 2.2, 3.5 and 3.5 fold downregulated respectively (Table 4.4, Section 4.2.2 and refer to Tables 4.5 – 4.9 Section 4.2.3 for GO enrichment results). Differential expression of *S100A4* was also detected with SYBR Green real-time qPCR analysis in the LDH of the rats. S100 proteins are involved in growth, differentiation, cell division and survival; the majority of the S100 proteins' functions are calcium-dependent, with the exception of a few calcium-independent functions (Santamaria-Kisiel et al., 2006). Due to their vast array of functions, S100 proteins are implicated in various physiological and pathological conditions such as inflammation, arthritis and inflammation-associated cancer (Gebhardt et al., 2006; Senolt et al., 2006; Foell et al., 2007). In the nervous system, S100 proteins have intracellular functions, where they are involved in the regulation of synaptic vesicle trafficking (Benfenati et al., 2004), cell survival and differentiation (Arcuri et al 2005). S100 proteins also have extracellular functions, where they interact mainly with RAGE (Donato, 2003). S100 proteins are released by glial cells following insults to the CNS. Furthermore, increased levels of S100 protein have been detected in serum in several neurological pathologies, including head injury, stroke and neurodegenerative diseases (Donato, 2001). S100 proteins are also part of the group of alarmins or ligands that bind to the TLR class of pattern recognition receptors which has been shown to contribute to chronic inflammation (Lotze et al., 2004; Scheibner et al., 2006; Okamura et al., 2001; Ohashi et al., 2000; Shelton et al., 2008).

S100A3 and S100A4 have been associated with cancer and are part of one of the subgroups that is associated with neoplasias (the formation of an abnormal mass of tissue) (Marenholz et al., 2004). S100A4 is secreted and act in a cytokine-like manner (Newton and Hogg 1998). Furthermore, high levels of S100A4 in white matter astrocytes were found to elicit a negative effect on astrocyte neurite growth, whereas extracellular application of S100A4 induced extensive growth of dorsal root ganglion cell neurites on white matter astrocytes (Fang et al., 2006). S100A4 was also found to display angiogenic effects (Ambartsumian et al.,

2001). Multimeric forms of S100 proteins appear to be associated with extracellular activity of these proteins. S100A4 (Novitskaya et al., 2000) is one of the assemblies that is larger than dimers and such polymeric forms of S100 proteins are proposed to trigger aggregation of the receptor RAGE, thereby activating intracellular signalling cascades (Moroz et al., 2002). As discussed earlier, RAGE cross-linking of peptides and proteins results in irreversible tissue damage (refer to Section 5.3.4) and activates cell types such as macrophages, monocytes, vascular smooth muscle cells and cardiac fibroblasts, which consequently generates reactive oxygen species (ROS). Interaction between RAGE and its ligands is believed to result in the activation of proinflammatory genes (refer to Section 5.3.4) (Bierhaus et al., 2001).

S100A9 is secreted and acts in a cytokine-like fashion. It is predominantly expressed in phagocytes and is strongly associated with proinflammatory functions (Ryckman et al., 2003). It primarily occurs in the extracellular space, where it acts as a chemotactic molecule to mediate proinflammatory signals (Marenholz et al., 2004). The S100A8/A9 heterodimer was shown to act as a chemotactic molecule in inflammation (Newton et al., 1998), and S100A9 has been implicated in inflammatory disorders (such as rheumatoid arthritis, chronic bronchitis, and cystic fibrosis).

S100 proteins display different effects in cultured neuronal and glial cells, depending on their extracellular concentrations. They elicit trophic effects when they are present in the nanomolar range, and toxic effects in the micromolar range (Adami et al., 2004; Reali et al., 2005; Businaro et al., 2006). Similarly, dose-dependent effects have been described for the effect of S100 proteins on learning and memory in rodents (Donato 2001). High doses of antibodies that bind to S100 proteins have been shown to impair LTP (Rebaudo et al., 2000) and induce amnesia for passive avoidance in chicks (O'Dowd et al., 1997), however at ultralow doses, these antibodies have the opposite effects (Epstein et al., 2003, 2006). High levels of S100 protein in the brain has detrimental effects on memory function, therefore it is thought that increased levels of S100 protein in the brain of Alzheimer's patients may be associated with some cognitive deficits associated with Alzheimer's disease (Korfias et al., 2006).

Numerous studies have suggested a role for S100 proteins in anxiety. In S100 protein knockout mice, decreased anxiety-related behaviour was evident in avoidance-approach tests, suggesting a role for these proteins in the modulation of fear and anxiety (Ackermann et al., 2006). Other studies have showed that restraint stress increases S100 protein levels in serum (Scaccianoce et al., 2004) and exposure to predator stress (odour) transiently increases S100 protein levels in CSF of adult rats (Margis et al., 2004). Furthermore, increased concentrations of S100b protein in serum were associated with anxiety-like behaviour in adult male rats following exposure to electric shocks and weekly sessions of situational reminders (Diehl et al., 2007).

Considering the literature discussed above, it is clear that the S100 proteins, especially *S100A4* and *S100A9*, are involved in inflammatory processes as well as in the modulation of fear and anxiety. *In the present study, downregulation of* S100A3, S100A4 *and* S100A9 *expression was detected in the FDW compared to the FSM group. In light of the cytokine-like actions of* S100A4 *and* S100A9 *and their proinflammatory functions, it is*

159

proposed that downregulation of these genes in the FDW group may have resulted in reduced levels of inflammation in the LDH. Furthermore, since S100A4 *is a RAGE ligand and RAGE cross-linking of peptides results in ROS generation as well as tissue damage, it is hypothesised that downregulation of* S100A4 *may have attenuated tissue damage in the LDH. It is therefore hypothesised that downregulation of* S100A4 *and* S100A9 *possibly contributed to the fear extinction process by decreasing neuroinflammation, attenuating tissue damage and possibly by supporting hippocampal memory and neurogenesis (through the mechanisms proposed in Section 5.3.3.3 and Fig. 5.3).*

5.3.6.10 *NPY*

Neuropeptide Y was another gene that was differentially expressed, with a 1.3 fold downregulation in the FDW compared to the FSM group (Table 4.4, Section 4.2.2 and refer to Tables 4.5 – 4.9 Section 4.2.3 for GO enrichment results). Even though *NPY* didn't have a big fold change in the current study, it was included in the discussion due to the fact that numerous studies have implicated NPY in anxiety disorders and PTSD (Munglani et al., 1996; Husum et al., 2002; Sah and Geracioti 2013; Serova et al., 2013). It is one of the most abundant and evolutionary conserved peptides in the CNS (Larhammar 1996a, 1996b). NPY has been shown to be involved in the regulation of hormone synthesis and release, feeding, memory, anti-anxiety action and circadian rhythms (Heilig and Widerlöv, 1995). Experimental studies in model organisms implicate NPY and its receptors in several pathological disorders, including obesity, anxiety-related disorders, depression and epilepsy or seizures (Klapstein and Colmers 1997; Munglani et al., 1996; Wahlestedt and Reis 1993). NPY modulates excitatory synaptic neurotransmission in the hippocampus by acting at Y2 receptors and subsequently inhibiting glutamate release onto pyramidal cells (Colmers and Bleakman 1994; Greber et al., 1994; Schwarzer et al., 1998). One of the most important properties of endogenous NPY is its anxiolytic effects (Heilig, 1995). Several animal models of fear or anxiety have illustrated that the effects of centrally administered NPY were similar to those observed with typical anxiolytic drugs (Heilig et al., 1989; Heilig et al., 1993; Broqua et al., 1995; Sajdyk et al., 1999).

In the present study, reduced levels of *NPY* gene expression were noted in the FDW animals relative to the FSM animals, which initially appeared to contradict the previously discussed literature. However, literature has also shown that, depending on the site of administration, NPY may have beneficial or detrimental effects on cognitive processing. They found that, when administered directly into the dorsal hippocampus, NPY administration resulted in enhanced learning and memory, whereas direct administration into the ventral hippocampus, amygdala and septum, resulted in amnestic effects (Redrobe et al., 1999; Flood et al., 1987, 1989). Furthermore, the endogenous response of NPY to stressful events and the effects of exogenous administration of NPY following stressful events appear to be different. A study by Krysiak et al. (2000) showed that conditioned fear resulted in increased levels of NPY in the amygdala, nucleus accumbens and the hypothalamus (Krysiak et al., 2000). Another study also found that single immobilisation stress increased mRNA levels of *NPY* in the superior cervical ganglia and stellate ganglia of the adrenal medulla of rats

(Nankova et al., 1996). In addition, repeated electroconvulsive stimulation has been shown to result in a long-term increase in hippocampal NPY neurotransmission (Husum et al., 2000; Madsen et al., 2000; Mathé et al., 1998; Greisen et al., 1997; Zachrisson et al., 1995; Mikkelsen et al., 1994).

A major link between the immune and nervous systems is the sympathetic nervous system (SNS) (Felten and Olschowka, 1987). Activation of the SNS results not only in the release of catecholamines (CA), such as NE and epinephrine, but also results in the release of NPY and other neuropeptides (Lundberg et al., 1989). This CA and NPY releasing pattern also occurs in sympathetic nerve fibers that terminate in lymphatic tissues (Felten et al., 1985), providing a functional anatomical link for a role of NPY in SNS-mediated immunomodulation. Indeed, studies have demonstrated various effects of NPY on several immunological parameters. Furthermore, stress is one of the physiological conditions that leads to SNS activation. The fact that NPY is released during stress via the SNS provides the basis for this peptide's function in stress-induced immune alterations (Bedoui et al., 2003).

NPY is also regarded as a neuroimmune transmitter that fine-tunes immunological functions (Bedoui et al., 2003). Studies have shown that NPY can directly increase the release of oxygen radicals (De la Fuente et al., 1993) to modulate inflammatory paw edema via interaction with Y1 and Y5 receptors (Dimitrijevic et al., 2002). It was also established that NPY elicits proinflammatory effects on antigen-presenting cells (Prod'homme et al., 2006). In mice neutrophils, NPY resulted in increased levels of IL-1β (De la Fuente et al., 2001) and in mice T cells; it resulted in increased levels of IFN-γ (Levite et al., 1998). This dysregulation in the levels of IL-1β and IFN-γ may possibly mediate decreased neurogenesis and hippocampal memory (through the mechanisms proposed in Section 5.3.3.3 and Fig. 5.3).

Therefore, we hypothesise that downregulation of NPY *expression in the FDW compared to FSM groups may have attenuated the detrimental effects of ROS and neuroinflammation on neurogenesis and memory processes in the LDH, possibly through regulating levels of IL-1β and IFN-γ. This may possibly have contributed to the extinction of conditioned fear.*

5.3.6.11 *MT2A*

Metallothionein 2A was downregulated about two-fold in the FDW compared to the FSM group (Table 4.4, Section 4.2.2 and refer to Tables 4.5 – 4.9 Section 4.2.3 for GO enrichment results). Although differential expression of this gene was not statistically significant in the SYBR Green real-time qPCR analysis in the LDH of the rats, a p-value of 0.06 indicated a trend towards a significant difference in expression (also showing lower levels of expression in FDW vs. FSM animals). Metallothionein (MT) is an enigmatic protein. Its physiological functions, especially in the CNS, are still under intense investigation 50 years after its discovery. Various studies have investigated this protein in order to associate its known biochemical properties of metal binding and free radical scavenging to the intricate workings of brain (West et al., 2008). To this end, *MT1* and *MT2* genes were found to be co-ordinately regulated by metals and glucocorticoids in mice (Searle et al., 1984; Yagle and Palmiter, 1985). Gene expression of *MT2* can be metal-induced by a

process that is mediated through the action of short cis-acting DNA sequences, which are metal-responsive elements. Expression can also be induced by glucocorticoid-responsive elements (GREs) which induce *MT2A* expression in response to glucocorticoids (Karin et al., 1984b; Kelly et al., 1997). It has been proposed that increased levels of MT2, in response to inflammatory factors, are likely facilitated by cytokines, such as IL-6, through signal transducer and activator of transcription (STAT) factors (Lee et al., 1999). STAT is one of the components of the JAK-STAT signalling pathway; this pathway transmits information from chemical signals outside the cell, through the cell membrane, and into gene promoters on the DNA, resulting in DNA transcription and activity in the cell (Aaronson and Horvath 2002). However, it is possible that not only IL-6 and STAT, but multiple stress, inflammation and oxidative stress signals participate in the induction of MT genes (West et al., 2008).

Results from knockout studies indicate that MT-1/2 null mice displayed impairment in spatial learning and memory. Upon further investigation, the authors found that the MT-1/2 knockout-induced impairment was not due to learning per se, but more likely due to impairments in memory or attention processes (Levin et al., 2006). Studies have also shown that the expression of MT-1/2 proteins is highly inducible in response to various stimuli, such as hormones, cytokines, metals, oxidative agents, inflammation and stress (Bremner, 1987b; Sato and Bremner, 1993).

MT2 plays an important function in the brain's response to different onslaughts and damage (Allan and Rothwell, 2001). Levels of MT-1/2 in the brain have been found to be upregulated by psychogenic stress (Hidalgo et al., 1990), by the administration of glutamate analogues (Acarin et al., 1999; Dalton et al., 1995), cryogenic injury (Penkowa et al., 1999a,c), or by stroke or ischemia (Campagne et al., 1999, 2000; Neal et al., 1996; Tang et al., 2002; Trendelenburg et al., 2002). In addition, levels of MT-1/2 in the brain have also been consistently reported to be increased in Alzheimer's disease (Duguid et al., 1989; Uchida, 1994; Adlard et al., 1998; Chuah and Getchell, 1999; Zambenedetti et al., 1998), multiple sclerosis (Lock et al., 2002; Penkowa et al., 2003b) and aging (Suzuki et al., 1994).

Since MT2A expression can be induced in response to glucocorticoids, it is hypothesised that in the current study, dysregulation of glucocorticoids, due to HPA axis alterations that occur in response to stress, may have resulted in increased *MT2A* expression. Furthermore, *MT2A* expression is proposed to be even further induced through inflammatory factors, such as IL-6 (through STAT, which is one of the components of the JAK-STAT signalling pathway). It has been proposed that MT2A is upregulated in order to manage neuronal damage (Penkowa et al., 2001, 2003a; Giralt et al., 2002b; Penkowa et al., 1999b; Penkowa and Hidalgo, 2000) and to decrease oxidative stress, inflammation and apoptosis in the CNS (Kang et al., 1997; Kondo et al., 1995; Lazo et al., 1995; Youn et al., 2002). However, the exact mechanism whereby these processes are facilitated remains to be fully elucidated (West et al., 2008). It is therefore also proposed that a fine balance in the amount of MT2A is probably required in order to maintain homeostasis in the CNS, therefore simple upregulation or downregulation may not fully explain the involvement of the gene in disease pathology, as is

the case with MT2A, where increased expression is observed in response to oxidative stress, however administration of MT2A elicits antioxidant properties (Andrews, 2000; Sato and Bremner, 1993). *Therefore, in the present study, the downregulation of* MT2A *in the FDW compared to FSM animals is hypothesised to be a marker of lower neuroinflammation and oxidative stress.* MT2A *is hypothesised to be downregulated as a result of the decreased levels of inflammation and inflammatory cytokines which can be attributed to the downregulation of other proinflammatory genes and ROS inducing agents (induced by co-administration of DCS and behavioural fear extinction, as discussed throughout this chapter).*

5.3.6.12 Summary of differential gene expression induced by co-administration of DCS and behavioural fear extinction

In summary, differential gene expression analyses in the current study revealed that one of the main effects of co-administration of intrahippocampal DCS and behavioural fear extinction was the downregulation of immune system genes and genes transcribing proinflammatory molecules as well as molecules involved in oxidative stress. By mediating the downregulation of these genes, DCS may have attenuated neuroinflammation and subsequent neuronal damage and impairment in hippocampal memory. Since differential expression of the aforementioned genes were detected between FDW and FSM animals, but not between CS and CD animals, it is hypothesised that the combination of behavioural fear extinction and DCS administration facilitated these expression changes. Furthermore, all of the genes discussed above, were also found to be significantly downregulated in the FDW vs. FSW groups (except *MMP9*). Genes that are differentially expressed between these groups (FDW vs. FSW) point to genes associated with DCS induced fear extinction pathway compared to the "natural" fear extinction pathway (seen in animals resilient to fear conditioning). It is therefore hypothesised that DCS-induced fear extinction possibly incorporates different genes and pathways compared to the "natural" processes of fear extinction. Concomitantly, these gene expression alterations may have mediated optimal neuronal functioning, plasticity, learning and memory which contributed to the fear extinction process in this animal model. This study therefore highlights the intimate connections between regulatory structures, specifically those of the neural and immune systems, and their importance in PTSD and anxiety disorder pathology and fear extinction.

5.3.7   SYBR Green real-time quantitative PCR gene expression analysis

Real-time qPCR is routinely used to verify next generation sequencing data (Nagalakshmi et al., 2008), however this technique is not without limitations. Technical aspects that could influence the final results include template quality, the reverse transcription (RT) step, subjectivity in data analysis and reporting (Bustin and Nolan 2004; Altar et al., 2009), selection of reference genes (Schmittgen and Zakrajsek 2000; Martínez-Beamonte et al., 2011), generation of false negatives and primer–dimers (Altar et al., 2009). It is also not known whether qPCR is the most effective means of validating RNAseq data: qPCR produces expression data relative to stably expressed reference genes, whereas RNA sequencing data (that generated a

sufficient number of reads) provides a more absolute representation of the transcriptome. Furthermore, next generation sequencing platforms have the capacity to generate millions of reads per sample, enabling this very sensitive technique to detect transcripts with low expression levels or slight differences in expression levels. Finally, next generation sequencing has the ultimate advantage of being able to identify novel transcripts (Git et al., 2010). However, since real-time qPCR is a much more cost-effective and simple screening method, its sensitivity to validate RNAseq results, in both LDH and blood samples of the rats, was investigated. Blood samples were investigated as they provide a less invasive means of obtaining genetic material and studies have shown comparable gene expression profiles in PBMCs and brain samples for certain genes (van Heerden et al., 2009).

Relative gene expression analysis was performed on the same LDH cDNA used for RNAseq as well as blood cDNA from these samples. A subset of genes was selected for real time qPCR validation. These genes were selected based on the fact that they were statistically significant differentially expressed between FDW and FSM animals (fold changes ≥ 1.3 and p < 0.05); furthermore, high fold changes were detected during RNAseq analysis and these genes have been implicated in fear, learning, memory and anxiety in previous research. Statistically significant differential expression of five (*CXCL13*, *CYBB*, *IL1RN*, *S100A4* and *TRH*) of the nine investigated genes could be detected using SYBR Green real-time qPCR in the rat LDH samples. Differential expression of *MMP9* was not detected in the LDH samples, but it was the only gene for which SYBR Green real-time qPCR could detect significant differential expression in the rat blood samples (Table 4.10). A trend towards a significant difference in expression levels between the groups was detected for *MT2A* (p = 0.06). Significant differential expression of *SPP1* and *NPY* could not be detected with SYBR Green real-time qPCR.

One explanation for not being able to detect differential expression for *SPP1* and *NPY* with SYBR Green real-time qPCR in the LDH might be the small sample sizes (six rats per group), resulting in reduced statistical power to detect differences. A possible explanation for not detecting differential expression of *CXCL13* and *MT2A* in the blood RNA, is the fact that expression of these genes are probably tissue-specific, since *CXCL13* and *MT2A* could not be amplified in blood cDNA, suggesting very low or undetectable levels of these genes in blood. Indeed, the online repository, nextprot BETA, indicated that, based on previous microarray data, mRNA expression of *CXCL13* has not been detected in blood (http://www.nextprot.org/db/entry/NX_O43927/expression#a-ts-line-322). However, the repository indicated that prior microarray studies have detected mRNA expression levels of *MT2A* in blood (http://www.nextprot.org/db/entry/NX_P02795/expression#a-ts-line-322).

Expression of the other genes in the blood cDNA was, however, detectable with SYBR Green real-time qPCR. Furthermore, gene expression differences in the brain (induced by intrahippocampal DCS administration) are not necessarily reflected in the blood (might be a tissue specific change), and lastly, the small sample size of six rats per group, reduced statistical power to detect differences.

These results indicate that qPCR can be used as a sensitive screening tool to detect differential expression of *CXCL13*, *CYBB*, *IL1RN*, *S100A4* and *TRH* in LDH samples and *MMP9* in blood samples between mal- and well- adapted animals in this specific animal model of PTSD. However, in the present study it was found that SYBR Green real-time qPCR will not necessarily detect all the differentially expressed genes identified in RNAseq analysis.

5.4 DNA Methylation Analysis

Epigenetic elements are one of the factors that mediate changes in gene expression. This study investigated whether differential DNA CpG island methylation facilitated the differential expression observed in the RNA sequencing data for *MT2A*, *NPY* and *TRH*. These genes were selected from the set of genes that were investigated with SYBR Green real-time PCR, based on the fact that they contained CpG islands and that they were associated with particular fear, anxiety and memory GO terms (any factors or phenotypes associated with anxiety/PTSD) initially identified during *BORG* analyses. Neuropeptide Y has also been well studied in anxiety disorders and PTSD, and differential methylation of *NPY* CpG islands have been investigated in obesity studies (Crujeiras et al., 2013); in the light of recent studies suggesting comorbidity of metabolic disorders and PTSD, *NPY* CpG island methylation was investigated since it could provide a potential link between the pathologies.

No differences in CpG island methylation levels in any of the genes were detected between the FDW and FSM groups. Methylation levels were between 0% and 5% in both groups (Fig. 4.13 Section 4.3). It can therefore be concluded that different levels of methylation of these CpG islands between the two treatment groups did not mediate the differential expression of these genes. However, other forms of methylation could still contribute to the observed differences in expression. The CpG islands investigated were all in close proximity to the TSS, therefore methylation of these islands would be expected to influence initiation of transcription. However, gene body methylation, which does not necessarily block transcription and may stimulate transcription elongation (Moarefi and Chedin 2011), may have mediated the observed differential expression. This warrants further investigation. It is also possible that some of the other differentially expressed genes that were not screened for differential DNA CpG island methylation were in fact regulated by CpG island methylation. This should be investigated in future studies. Furthermore, other epigenetic regulators could have facilitated the observed differential gene expression, such as acetylation, methylation, phosphorylation, ubiquitination and sumoylation of histone proteins and non-coding RNA-mediated alterations (such as micro-RNAs (miRNAs) and small interfering RNAs (siRNAs)) (Yehuda and Bierer 2009). The effect of differentially expressed miRNAs on gene expression was investigated in the present study, and will be discussed in the following section.

5.5 MicroRNA expression analysis

MicroRNAs extracted from the LDH of the rats were sequenced by means of next generation sequencing technologies to identify miRNAs that were differentially expressed between the FDW and FSM animals. Differentially expressed miRNAs were further investigated to identify miRNAs that may have mediated the differential gene expression observed between FDW and FSM animals.

5.5.1   Differential miRNA expression

The overall quality of the sequencing reads that were generated from the miRNA cDNA was very high. However, when these reads were mapped to the reference genome there were four FSM and one FDW sample that had very few reads that could be mapped to the reference genome (Table 4.11 Section 4.4.1). This could not be attributed to the quality of the sequencing reads or the number of reads generated (Fig. 4.14). The Basic Local Alignment Search Tool (BLAST) was used to determine the origin of reads that could not be mapped to the reference genome. Results indicated that the RNA PCR primer and certain RNA PCR Primer Indexes formed a duplex which was subsequently amplified and sequenced. This was probably due to the limited amount of available miRNAs in the LDH which could be amplified, together with the fact that the PCR cycle numbers were increased during the adapted protocol. Due to the very limited starting amount of miRNAs in the LDH, the adapted protocol was one of the only means of obtaining enough material to sequence. Indeed, the protocol was effective in amplifying six samples that could be sequenced and mapped sufficiently to the reference genome. It was, however, not as successful in the other six samples.

Although this did not affect the GFOLD analysis itself, as the program only incorporates mapped reads in its analysis, due to the differences of mappable reads between the samples, it is likely to have resulted in sub-optimal statistical power to detect differentially expressed miRNAs. When the analysis was repeated using only the samples that had a higher number of mappable reads, the same results were generated by GFOLD, therefore providing more confidence in the results generated.

GFOLD analyses detected 23 miRNAs that were differentially expressed between FDW and FSM groups (Table 4.12, Section 4.4.1). Of these, three miRNAs were overexpressed in the FDW group compared to the FSM group. Typically, if a miRNA is upregulated, the expression of its direct mRNA target (based on sequence homology) is expected to be downregulated (due to endonucleotic cleavage or repression of translation or deadenylation of the mRNA target) (Kuss and Chen 2008). There is also the possibility of indirect targets, however we identified the mRNA targets of the differentially expressed miRNAs solely on sequence similarity, and therefore indirect targets were not included or investigated in this study. Subsequently, the study only focussed on those miRNAs that were upregulated in the FDW group that could mediate the downregulation of the genes identified in the FDW.

However, pairing of the differentially expressed miRNA data and the biologically relevant differentially expressed genes (using IPA target filter and manual searches), identified miRNAs that were downregulated and predicted targeted genes that were also downregulated in this study (which is not in line with the basic theory of miRNA regulated gene expression, where downregulation of the miRNA usually results in

upregulation of the target gene) (Table 4.14, Section 4.4.2). This could be attributed to the fact that these miRNA:mRNA interactions are predicted mostly based on sequence similarity and do not take different biological or environmental factors (such as tissue differences, developmental stages, stress exposure or drug administration) into account. Since miRNA expression differs between cell types, tissues, developmental stages (Brown and Naldini 2009; Tang et al., 2007; Krichevsky et al., 2003), in response to stress exposure (Rinaldi et al., 2010; Meerson et al., 2010) or drug administration (Zhou et al., 2009) and also due to the complex nature of miRNA:mRNA interactions (where one miRNA can target numerous genes and one gene can be targeted by numerous miRNAs), it could be hypothesised that, even though those miRNAs are predicted to target those genes, in the context of fear conditioning and extinction of adult male Sprague Dawley rats, those miRNAs may not target those predicted mRNA targets in the LDH following DCS administration. Furthermore, other factors also influence the expression levels of genes, and even if some of these downregulated miRNAs possibly resulted in upregulation of their mRNA target genes, other factors, such as DNA methylation and posttranscriptional modifications of histone proteins, including acetylation, methylation, phosphorylation, ubiquitination and sumoylation, could ultimately have resulted in downregulation of these genes.

5.5.1.1 Functions of differentially expressed miRNAs based on functions of their mRNA targets

After identification of differentially expressed miRNAs in the LDH of the rats, it was important to determine the possible functions of these miRNAs, especially in the context of fear and anxiety. These functions can be deduced based on the functions of their predicted mRNA targets or by consulting the literature for previously described functions. One of the main associated functions, as predicted by IPA, was reproductive system disease (Table 4.13, Section 4.4.2), with 11 miRNAs that had predicted mRNA targets that were associated with this function. As mentioned above, these predictions mostly rely on sequence similarity and free energy, and do not take the biological context into account. The reason for the particular enrichment of reproductive system disease is not clear, as there is not direct connection with the current PTSD animal model. It was also not know whether the rats in the current study suffered from any reproductive system diseases.

Another function that was associated with the predicted mRNA targets of differentially expressed miRNAs identified in the LDH was cardiovascular system development and function (Table 4.13, Section 4.4.2). With reference to the model described in Fig. 5.1 (Section 5.1) and previously published research, other disease pathologies that may develop due to chronic stress are atherosclerosis and cardiovascular disease (Kang et al., 2006; Boscarino et al., 2008). MiRNAs are expressed in a temporal-spatial fashion in the cardiovascular system. They have recently emerged as important modulators of cardiovascular development (Cordes and Srivastava 2009) and angiogenesis (Suárez and Sessa 2009). Dysregulation of miRNA expression is associated with cardiac hypertrophy, cardiac arrhythmias and arterial stenosis (Latronico and Condorelli 2009). One of the differentially expressed miRNAs identified in the current study that was associated with cardiovascular system development and function is rno-mir-10b-5p. Research has shown that

downregulation of miR-10a in athero-susceptible regions in swine may contribute to endothelial proinflammatory signalling, which could ultimately result in atherosclerosis (Fang et al., 2010). In the present study this miRNA was downregulated in the FDW animals. It can therefore be hypothesised that DCS not only decreases neuroinflammation (which possibly contributed to hippocampal memory and fear extinction), but may also decrease endothelial proinflammatory signalling and lower the possibility of atherosclerosis. This reduction in proinflammatory signalling could also, in turn, possibly contribute to lower neuroinflammation (if these proinflammatory signalling molecules are able to cross the BBB).

Together, these results indicate that several miRNAs that were found to be downregulated in the FDW group are associated with cardiovascular system development and function, and have been previously described to be associated with numerous cardiovascular diseases. Although these miRNAs are not directly implicated in fear extinction processes, they shed light on how fear conditioning and stress alters miRNA expression which could modulate the development of secondary pathologies, such as cardiovascular disease.

5.5.1.2 Functions of differentially expressed miRNAs implicated in fear extinction or CNS functions

Studies that have detected differential expression of the same miRNAs found to be differentially expressed in the present study were studies that investigated (i) the effects of antidepressants (rno-mir-144-3p) (Zhou et al., 2009) or electroconvulsive shock therapy (ECT) on miRNA expression in a maternal separation model (rno-mir-187-3p, rno-mir-33-5p, rno-mir-144-3p, rno-miR-10b-5p, rno-mir-339-3p, rno-mir-193-3p, rno-miR-219-1-5p and rno-mir-451-5p) (O'Connor et al., 2013), (ii) miRNAs that tagged SNPs that were found to be associated with panic disorder (rno-mir-488-3p and rno-mir-339-3p) (Muiños-Gimeno et al., 2011), or (iii) comparative expression profiles of miRNAs in neural stem cells and motor neurons in rat embryonic spinal cord (rno-mir-31a-5p) (Wei et al., 2010) (Table 4.12, Section 4.4.1).

Depression is one of the most prevalent co-morbid disorders in PTSD and other anxiety disorders. Expression profiles of miRNAs in the current study that correlated with expression profiles of miRNAs in studies that have investigated antidepressant treatments; where ECT, ketamine and the SSRI, fluoxetine resulted in the differential expression of miRNAs such as rno-mir-187-3p, rno-mir-144-3p, rno-miR-10b-5p, rno-mir-193-3p and rno-mir-451-5p (O'Connor et al., 2013). These results may point to miRNAs that could be targeted in the treatment of co-morbid depression. *Since co-administration of DCS and behavioural fear extinction regulated miRNA expression in a similar fashion as certain antidepressant therapies (such as ECT, ketamine and the SSRI, fluoxetine), it is hypothesised that differential expression of these miRNAs could possibly facilitate the antidepressant effects of DCS previously reported in other studies* (Crane 1959, 1961; Zarate and Manji 2008; Heresco-Levy et al., 2013; Poleszak et al., 2013). D-cycloserine could therefore be an attractive therapeutic agent for patients exhibiting both PTSD and comorbid depression.

Overexpression of miR-144, the miRNA that forms a cluster with miR451 (another downregulated miRNA detected in this study) in primary erythroid cells, has been found to significantly increase sensitivity to

oxidative stress (Sangokoya et al., 2010). *The current study detected decreased levels of expression of rno-miR-144-3p in the LDH of FDW animals compared to FSM animals. The co-administration of DCS and behavioural fear extinction possibly resulted in reduced sensitivity to oxidative stress in the brain, which may have been mediated by decreased levels rno-miR-144-3p, therefore protecting the CNS from oxidative damage and cell injury, which contributed to optimal neuronal function* (Lisowski et al., 2013). *Downregulation of rno-miR-144-3p by co-administration of DCS and behavioural fear extinction may therefore possibly have contributed to fear extinction (possibly facilitated through increased consolidation and retrieval of the extinction memories as DCS was infused into the hippocampus, which is a vital region in consolidation and retrieval of the extinction memories).*

One of the miRNAs that had a specific function relating to fear extinction, based on previous literature, was rno-mir-451-5p. Expression of this miRNA was previously correlated with behavioural measures for exploration on the elevated plus maze task and learning and memory measures (Parsons et al., 2008). The authors compared miRNA expression profiles between four inbred mouse strains, A/J, BALB/cJ, C57BL/ 6J, and DBA/2J, in the hippocampus. The authors did not, however, specify whether up- or downregulation of this miRNA correlated with increased or decreased exploration and learning and memory. In the current study, rno-mir-451 was significantly downregulated in the FDW group compared to the FSM group. *We therefore hypothesise that, in the current study, downregulation of rno-mir-451 possibly facilitated increased exploration and improved learning and memory. One of the mechanisms whereby DCS thus facilitates fear extinction may be through downregulation of rno-mir-451.*

One of the three miRNAs that were upregulated in the FDW group compared to the FSM group was rno-mir-31a-5p. This miRNA was predicted to target the differentially expressed genes, *IL1RN*, *RAC2* and *HMOX*, from the RNAseq data (Table 4.14 Section 4.4.2). A study that investigated miRNA expression in the hippocampi of different inbred mice (C57BL/6 J x DBA/2 J recombinant inbred mice (BXD)) found significant correlations between miR-31 (previous nomenclature mir-31a) expression with both blood ethanol concentration and a measure of anxiety (as measured by the open field test and light-dark box measures), suggesting that this miRNA may be involved in alcohol- or anxiety-related traits (it was, however, not clear if up or downregulation of the miRNA was associated with the anxiety, and in which strains). KEGG pathway analysis revealed that miR-31 may possibly play a role in adult neurogenesis and axon guidance (Parsons et al., 2012). *In the current study it is hypothesised that co-administration of DCS and behavioural fear extinction possibly resulted in rno-mir-31a upregulation in the FDW group compared to the FSM group. This miRNA may have targeted its predicted mRNA target,* IL1RN*, which may have facilitated the downregulation of this gene which, in turn, possibly facilitated neurogenesis, hippocampal memory and potentially fear extinction (through means discussed in Sections 5.3.3.3 and 5.3.6.5 and Fig. 5.2 Section 5.3.3.2).* Furthermore, differential expression of this miRNA may be involved in comorbid substance-related disorders (possibly by regulating genes that are associated with both substance related disorders and anxiety disorders). This hypothesis warrants further investigation.

In the current study, rno-mir-19b-1-3p was also downregulated in the FDW compared to the FSW group. This miRNA forms part of the miR-17~92 cluster and was found to ubiquitously regulate B-cell, T cell and monocyte development (Xiao et al., 2008). Xiao and colleagues found that increased miR-17~92 expressions in mice lymphocytes resulted in lymphoproliferative disease and autoimmunity. *In the present study, co-administration of DCS and behavioural fear extinction resulted in decreased levels of rno-mir-19b-1-3p, decreasing possible autoimmune responses which could have caused inflammation and subsequent tissue destruction.* This corresponds with our previous gene expression findings where the autoimmune disease, systemic lupus erythematosus, was one of the enriched pathways associated with our biologically relevant differentially expressed genes, together with findings that DCS downregulates immune system genes and proinflammatory molecules that facilitate neuroinflammation. *Therefore one of the possible mechanism utilised by DCS to facilitate the downregulation of immune-related genes and hamper neuroinflammation, could be through downregulation of rno-mir-19b-1-3p.*

5.5.1.3 Differentially expressed miRNAs that may have facilitated the observed gene expression changes

Two miRNAs were upregulated and were predicted to target genes that were found to be downregulated in the FDW vs. FSM animals in the current study. Rno-mir-31a-5p was predicted (with moderate confidence, as indicated by the prediction programs) to target *HMOX1*, *RAC2* and *IL1RN,* and rno-mir-187-3p was predicted (with moderate confidence) to target *CD4*, *CD44*, *S100A4* and *A2M* and a highly confident prediction was made for *C1S* to be targeted by rno-mir-187-3p (Table 4.14 and Fig. 4.15, Section 4.4.2). Previous literature has shown that one of the mechanisms whereby certain antidepressant drugs elicit their effect is by regulating the levels of miRNAs (Baudry et al., 2010). For example, the administration of the SSRI fluoxetine (Prozac) increased the levels of miR-16 in serotonergic raphe nuclei, which resulted in reduced SERT expression (Baudry et al., 2010). It is therefore hypothesised that administration of DCS modulated the expression of rno-mir-31a-5p and rno-mir-187-3p; the upregulation of these two miRNAs may subsequently have resulted in the downregulation of their direct mRNA targets, which possibly facilitated or contributed to the fear extinction process, mostly by downregulating genes that mediate neuroinflammation, oxidative stress and/or neuronal damage, thereby improving neurogenesis and hippocampal memory (through mechanisms described in Section 5.3.3.3 and Fig. 5.3).

The remaining differentially expressed miRNAs may possibly also have been involved in the modulation of the observed differential gene expression, through targeting genes that indirectly affected the expression of the genes detected to be differentially expressed in this study. This is however difficult to investigate as it is not based on sequence complementarity, but pertains to genes that regulate the expression of other genes. Furthermore, these miRNA may possibly also have targeted genes that were differentially expressed in the current study, but that were not regarded as biologically relevant by the *BORG* analysis tool.

In order to determine if predicted mRNA targets are true targets, *in vitro* functional analyses should be performed. In the current study such analysis was performed (Section 4.4.4) and will be discussed in Section 5.5.2.

5.5.1.4 SYBR Green analysis of rno-miRNA-31a-5p in LDH brain and blood miRNA samples

The upregulated miRNA, rno-miRNA-31a-5p, was selected for SYBR Green real-time qPCR analysis, to determine if this technique would be sensitive enough to detect the slight change in expression level in LDH and blood samples between the FDW and FSM groups. Rno-miRNA-31a-5p was selected as it was one of two miRNAs that were upregulated (therefore it possibly has the ability to downregulate genes identified in the RNAseq) and it targeted *IL1RN*, a gene that was more than four-fold downregulated in FDW vs. FSM LDH.

Results indicated that SYBR Green real-time qPCR analysis was not able to detect differential expression of rno-miRNA-31a-5p in the LDH or blood samples of the rats. This could be attributed to the fact that the SYBR Green real-time qPCR may not be sensitive enough to detect such subtle differences in miRNA expression (1.1 fold upregulated), furthermore, sophisticated data analysis tools (GFOLD) had to be employed in order to detect this slight difference in expression. GFOLD was used due to the fact that *Cuffdiff* was not able to detect differential expression between the groups, possibly due to the differences in mappable reads between samples (as discussed in Section 5.5.1). It is also possible that this expression difference induced by DCS is tissue-specific and not detectable in the blood. Once again, the sample size used in this analysis was limited, with six animals per comparison group, resulting in less statistical power.

5.5.2   Functional analysis of miRNA-target interaction

The predicted interaction between rno-miR-31a-5p and *IL1RN* was selected for functional verification analysis in light of the involvement of *IL1RN* in learning and long-term memory, and the possible association between miR-31a-5p with anxiety-related traits. Co-transfection of a promoter clone containing the 3' UTR sequence of *IL1RN* and the rno-miR-31a-5p precursor clone resulted in significantly lower luciferase activity compared to co-transfection of the miRNA precursor and a scrambled target region (Fig. 4.17 Section 4.4.4). This suggests that *IL1RN* was a target of rno-miR-31a-5p. However, when luciferase signal was compared to that of the other controls, namely the scrambled miRNA precursor clone + *IL1RN* target region, and only the *IL1RN* target region alone without any miRNA precursor, there was no statistically significant difference in luciferase activity.

Although the luciferase signal for the co-transfection between rno-miR-31a-5p and *IL1RNI* was clearly lower compared to the negative controls, this difference did not reach statistical significance. This suggests that miR-31a-5p may be endogenously expressed in this human embryonic kidney cell line, which targeted the *IL1RN* region, due to the fact that transfection of the *IL1RN* target region without rno-miR-31a-5p also resulted in decreased luciferase signal. Also, other miRNAs that are endogenously expressed in HEK 293 cells that also target *IL1RN* (since one gene can be targeted by numerous miRNAs) could have resulted in lower luciferase signal (in the control transfection with only the *IL1RN* target region in the absence of rno-miR-31a-5p). Also, the experiment was repeated on a separate occasion, therefore two biological repeats

172

were performed (the results presented are the means of the data from the two experiments), therefore the small sample size limited the statistical power of the analyses. Furthermore, the interaction between rno-miR-31a-5p and *IL1RN* may be more pronounced in the presence of DCS or perhaps other cell stressors associated with physical stress (oxidative stress, inflammation, cell stress etc.), since this miRNA was found to be upregulated in the FDW group. Future studies could repeat the experiment in the presence of DCS.

5.5.3   Limitations of the study

This PTSD animal model provided the opportunity to perform brain-specific genetic analyses in order to determine the molecular mechanisms involved in DCS-facilitated fear extinction. It furthermore provided the opportunity to correlate gene and miRNA expression results between brain and blood samples. However, due to the fact that the blood was stored in RNA*later*, DNA extraction proved to be problematic and DNA could not be extracted from these samples to correlate DNA methylation results between brain and blood samples. Future studies could store a subset of the blood in such a way that would enable DNA extraction or DNA extraction should be performed immediately after blood collection.

Another limitation to the study was the limited amounts of miRNAs that could be obtained from the LDH samples. This brain region is quite small and in the present study DNA, RNA and miRNAs were extracted simultaneously from one LDH sample. These small amounts of miRNA starting material also gave rise to another problem, the formation of duplexes between the RNA PCR primer and the RNA PCR Primer Indexes, these duplexes were amplified and sequenced. This in turn resulted in the limited amount of miRNAseq reads that could be mapped to the reference genome. This resulted in sub-optimal statistical power to detect differentially expressed miRNAs. Furthermore, the number of reads generated during sequencing was also relatively low; higher read counts would increase the ability to detect a larger number of differentially expressed miRNAs as well as miRNAs that are expressed at low levels. On the other hand, the fact that the analyses included six biological repeats (six animals per subgroup) provided some strength to the analyses. It should however be kept in mind that this was a pilot study to determine whether sufficient amounts of miRNAs could be amplified from such limited starting amounts and that it can be sequenced to identify differentially expressed miRNAs that may have mediated the differential gene expression. Therefore sequencing was performed on the MiSeq system which only yielded an average of 1.9 million reads per sample. Despite these limitations, 23 differentially expressed miRNAs could be identified and follow-up sequencing will be performed on a higher throughput platform in order to verify these results, increase the read depth and facilitate the detection of a larger number of differentially expressed miRNAs as well as miRNAs expressed at low levels.

Another limitation was the small sample numbers in the sub-groups that were used for SYBR Green real time qPCR analyses. Comparing six FDW and six FSM animals with the statistical analyses used for the SYBR Green real time qPCR resulted in less statistical power. Another factor that should be kept in mind is that this study only investigated gene expression and epigenetic profiles in the LDH of these rats, therefore future studies that perform similar investigations in other brain regions should be carried out in order to

obtain a holistic and comprehensive representation of the effects of intrahippocampally administered DCS throughout the brain and how that might facilitate fear extinction. There are also other epigenetic mechanisms that could have contributed to the DCS-induced differential gene expression, such as posttranscriptionional modifications of histone proteins (acetylation, phosphorylation, ubiquitination and sumoylation) and other non-coding RNA-mediated alterations (such as long noncoding (lcnRNAs) which could be investigated in future studies to determine if they may have contributed to the differential gene expression detected in the LDH.

6. Conclusion

Currently available pharmacotherapies for the treatment of PTSD and stress-related disorders are limited and while there are effective treatments, they still fall short with regard to optimal efficacy and tolerability. It is therefore important to focus efforts towards the identification of novel anxiolytic drugs and to have a thorough understanding of their molecular mechanisms. D-cycloserine has been shown to be effective in facilitating fear extinction in animals and humans. It is a tuberculstatic drug that is not approved by the FDA for the indication of PTSD but is currently being studied.

This study investigated the molecular mechanisms whereby DCS facilitates fear extinction in an animal model of PTSD. By comparing gene expression profiles between FDW and FSM animals, we were able to identify 42 genes that were downregulated by the drug and that may underlie the molecular mechanism of DCS. One of the main effects of co-administration of intrahippocampal DCS and behavioural fear extinction was the downregulation of immune system genes and genes transcribing proinflammatory and oxidative stress molecules. These molecules mediate neuroinflammation and subsequently cause neuronal damage; DCS thereby attenuates neuroinflammation and subsequent neuronal damage. Additionally, DCS also regulates genes involved in learning and memory processes. Concomitantly, these gene expression alterations mediate optimal neuronal functioning, plasticity, learning and memory which contribute to the fear extinction process. Interestingly, some of the genes that were downregulated by DCS have been associated with conditions that co-occur and that have been found to be associated with PTSD, such as metabolic diseases, cardiovascular disease, Alzheimer's and Parkinson's disease. This indicates that DCS may not only be effective in treating PTSD, but also some of its co-morbid disorders. Indeed studies have also shown the efficacy of DCS in the treatment of other psychiatric disorders.

Future studies could investigate if differentially expressed genes identified in this animal model may be extrapolated to human samples to determine if similar genes, biological processes and pathways are involved in fear extinction processes in humans. Furthermore, SYBR Green real-time qPCR could be used to perform gene expression analysis in other biologically relevant differentially expressed genes identified in this study. This could be followed by CpG island identification of those genes detected with SYBR Green real-time qPCR to investigate additional CpG islands for DNA methylation and to determine if this may have mediated expression changes in other biologically relevant genes identified during RNAseq. Other techniques could also be employed to investigate DNA methylation, such as using methyl sensitive enzymes, DNA methylation arrays or bisulfite sequencing.

MicroRNA expression profiles revealed 23 miRNAs that were differentially expressed between the FDW and FSM animals. Functional analysis suggested that DCS induced differential gene expression might be mediated through miRNAs. This study therefore proposes that the co-administration of DCS and behavioural fear extinction was able to facilitate fear extinction in this animal model by regulating genes and miRNAs

175

involved in processes, such as neuroinflammation, learning and memory. In turn, this may have minimised neuronal damage and ensured optimal learning and memory functioning during the fear extinction process.

Future studies should include identification of potentially novel miRNAs that may be involved in the fear extinction process (by employing different bioinformatics techniques on unmapped sequences). Differentially expressed miRNAs identified in this animal model could be extrapolated to human samples to determine if similar miRNA families play a role in human fear extinction.

This study elucidated some of the mechanisms that underlie DCS induced fear extinction thereby broadening our understanding of the fear extinction process through novel insights into gene and miRNA expression profiles, molecular functions and biological pathways that are involved in fear extinction. Furthermore, these results may point to novel biological targets that could be explored as therapeutic drug targets (e.g. epigenetic drugs) in the pharmacological management of PTSD.

Appendix I

Buffers and solutions

1. Electrophoresis stock solutions

SB Buffer (20x stock)

di-Sodium tetraborate decahydrate	38.14 g

ddH$_2$O to a final volume of 1 litre

Bromophenol Blue

Bromophenol blue	0.2 % (w/v)
Glycerol	50 %
Tris (pH 8)	10 mM

Ethidium Bromide

Ethidium bromide	500 mg
ddH2O	50 ml

TE buffer (10 x stock)

Tris OH	0.1 M (pH 8)
EDTA (pH 8)	0.01 M
ddH$_2$O	150 ml

2. Gels

1 % Agarose gel

Agarose	1 g
SB Buffer (1 x)	100 ml

3. Eukaryotic cell culture media

Complete growth media

DMEM (4.5 g/L, with L-glutamine)	196 ml
Foetal calf serum	20 ml
Penstrep	2 ml

Pre-warm to 37 °C before use

Serum-free media

DMEM (4.5 g/L, with L-glutamine)   100 ml

Pre-warm to 37 °C before use

Appendix II

Differentially expressed gene between sub-groups

The focus groups of the current study was FDW vs. FSM; genes differentially expressed between these two groups might possibly be involved in DCS-induced fear extinction. However, other groups were also compared during the *Cuffdiff* analysis and their results are depicted in the table below (Table II.1). Negative fold changes imply that gene expression levels in the first group is lower than that in the second group; positive fold changes imply that gene expression levels in the first group is higher than that in the second group. Genes in bold in the FDW vs. FSW comparison are genes that were also downregulated in the FDW vs. FSM groups.

Table II.1: Biologically significant differentially expressed genes between other experimental sub-groups. Biologically significant genes that were differentially expressed between FSM and CS groups (as identified by BORG analyses); negative fold changes indicate that genes were down-regulated in the FDW group compared to the FSM group.

FSM vs. CS		
Gene	**Name**	**Fold change**
Cxcl13	C-X-C motif chemokine 13	-6.2112
FSW vs. FSM		
Gene	**Name**	**Fold change**
Trh	Thyrotropin releasing hormone	3.46987
Serpina3n	Serine (or cysteine) peptidase inhibitor, clade A, member 3N	4.68017
Cxcl13	Chemokine (C-X-C motif) ligand 13	5.39096
FDW vs. FDM		
Gene	**Name**	**Fold change**
Htr2c	5-hydroxytryptamine (serotonin) receptor 2C	2.39421
Tacr3	Tachykinin receptor 3	2.70281
FDW vs. FSW		
Gene	**Name**	**Fold change**
Spp1	**Secreted phosphoprotein 1**	**-8.51347**
Clec7a	**C-type lectin domain family 7, member A**	**-7.45662**
Msr1	**Macrophage scavenger receptor 1**	**-6.00211**
Ccl2	Chemokine (C-C motif) ligand 2	-5.90184
Mapk13	Mitogen-activated protein kinase 13	-5.7124
Il1rn	**Interleukin 1 receptor antagonist**	**-5.40918**
Ccl3	Chemokine (C-C motif) ligand 3	-5.23823
Il1b	Interleukin 1, beta	-5.20524
Trh	**Thyrotropin releasing hormone**	**-5.19366**

Cd8a	**CD8a molecule**	**-4.9112**
Gpnmb	**Glycoprotein (transmembrane) nmb**	**-4.82344**
Serpine1	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	-4.77003
Gdf15	Growth differentiation factor 15	-4.64086
Cybb	**Cytochrome b-245, beta polypeptide**	**-4.50854**
Hspb1	Heat shock 27kDa protein 1	-4.45489
S100a9	**S100 calcium binding protein A9**	**-4.44037**
Cd36	CD36 molecule (thrombospondin receptor)	-4.43935
Cd14	CD14 molecule	-4.42828
S100a4	**S100 calcium binding protein A4**	**-4.26916**
Lbp	**Lipopolysaccharide binding protein**	**-4.2255**
Bcl3	B-cell CLL/lymphoma 3	-3.96187
Pf4	Platelet factor 4	-3.90474
A2m	**Alpha-2-macroglobulin**	**-3.66365**
Vim	**Vimentin**	**-3.61174**
F5	Coagulation factor V (proaccelerin, labile factor)	-3.52583
Ctsc	**Cathepsin C**	**-3.48689**
Ncf1	**Neutrophil cytosolic factor 1**	**-3.45246**
Mt2A	**Metallothionein 2A**	**-3.42771**
Slc11a1	Solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1	-3.42324
Tspo	**Translocator protein**	**-3.2462**
Tagln	Transgelin	-3.17222
Cd44	**Cd44 molecule**	**-3.15101**
C3ar1	Complement component 3a receptor 1	-3.13968
Mt1a	Metallothionein 1A	-3.09502
Fcgr2b	Fc fragment of IgG, low affinity IIb, receptor (CD32)	-3.00999
Ptprc	**Protein tyrosine phosphatase receptor type, C**	**-2.99249**
Prlhr	Prolactin releasing hormone receptor	-2.97606
Pttg1	Pituitary tumor-transforming 1	-2.95509
Cp	**Ceruloplasmin (glycoprotein)**	**-2.95249**
Spta1	Spectrin, alpha, erythrocytic 1 (elliptocytosis 2)	-2.92093
Cd4	**Cd4 molecule**	**-2.91443**
Hpgds	Hematopoietic prostaglandin D synthase	-2.7878
Fes	Feline sarcoma oncogene	-2.68594
Gldn	Gliomedin	-2.67487
Cd300a	CD300a molecule	-2.65669
Cd86	CD86 molecule	-2.6457
Espl1	Extra spindle pole bodies homolog 1 (S. cerevisiae)	-2.63542

S100a3	**S100 calcium binding protein A3**	**-2.62302**
Cdc20	Cell division cycle 20	-2.62129
S100a10	S100 calcium binding protein A10	-2.53721
Fcer1g	**Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide**	**-2.53653**
Fermt3	Fermitin family member 3	-2.53267
C1qa	**Complement component 1, q subcomponent, A chain**	**-2.50657**
Spi1	Spleen focus forming virus (SFFV) proviral integration oncogene spi1	-2.49307
Tyrobp	TYRO protein tyrosine kinase binding protein	-2.47804
Irf8	Interferon regulatory factor 8	-2.45074
Pycard	PYD and CARD domain containing	-2.42513
Tlr2	Toll-like receptor 2	-2.37908
Itga5	Integrin, alpha 5 (fibronectin receptor, alpha polypeptide)	-2.31441
Mafb	V-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian)	-2.30377
Tnfrsf1b	Tumor necrosis factor receptor superfamily, member 1B	-2.2537
Lcp1	**Plastin-2**	**-2.24029**
Lgals1	Lectin, galactoside-binding, soluble, 1	-2.22248
Cd74	**Cd74 molecule, major histocompatibility complex, class II invariant chain**	**-2.2108**
Gadd45a	Growth arrest and DNA-damage-inducible, alpha	-2.20226
B4galt1	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 1	-2.06512
Nlrp3	NLR family, pyrin domain containing 3	-1.97888
Rbp1	Retinol binding protein 1, cellular	-1.96298
Grn	**Granulin**	**-1.89918**
Tnfrsf1a	Tumor necrosis factor receptor superfamily, member 1A	-1.88991
Serpinf1	Serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1	-1.87216
Tlr7	Toll-like receptor 7	-1.8502
Itgam	Integrin, alpha M (complement component 3 receptor 3 subunit)	-1.846
Syk	Spleen tyrosine kinase	-1.83038
Pla2g4a	Phospholipase A2, group IVA (cytosolic, calcium-dependent)	-1.80892
Lyn	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog	-1.78778
Tgfb1	Transforming growth factor, beta 1	-1.71818
Ucp2	Uncoupling protein 2 (mitochondrial, proton carrier)	-1.63483
Csf1r	**Colony stimulating factor 1 receptor**	**-1.62767**
Shc1	SHC (Src homology 2 domain containing) transforming protein 1	-1.61646
Tf	Transferrin	-1.59159
Npy	**Neuropeptide Y**	**-1.55933**
Fabp7	**Fatty acid binding protein 7, brain**	**-1.55747**
Tmem176b	**Transmembrane protein 176B**	**-1.53798**
Flna	Filamin A, alpha	-1.51243

Ptafr	Platelet-activating factor receptor	-1.50641
Gsn	**Gelsolin**	**-1.48154**
Cd38	CD38 molecule	-1.43933
Tgm2	**Transglutaminase 2, C polypeptide**	**-1.4324**
Nes	Nestin	-1.42423
Ncan	Neurocan	-1.42355
Ctsl1	Cathepsin L1	-1.42159
Il17ra	Interleukin 17 receptor A	-1.42145
Ifngr1	Interferon gamma receptor 1	-1.39074
Npc2	Niemann-Pick disease, type C2	-1.34291
Cxcl13	**Chemokine (C-X-C motif) ligand 13**	**-10.7018**

Bibliography

Aaronson, D. S. & Horvath, C. M. (2002) A road map for those who don't know JAK-STAT. Science, 296, 1653-1655.

Abelson, J. L., Le, M. J. & Bichet, D. G. (2001) Dose response of arginine vasopressin to the CCK-B agonist pentagastrin. Neuropsychopharmacology, 24, 161-169.

Abu-Elneel, K., Liu, T., Gazzaniga, F. S., Nishimura, Y., Wall, D. P., Geschwind, D. H., Lao, K. & Kosik, K. S. (2008) Heterogeneous dysregulation of microRNAs across the autism spectrum. Neurogenetics, 9, 153–161

Abush, H. & Akirav, I. (2012) Short- and long-term cognitive effects of chronic cannabinoids administration in late-adolescence rats. PLoS One, 7, e31731.

Acarin, L., Gonzalez, B., Hidalgo, J., Castro, A. J. & Castellano, B. (1999) Primary cortical glial reaction versus secondary thalamic glial response in the excitotoxically injured young brain: astroglial response and metallothionein expression. Neuroscience, 92, 827-839.

Ackard, D. M. & Neumark-Sztainer, D. (2002) Date violence and date rape among adolescents: associations with disordered eating behaviors and psychological health. Child Abuse Negl., 26, 455-473.

Ackermann, G. E., Marenholz, I., Wolfer, D. P., Chan, W. Y., Schafer, B., Erne, P. & Heizmann, C. W. (2006) S100A1-deficient male mice exhibit increased exploratory activity and reduced anxiety-related responses. Biochim.Biophys.Acta, 1763, 1307-1319.

Adamec, R., Fougere, D. & Risbrough, V. (2010) CRF receptor blockade prevents initiation and consolidation of stress effects on affect in the predator stress model of PTSD. Int.J Neuropsychopharmacol., 13, 747-757.

Adamec, R. E., Burton, P., Shallow, T. & Budgell, J. (1999) NMDA receptors mediate lasting increases in anxiety-like behavior produced by the stress of predator exposure--implications for anxiety associated with posttraumatic stress disorder. Physiol Behav., 65, 723-737.

Adami, C., Bianchi, R., Pula, G. & Donato, R. (2004) S100B-stimulated NO production by BV-2 microglia is independent of RAGE transducing activity but dependent on RAGE extracellular domain. Biochim.Biophys.Acta, 1742, 169-177.

Aderem, A. & Underhill, D. M. (1999) Mechanisms of phagocytosis in macrophages. Annu.Rev.Immunol., 17, 593-623.

Adlard, P. A., West, A. K. & Vickers, J. C. (1998) Increased density of metallothionein I/II-immunopositive cortical glial cells in the early stages of Alzheimer's disease. Neurobiol.Dis., 5, 349-356.

Agrawal, S. M., Lau, L. & Yong, V. W. (2008) MMPs in the central nervous system: where the good guys go bad. Semin.Cell Dev.Biol., 19, 42-51.

Aguilera, G. & Rabadan-Diehl, C. (2000) Vasopressinergic regulation of the hypothalamic-pituitary-adrenal axis: implications for stress adaptation. Regul.Pept., 96, 23-29.

Akbarian, S. & Huang, H. S. (2009) Epigenetic regulation in human brain-focus on histone lysine methylation. Biol.Psychiatry, 65, 198-203.

Akirav, I. (2011) The role of cannabinoids in modulating emotional and non-emotional memory processes in the hippocampus. Front Behav.Neurosci., 5, 34.

Albert, M. L., Sauter, B. & Bhardwaj, N. (1998) Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. Nature, 392, 86-89.

Alfonso, J., Frick, L. R., Silberman, D. M., Palumbo, M. L., Genaro, A. M. & Frasch, A. C. (2006) Regulation of hippocampal gene expression is conserved in two species subjected to different stressors and antidepressant treatments. Biol.Psychiatry, 59, 244-251.

Allan, S. M. & Rothwell, N. J. (2001) Cytokines and acute neurodegeneration. Nat.Rev.Neurosci., 2, 734-744.

Allen, L. A. & Aderem, A. (1996) Mechanisms of phagocytosis. Curr.Opin.Immunol., 8, 36-40.

Allo, M., Schor, I. E., Munoz, M. J., de la, M. M., Agirre, E., Valcarcel, J., Eyras, E. & Kornblihtt, A. R. (2010) Chromatin and alternative splicing. Cold Spring Harb.Symp.Quant.Biol., 75, 103-111.

Aloisi, F., Columba-Cabezas, S., Franciotta, D., Rosicarelli, B., Magliozzi, R., Reynolds, R., Ambrosini, E., Coccia, E., Salvetti, M. & Serafini, B. (2008) Lymphoid chemokines in chronic neuroinflammation. J Neuroimmunol., 198, 106-112.

Alonso, M., Vianna, M. R., Depino, A. M., Mello e Souza, Pereira, P., Szapiro, G., Viola, H., Pitossi, F., Izquierdo, I. & Medina, J. H. (2002) BDNF-triggered events in the rat hippocampus are required for both short- and long-term memory formation. Hippocampus, 12, 551-560.

Altar, C. A., Vawter, M. P. & Ginsberg, S. D. (2009) Target identification for CNS diseases by transcriptional profiling. Neuropsychopharmacology, 34, 18-54.

Amara, U., Rittirsch, D., Flierl, M., Bruckner, U., Klos, A., Gebhard, F., Lambris, J. D. & Huber-Lang, M. (2008) Interaction between the coagulation and complement system. Adv.Exp.Med.Biol., 632, 71-79.

Amatori, S., Bagaloni, I., Donati, B. & Fanelli, M. (2010) DNA demethylating antineoplastic strategies: a comparative point of view. Genes Cancer, 1, 197-209.

Ambartsumian, N., Klingelhofer, J., Grigorian, M., Christensen, C., Kriajevska, M., Tulchinsky, E., Georgiev, G., Berezin, V., Bock, E., Rygaard, J., Cao, R., Cao, Y. & Lukanidin, E. (2001) The metastasis-associated Mts1(S100A4) protein could act as an angiogenic factor. Oncogene, 20, 4685-4695.

Amstadter, A. B., Nugent, N. R. & Koenen, K. C. (2009) Genetics of PTSD: Fear Conditioning as a Model for Future Research. Psychiatr.Ann., 39, 358-367.

Andero, R. & Ressler, K. J. (2012) Fear extinction and BDNF: translating animal models of PTSD to the clinic. Genes Brain Behav., 11, 503-512.

Andersen, J. K. (2004) Oxidative stress in neurodegeneration: cause or consequence? Nat.Med., 10 Suppl, S18-S25.

Anderson, G., Berk, M., Dodd, S., Bechter, K., Altamura, A. C., Dell'osso, B., Kanba, S., Monji, A., Fatemi, S. H., Buckley, P., Debnath, M., Das, U. N., Meyer, U., Muller, N., Kanchanatawan, B. & Maes, M. (2013) Immuno-inflammatory, oxidative and nitrosative stress, and neuroprogressive pathways in the etiology, course and treatment of schizophrenia. Prog.Neuropsychopharmacol.Biol.Psychiatry, 42, 1-4.

Andreazza, A. C., Kapczinski, F., Kauer-Sant'Anna, M., Walz, J. C., Bond, D. J., Goncalves, C. A., Young, L. T. & Yatham, L. N. (2009) 3-Nitrotyrosine and glutathione antioxidant system in patients in the early and late stages of bipolar disorder. J Psychiatry Neurosci., 34, 263-271.

Andrews, G. K. (2000) Regulation of metallothionein gene expression by oxidative stress and metal ions. Biochem.Pharmacol., 59, 95-104.

Anisman, H. & Zacharko, R. M. (1992) Depression as a consequence of inadequate neurochemical adaptation in response to stressors. Br.J Psychiatry Suppl, 36-43.

Ansorge, W. J. (2009) Next-generation DNA sequencing techniques. N.Biotechnol., 25, 195-203.

Antelman, S. M. (1988). Time-dependent sensitization as the cornerstone for a new approach to pharmacotherapy: drugs as foreign/stressful stimuli. Drug Development Research, 14, 1–30.

Arcuri, C., Bianchi, R., Brozzi, F. & Donato, R. (2005) S100B increases proliferation in PC12 neuronal cells and reduces their responsiveness to nerve growth factor via Akt activation. J Biol.Chem., 280, 4402-4414.

Arranz, L., Guayerbas, N. & De la, F. M. (2007) Impairment of several immune functions in anxious women. J Psychosom.Res, 62, 1-8.

Asmundson, G. J., Bonin, M. F., Frombach, I. K. & Norton, G. R. (2000) Evidence of a disposition toward fearfulness and vulnerability to posttraumatic stress in dysfunctional pain patients. Behav.Res Ther., 38, 801-812.

Aubry, J. M., Bartanusz, V., Jezova, D., Belin, D. & Kiss, J. Z. (1999) Single stress induces long-lasting elevations in vasopressin mRNA levels in CRF hypophysiotrophic neurones, but repeated stress is required to modify AVP immunoreactivity. J Neuroendocrinol., 11, 377-384.

Aurora, R. N., Zak, R. S., Auerbach, S. H., Casey, K. R., Chowdhuri, S., Karippot, A., Maganti, R. K., Ramar, K., Kristo, D. A., Bista, S. R., Lamm, C. I. & Morgenthaler, T. I. (2010) Best practice guide for the treatment of nightmare disorder in adults. J Clin.Sleep Med., 6, 389-401.

Awad, H., Hubert, G. W., Smith, Y., Levey, A. I. & Conn, P. J. (2000) Activation of metabotropic glutamate receptor 5 has direct excitatory effects and potentiates NMDA receptor currents in neurons of the subthalamic nucleus. J Neurosci., 20, 7871-7879.

Babenko, O., Golubov, A., Ilnytskyy, Y., Kovalchuk, I. & Metz, G. A. (2012) Genomic and epigenomic responses to chronic stress involve miRNA-mediated programming. PLoS One, 7, e29441.

Bachmann, A. W., Sedgley, T. L., Jackson, R. V., Gibson, J. N., Young, R. M. & Torpy, D. J. (2005) Glucocorticoid receptor polymorphisms and post-traumatic stress disorder. Psychoneuroendocrinology, 30, 297-306.

Baek, D., Villen, J., Shin, C., Camargo, F. D., Gygi, S. P. & Bartel, D. P. (2008) The impact of microRNAs on protein output. Nature, 455, 64-71.

Baek, D., Villen, J., Shin, C., Camargo, F. D., Gygi, S. P. & Bartel, D. P. (2008) The impact of microRNAs on protein output. Nature, 455, 64-71.

Bailey, D. J., Kim, J. J., Sun, W., Thompson, R. F. & Helmstetter, F. J. (1999) Acquisition of fear conditioning in rats requires the synthesis of mRNA in the amygdala. Behav.Neurosci., 113, 276-282.

Bailey, J. N., Goenjian, A. K., Noble, E. P., Walling, D. P., Ritchie, T. & Goenjian, H. A. (2010) PTSD and dopaminergic genes, DRD2 and DAT, in multigenerational families exposed to the Spitak earthquake. Psychiatry Res, 178, 507-510.

Baisley, S. K., Cloninger, C. L. & Bakshi, V. P. (2011) Fos expression following regimens of predator stress versus footshock that differentially affect prepulse inhibition in rats. Physiol Behav., 104, 796-803.

Bajor, L. A., Ticlea, A. N. & Osser, D. N. (2011) The Psychopharmacology Algorithm Project at the Harvard South Shore Program: an update on posttraumatic stress disorder. Harv.Rev.Psychiatry, 19, 240-258.

Baker, D. G., Diamond, B. I., Gillette, G., Hamner, M., Katzelnick, D., Keller, T., Mellman, T. A., Pontius, E., Rosenthal, M. & Tucker, P. (1995) A double-blind, randomized, placebo-controlled, multi-center study of brofaromine in the treatment of post-traumatic stress disorder. Psychopharmacology (Berl), 122, 386-389.

Baker, D.G., Geracioti, T.D.J., Kasckow, J.W., Zoumakis, E. & Chrousos, G.P. (2003) Cytokines and post traumatic stress disorder. In: Kronfol, Z. (Ed.), Cytokines and Mental Health (Neurobiological Foundation of Aberrant Behaviors). Kluwer Academic Publishers, Boston; London, 301-338.

Baker, D. G., West, S. A., Nicholson, W. E., Ekhator, N. N., Kasckow, J. W., Hill, K. K., Bruce, A. B., Orth, D. N. & Geracioti, T. D., Jr. (1999) Serial CSF corticotropin-releasing hormone levels and adrenocortical activity in combat veterans with posttraumatic stress disorder. Am J Psychiatry, 156, 585-588.

Baker, D. G., Nievergelt, C. M. & O'Connor, D. T. (2012) Biomarkers of PTSD: neuropeptides and immune signaling. Neuropharmacology, 62, 663-673.

Baker, J. D. & Azorlosa, J. L. (1996) The NMDA antagonist MK-801 blocks the extinction of Pavlovian fear conditioning. Behav.Neurosci., 110, 618-620.

Ballabio, A. & Gieselmann, V. (2009) Lysosomal disorders: from storage to cellular damage. Biochim.Biophys.Acta, 1793, 684-696.

Balschun, D., Wetzel, W., del, R. A., Pitossi, F., Schneider, H., Zuschratter, W. & Besedovsky, H. O. (2004) Interleukin-6: a cytokine to forget. FASEB J, 18, 1788-1790.

Bannerman, D. M., Rawlins, J. N., McHugh, S. B., Deacon, R. M., Yee, B. K., Bast, T., Zhang, W. N., Pothuizen, H. H. & Feldon, J. (2004) Regional dissociations within the hippocampus--memory and anxiety. Neurosci.Biobehav.Rev., 28, 273-283.

Barbash, S., Hanin, G. & Soreq, H. (2013) Stereotactic injection of microRNA-expressing lentiviruses to the mouse hippocampus ca1 region and assessment of the behavioural outcome. J Vis.Exp., e50170.

Bartel, D. P. (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell, 116, 281-297.

Bartoli, F., Carra, G., Crocamo, C., Carretta, D. & Clerici, M. (2013) Metabolic Syndrome in People Suffering from Posttraumatic Stress Disorder: A Systematic Review and Meta-Analysis. Metab Syndr.Relat Disord..

Baudry, A., Mouillet-Richard, S., Schneider, B., Launay, J. M. & Kellermann, O. (2010) miR-16 targets the serotonin transporter: a new facet for adaptive responses to antidepressants. Science, 329, 1537-1541.

Bauer, M. E., Wieck, A., Lopes, R. P., Teixeira, A. L. & Grassi-Oliveira, R. (2010) Interplay between neuroimmunoendocrine systems during post-traumatic stress disorder: a minireview. Neuroimmunomodulation., 17, 192-195.

Baxter, A. J., Scott, K. M., Vos, T. & Whiteford, H. A. (2013) Global prevalence of anxiety disorders: a systematic review and meta-regression. Psychol.Med., 43, 897-910.

Baylin, S. B. & Herman, J. G. (2000) DNA hypermethylation in tumorigenesis: epigenetics joins genetics. Trends Genet., 16, 168-174.

Beach, S. R., Brody, G. H., Todorov, A. A., Gunter, T. D. & Philibert, R. A. (2010) Methylation at SLC6A4 is linked to family history of child abuse: an examination of the Iowa Adoptee sample. Am J Med.Genet.B Neuropsychiatr.Genet., 153B, 710-713.

Beck, J. G., Freeman, J. B., Shipherd, J. C., Hamblen, J. L. & Lackner, J. M. (2001) Specificity of Stroop interference in patients with pain and PTSD. J Abnorm.Psychol., 110, 536-543.

Becker, M. E., Hertzberg, M. A., Moore, S. D., Dennis, M. F., Bukenya, D. S. & Beckham, J. C. (2007) A placebo-controlled trial of bupropion SR in the treatment of chronic posttraumatic stress disorder. J Clin.Psychopharmacol., 27, 193-197.

Beckham, J. C., Crawford, A. L., Feldman, M. E., Kirby, A. C., Hertzberg, M. A., Davidson, J. R. & Moore, S. D. (1997) Chronic posttraumatic stress disorder and chronic pain in Vietnam combat veterans. J Psychosom.Res, 43, 379-389.

Bedoui, S., Kawamura, N., Straub, R. H., Pabst, R., Yamamura, T. & von, H. S. (2003) Relevance of neuropeptide Y for the neuroimmune crosstalk. J Neuroimmunol., 134, 1-11.

Bekris, S., Antoniou, K., Daskas, S. & Papadopoulou-Daifoti, Z. (2005) Behavioural and neurochemical effects induced by chronic mild stress applied to two different rat strains. Behav.Brain Res, 161, 45-59.

Bellmann, C., Miller, D. W., Mehltretter, K., Schutt, F., Jorzik, J., Unnebrink, K. & Holz, F. G. (2003) Digital analysis of choroidal neovascularisation in consecutive fluorescein angiograms for use in longitudinal clinical trials. Br.J Ophthalmol., 87, 890-892.

Ben, D. D., Reznick, A. Z., Srouji, S. & Livne, E. (2008) Exposure to pro-inflammatory cytokines upregulates MMP-9 synthesis by mesenchymal stem cells-derived osteoprogenitors. Histochem.Cell Biol., 129, 589-597.

Benfenati, F., Ferrari, R., Onofri, F., Arcuri, C., Giambanco, I. & Donato, R. (2004) S100A1 codistributes with synapsin I in discrete brain areas and inhibits the F-actin-bundling activity of synapsin I. J Neurochem., 89, 1260-1270.

Bennett, A. J., Lesch, K. P., Heils, A., Long, J. C., Lorenz, J. G., Shoaf, S. E., Champoux, M., Suomi, S. J., Linnoila, M. V. & Higley, J. D. (2002) Early experience and serotonin transporter gene variation interact to influence primate CNS function. Mol.Psychiatry, 7, 118-122.

Bergstrom, A., Jayatissa, M. N., Mork, A. & Wiborg, O. (2008) Stress sensitivity and resilience in the chronic mild stress rat model of depression; an in situ hybridization study. Brain Res, 1196, 41-52.

Berlin, H. A. (2007) Antiepileptic drugs for the treatment of post-traumatic stress disorder. Curr.Psychiatry Rep., 9, 291-300.

Berman, D. E. & Dudai, Y. (2001) Memory extinction, learning anew, and learning the new: dissociations in the molecular machinery of learning in cortex. Science, 291, 2417-2419.

Besedovsky, H. O. & del, R. A. (2011) Central and peripheral cytokines mediate immune-brain connectivity. Neurochem.Res, 36, 1-6.

Betel, D., Wilson, M., Gabow, A., Marks, D. & Sander, C. The microRNA.org resource: targets and expression. Nucleic Acids Research 36, 149-153. 2008.

Betel, D., Koppal, A., Agius, P., Sander, C. & Leslie, C. (2010) Comprehensive modeling of microRNA targets predicts functional non-conserved and non-canonical sites. Genome Biol., 11, R90.

Beveridge, N. J., Tooney, P. A., Carroll, A. P., Gardiner, E., Bowden, N., Scott, R. J., Tran, N., Dedova, I. & Cairns, M. J. (2008) Dysregulation of miRNA 181b in the temporal cortex in schizophrenia. Hum.Mol.Genet., 17, 1156-1168.

Beveridge, N. J., Gardiner, E., Carroll, A. P., Tooney, P. A. & Cairns, M. J. (2010) Schizophrenia is associated with an increase in cortical microRNA biogenesis. Mol.Psychiatry, 15, 1176-1189.

Bhat, R. & Steinman, L. (2009) Innate and adaptive autoimmunity directed to the central nervous system. Neuron, 64, 123-132.

Bhutani, N., Brady, J. J., Damian, M., Sacco, A., Corbel, S. Y. & Blau, H. M. (2010) Reprogramming towards pluripotency requires AID-dependent DNA demethylation. Nature, 463, 1042-1U57.

Bierhaus, A., Hofmann, M. A., Ziegler, R. & Nawroth, P. P. (1998) AGEs and their interaction with AGE-receptors in vascular disease and diabetes mellitus. I. The AGE concept. Cardiovasc.Res, 37, 586-600.

Bierhaus, A., Schiekofer, S., Schwaninger, M., Andrassy, M., Humpert, P. M., Chen, J., Hong, M., Luther, T., Henle, T., Kloting, I., Morcos, M., Hofmann, M., Tritschler, H., Weigle, B., Kasper, M., Smith, M., Perry, G., Schmidt, A. M., Stern, D. M., Haring, H. U., Schleicher, E. & Nawroth, P. P. (2001) Diabetes-associated sustained activation of the transcription factor nuclear factor-kappaB. Diabetes, 50, 2792-2808.

Binder, E. B., Bradley, R. G., Liu, W., Epstein, M. P., Deveau, T. C., Mercer, K. B., Tang, Y., Gillespie, C. F., Heim, C. M., Nemeroff, C. B., Schwartz, A. C., Cubells, J. F. & Ressler, K. J. (2008) Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. JAMA, 299, 1291-1305.

Bjornsson, H. T., Fallin, M. D. & Feinberg, A. P. (2004) An integrated epigenetic and genetic approach to common human disease. Trends Genet., 20, 350-358.

Black, P. H. (2006) The inflammatory consequences of psychologic stress: relationship to insulin resistance, obesity, atherosclerosis and diabetes mellitus, type II. Med.Hypotheses, 67, 879-891.

Blacker, D., Wilcox, M. A., Laird, N. M., Rodes, L., Horvath, S. M., Go, R. C., Perry, R., Watson, B., Jr., Bassett, S. S., McInnis, M. G., Albert, M. S., Hyman, B. T. & Tanzi, R. E. (1998) Alpha-2 macroglobulin is genetically associated with Alzheimer disease. Nat.Genet., 19, 357-360.

Blanchard, D. C. & Blanchard, R. J. (1988) Ethoexperimental approaches to the biology of emotion. Annu.Rev.Psychol., 39, 43-68.

Blanchard, D. C., Griebel, G. & Blanchard, R. J. (2003) The Mouse Defense Test Battery: pharmacological and behavioural assays for anxiety and panic. Eur.J Pharmacol., 463, 97-116.

Blanchard, E. B. (1990) Elevated basal levels of cardiovascular responses in Vietnam veterans with PTSD: a health problem in the making? J Anxiety Disord, 4, 233–7.

Blanchard, R. J., Griebel, G., Henrie, J. A. & Blanchard, D. C. (1997) Differentiation of anxiolytic and panicolytic drugs by effects on rat and mouse defense test batteries. Neurosci.Biobehav.Rev., 21, 783-789.

Blanquet, P. R. (2000) Identification of two persistently activated neurotrophin-regulated pathways in rat hippocampus. Neuroscience, 95, 705-719.

Blaszczyk, J. W., Tajchert, K., Lapo, I. & Sadowski, B. (2000) Acoustic startle and open-field behavior in mice bred for magnitude of swim analgesia. Physiol Behav., 70, 471-476.

Bliss, T. V. & Lomo, T. (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol, 232, 331-356.

Bliss, T. V. & Collingridge, G. L. (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature, 361, 31-39.

Bockaert, J. & Pin, J. P. (1999) Molecular tinkering of G protein-coupled receptors: an evolutionary success. EMBO J, 18, 1723-1729.

Bockaert, J., Claeysen, S., Becamel, C., Pinloche, S. & Dumuis, A. (2002) G protein-coupled receptors: dominant players in cell-cell communication. Int.Rev.Cytol., 212, 63-132.

Boler, J., Enzmann, F., Folkers, K., Bowers, C. Y. & Schally, A. V. (1969) The identity of chemical and hormonal properties of the thyrotropin releasing hormone and pyroglutamyl-histidyl-proline amide. Biochem.Biophys.Res Commun., 37, 705-710.

Bonefeld, B. E., Elfving, B. & Wegener, G. (2008) Reference genes for normalization: a study of rat brain tissue. Synapse , 62, 302-309.

Bonne, O., Vythilingam, M., Inagaki, M., Wood, S., Neumeister, A., Nugent, A. C., Snow, J., Luckenbaugh, D. A., Bain, E. E., Drevets, W. C. & Charney, D. S. (2008) Reduced posterior hippocampal volume in posttraumatic stress disorder. J Clin.Psychiatry, 69, 1087-1091.

Booth, M. J., Branco, M. R., Ficz, G., Oxley, D., Krueger, F., Reik, W. & Balasubramanian, S. (2012) Quantitative sequencing of 5-methylcytosine and 5-hydroxymethylcytosine at single-base resolution. Science, 336, 934-937.

Borsini, F., Podhorna, J. & Marazziti, D. (2002) Do animal models of anxiety predict anxiolytic-like effects of antidepressants? Psychopharmacology (Berl), 163, 121-141.

Boscarino, J. A. (2006) Posttraumatic stress disorder and mortality among U.S. Army veterans 30 years after military service. Ann.Epidemiol., 16, 248-256.

Boscarino, J. A. (2008) A prospective study of PTSD and early-age heart disease mortality among Vietnam veterans: implications for surveillance and prevention. Psychosom.Med., 70, 668-676.

Boscarino, J. A., Erlich, P. M., Hoffman, S. N., Rukstalis, M. & Stewart, W. F. (2011) Association of FKBP5, COMT and CHRNA5 polymorphisms with PTSD among outpatients at risk for PTSD. Psychiatry Res, 188, 173-174.

Boscarino, J. A., Erlich, P. M., Hoffman, S. N. & Zhang, X. (2012) Higher FKBP5, COMT, CHRNA5, and CRHR1 allele burdens are associated with PTSD and interact with trauma exposure: implications for neuropsychiatric research and treatment. Neuropsychiatr.Dis.Treat., 8, 131-139.

Boscarino, J. A., Kirchner, H. L., Hoffman, S. N. & Erlich, P. M. (2013) Predicting PTSD using the New York Risk Score with genotype data: potential clinical and research opportunities. Neuropsychiatr.Dis.Treat., 9, 517-527.

Botreau, F., Paolone, G. & Stewart, J. (2006) d-Cycloserine facilitates extinction of a cocaine-induced conditioned place preference. Behav.Brain Res, 172, 173-178.

Bourin, M. & Hascoet, M. (2003) The mouse light/dark box test. Eur.J Pharmacol., 463, 55-65.

Bouton, M. E. (2002) Context, ambiguity, and unlearning: sources of relapse after behavioural extinction. Biol.Psychiatry, 52, 976-986.

Boyce, S., Wyatt, A., Webb, J. K., O'Donnell, R., Mason, G., Rigby, M., Sirinathsinghji, D., Hill, R. G. & Rupniak, N. M. (1999) Selective NMDA NR2B antagonists induce antinociception without motor dysfunction: correlation with restricted localisation of NR2B subunit in dorsal horn. Neuropharmacology, 38, 611-623.

Boyko, E. J., Jacobson, I. G., Smith, B., Ryan, M. A., Hooper, T. I., Amoroso, P. J., Gackstetter, G. D., Barrett-Connor, E. & Smith, T. C. (2010) Risk of diabetes in U.S. military service members in relation to combat deployment and mental health. Diabetes Care, 33, 1771-1777.

Brady, K., Pearlstein, T., Asnis, G. M., Baker, D., Rothbaum, B., Sikes, C. R. & Farfel, G. M. (2000) Efficacy and safety of sertraline treatment of posttraumatic stress disorder: a randomized controlled trial. JAMA, 283, 1837-1844.

Brandes, M., Legler, D. F., Spoerri, B., Schaerli, P. & Moser, B. (2000) Activation-dependent modulation of B lymphocyte migration to chemokines. Int.Immunol., 12, 1285-1292.

Braun, P., Greenberg, D., Dasberg, H. & Lerer, B. (1990) Core symptoms of posttraumatic stress disorder unimproved by alprazolam treatment. J Clin.Psychiatry, 51, 236-238.

Bredy, T. W., Wu, H., Crego, C., Zellhoefer, J., Sun, Y. E. & Barad, M. (2007) Histone modifications around individual BDNF gene promoters in prefrontal cortex are associated with extinction of conditioned fear. Learn.Mem., 14, 268-276.

Bremner, I. (1987) Nutritional and physiological significance of metallothionein. Experientia Suppl, 52, 81-107.

Bremner, J. D., Southwick, S. M., Darnell, A. & Charney, D. S. (1996) Chronic PTSD in Vietnam combat veterans: course of illness and substance abuse. Am J Psychiatry, 153, 369-375.

Bremner, J. D., Licinio, J., Darnell, A., Krystal, J. H., Owens, M. J., Southwick, S. M., Nemeroff, C. B. & Charney, D. S. (1997) Elevated CSF corticotropin-releasing factor concentrations in posttraumatic stress disorder. Am J Psychiatry, 154, 624-629.

Bremner, J. D., Vermetten, E., Schmahl, C., Vaccarino, V., Vythilingam, M., Afzal, N., Grillon, C. & Charney, D. S. (2005) Positron emission tomographic imaging of neural correlates of a fear acquisition and extinction paradigm in women with childhood sexual-abuse-related post-traumatic stress disorder. Psychol.Med., 35, 791-806.

Brennecke, J., Stark, A., Russell, R. B. & Cohen, S. M. (2005) Principles of microRNA-target recognition. PLoS Biol., 3, e85.

Breslau, N., Kessler, R. C., Chilcoat, H. D., Schultz, L. R., Davis, G. C. & Andreski, P. (1998) Trauma and posttraumatic stress disorder in the community: the 1996 Detroit Area Survey of Trauma. Arch.Gen.Psychiatry, 55, 626-632.

Brewerton, T. D., Dansky, B. S., Kilpatrick, D. G., & O'Neil, P. M. (1999). Bulimia nervosa, PTSD and "forgetting": Results from the National Women's Study. In L. M. Williams, & V. L. Banyard (Eds.), Trauma and memory. Durham: Sage Publications. 127–138

Brettschneider, J., Czerwoniak, A., Senel, M., Fang, L., Kassubek, J., Pinkhardt, E., Lauda, F., Kapfer, T., Jesse, S., Lehmensiek, V., Ludolph, A. C., Otto, M. & Tumani, H. (2010) The chemokine CXCL13 is a prognostic marker in clinically isolated syndrome (CIS). PLoS One, 5, e11986.

Brewerton, T. D., Lydiard, R. B., Herzog, D. B., Brotman, A. W., O'Neil, P. M. & Ballenger, J. C. (1995) Comorbidity of axis I psychiatric disorders in bulimia nervosa. J Clin.Psychiatry, 56, 77-80.

Brewerton, T. D. (2007) Eating disorders, trauma, and comorbidity: focus on PTSD. Eat.Disord., 15, 285-304.

Broekman, B. F., Olff, M. & Boer, F. (2007) The genetic background to PTSD. Neurosci.Biobehav.Rev., 31, 348-362.

Broqua, P., Wettstein, J. G., Rocher, M. N., Gauthier-Martin, B. & Junien, J. L. (1995) Behavioural effects of neuropeptide Y receptor agonists in the elevated plus-maze and fear-potentiated startle procedures. Behav.Pharmacol., 6, 215-222.

Brown, B. D. & Naldini, L. (2009) Exploiting and antagonizing microRNA regulation for therapeutic and experimental applications. Nat.Rev.Genet., 10, 578-585.

Brown, D. I. & Griendling, K. K. (2009) Nox proteins in signal transduction. Free Radic.Biol.Med., 47, 1239-1253.

Brown, G. D., Taylor, P. R., Reid, D. M., Willment, J. A., Williams, D. L., Martinez-Pomares, L., Wong, S. Y. & Gordon, S. (2002) Dectin-1 is a major beta-glucan receptor on macrophages. J Exp.Med., 196, 407-412.

Brown, M. W. & Aggleton, J. P. (2001) Recognition memory: what are the roles of the perirhinal cortex and hippocampus? Nat.Rev.Neurosci., 2, 51-61.

bu-Elneel, K., Liu, T., Gazzaniga, F. S., Nishimura, Y., Wall, D. P., Geschwind, D. H., Lao, K. & Kosik, K. S. (2008) Heterogeneous dysregulation of microRNAs across the autism spectrum. Neurogenetics. , 9, 153-161.

Buchanan, J. B., Sparkman, N. L., Chen, J. & Johnson, R. W. (2008) Cognitive and neuroinflammatory consequences of mild repeated stress are exacerbated in aged mice. Psychoneuroendocrinology, 33, 755-765.

Buckley, T. C. & Kaloupek, D. G. (2001) A meta-analytic examination of basal cardiovascular activity in posttraumatic stress disorder. Psychosom.Med., 63, 585-594.

Bulik, C. M., Sullivan, P. F., Wade, T. D. & Kendler, K. S. (2000) Twin studies of eating disorders: a review. Int.J Eat.Disord., 27, 1-20.

Burdo, T. H., Ellis, R. J. & Fox, H. S. (2008) Osteopontin is increased in HIV-associated dementia. J Infect.Dis., 198, 715-722.

Burgos-Robles, A., Vidal-Gonzalez, I., Santini, E. & Quirk, G. J. (2007) Consolidation of fear extinction requires NMDA receptor-dependent bursting in the ventromedial prefrontal cortex. Neuron, 53, 871-880.

Burnes, D. P. & Burnette, D. (2013) Broadening the etiological discourse on Alzheimer's disease to include trauma and posttraumatic stress disorder as psychosocial risk factors. J Aging Stud., 27, 218-224.

Businaro, R., Leone, S., Fabrizi, C., Sorci, G., Donato, R., Lauro, G. M. & Fumagalli, L. (2006) S100B protects LAN-5 neuroblastoma cells against Abeta amyloid-induced neurotoxicity via RAGE engagement at low doses but increases Abeta amyloid neurotoxicity at high doses. J Neurosci.Res, 83, 897-906.

Bustin, S. A. & Nolan, T. (2004) Pitfalls of quantitative real-time reverse-transcription polymerase chain reaction. J Biomol.Tech., 15, 155-166.

Butterfield, M. I., Becker, M. E., Connor, K. M., Sutherland, S., Churchill, L. E. & Davidson, J. R. (2001) Olanzapine in the treatment of post-traumatic stress disorder: a pilot study. Int.Clin.Psychopharmacol., 16, 197-203.

Caffe, A. R., van Leeuwen, F. W. & Luiten, P. G. (1987) Vasopressin cells in the medial amygdala of the rat project to the lateral septum and ventral hippocampus. J Comp Neurol., 261, 237-252.

Cain, C. K., Blouin, A. M. & Barad, M. (2002) L-type voltage-gated calcium channels are required for extinction, but not for acquisition or expression, of conditional fear in mice. J Neurosci., 22, 9113-9121.

Cain, C. K., Godsil, B. P., Jami, S. & Barad, M. (2005) The L-type calcium channel blocker nifedipine impairs extinction, but not reduced contingency effects, in mice. Learn.Mem., 12, 277-284.

Cameron, E. E., Bachman, K. E., Myohanen, S., Herman, J. G. & Baylin, S. B. (1999) Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. Nat.Genet., 21, 103-107.

Cammarota, M., Bevilaqua, L. R., Barros, D. M., Vianna, M. R., Izquierdo, L. A., Medina, J. H. & Izquierdo, I. (2005) Retrieval and the extinction of memory. Cell Mol.Neurobiol., 25, 465-474.

Camon, E., Magrane, M., Barrell, D., Lee, V., Dimmer, E., Maslen, J., Binns, D., Harte, N., Lopez, R. & Apweiler, R. (2004) The Gene Ontology Annotation (GOA) Database: sharing knowledge in Uniprot with Gene Ontology. Nucleic Acids Res, 32, D262-D266.

Campagne, M. V., Thibodeaux, H., van, B. N., Cairns, B. & Lowe, D. G. (2000) Increased binding activity at an antioxidant-responsive element in the metallothionein-1 promoter and rapid induction of metallothionein-1 and -2 in response to cerebral ischemia and reperfusion. J Neurosci., 20, 5200-5207.

Campos, A. C., Ferreira, F. R., da, S. W., Jr. & Guimaraes, F. S. (2013) Predator threat stress promotes long lasting anxiety-like behaviors and modulates synaptophysin and CB1 receptors expression in brain areas associated with PTSD symptoms. Neurosci.Lett., 533, 34-38.

Cantor, H. (1995) The role of Eta-1/osteopontin in the pathogenesis of immunological disorders. Ann.N.Y.Acad.Sci., 760, 143-150.

Cantor, H. & Shinohara, M. L. (2009) Regulation of T-helper-cell lineage development by osteopontin: the inside story. Nat.Rev.Immunol., 9, 137-141.

Carey, P., Suliman, S., Ganesan, K., Seedat, S. & Stein, D. J. (2012) Olanzapine monotherapy in posttraumatic stress disorder: efficacy in a randomized, double-blind, placebo-controlled study. Hum.Psychopharmacol., 27, 386-391.

Casciola-Rosen, L. A., Anhalt, G. & Rosen, A. (1994) Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. J Exp.Med., 179, 1317-1330.

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., McClay, J., Mill, J., Martin, J., Braithwaite, A. & Poulton, R. (2003) Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science, 301, 386-389.

Castellano, L., Giamas, G., Jacob, J., Coombes, R. C., Lucchesi, W., Thiruchelvam, P., Barton, G., Jiao, L. R., Wait, R., Waxman, J., Hannon, G. J. & Stebbing, J. (2009) The estrogen receptor-alpha-induced microRNA signature regulates itself and its transcriptional response. Proc.Natl.Acad.Sci.U.S.A, 106, 15732-15737.

Chabas, D., Baranzini, S. E., Mitchell, D., Bernard, C. C., Rittling, S. R., Denhardt, D. T., Sobel, R. A., Lock, C., Karpuj, M., Pedotti, R., Heller, R., Oksenberg, J. R. & Steinman, L. (2001) The influence of the proinflammatory cytokine, osteopontin, on autoimmune demyelinating disease. Science, 294, 1731-1735.

Challen, G. A., Sun, D., Jeong, M., Luo, M., Jelinek, J., Berg, J. S., Bock, C., Vasanthakumar, A., Gu, H., Xi, Y., Liang, S., Lu, Y., Darlington, G. J., Meissner, A., Issa, J. P., Godley, L. A., Li, W. & Goodell, M. A. (2012) Dnmt3a is essential for hematopoietic stem cell differentiation. Nat.Genet., 44, 23-31.

Champagne, F. A., Francis, D. D., Mar, A. & Meaney, M. J. (2003) Variations in maternal care in the rat as a mediating influence for the effects of environment on development. Physiol Behav., 79, 359-371.

Champagne, F. A. (2008) Epigenetic mechanisms and the transgenerational effects of maternal care. Front Neuroendocrinol., 29, 386-397.

Chan, J. A., Krichevsky, A. M. & Kosik, K. S. (2005) MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Res, 65, 6029-6033.

Chandrasekar, V. & Dreyer, J. L. (2009) microRNAs miR-124, let-7d and miR-181a regulate cocaine-induced plasticity. Mol.Cell Neurosci., 42, 350-362.

Chang, S., Johnston, R. J., Jr., Frokjaer-Jensen, C., Lockery, S. & Hobert, O. (2004) MicroRNAs act sequentially and asymmetrically to control chemosensory laterality in the nematode. Nature, 430, 785-789.

Chang, S. C., Koenen, K. C., Galea, S., Aiello, A. E., Soliven, R., Wildman, D. E. & Uddin, M. (2012) Molecular variation at the SLC6A3 locus predicts lifetime risk of PTSD in the Detroit Neighborhood Health Study. PLoS One, 7, e39184.

Charmandari, E., Tsigos, C. & Chrousos, G. (2005) Endocrinology of the stress response. Annu.Rev.Physiol, 67, 259-284.

Charney, D. S., Deutch, A. Y., Krystal, J. H., Southwick, S. M. & Davis, M. (1993) Psychobiologic mechanisms of posttraumatic stress disorder. Arch.Gen.Psychiatry, 50, 295-305.

Chen, J. A., Keller, S. M., Zoellner, L. A. & Feeny, N. C. (2013) "How will it help me?": reasons underlying treatment preferences between sertraline and prolonged exposure in posttraumatic stress disorder. J Nerv.Ment.Dis., 201, 691-697.

Chen, K. & Rajewsky, N. (2006) Natural selection on human microRNA binding sites inferred from SNP data. Nat.Genet., 38, 1452-1456.

Chen, W. G., Chang, Q., Lin, Y., Meissner, A., West, A. E., Griffith, E. C., Jaenisch, R. & Greenberg, M. E. (2003) Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. Science, 302, 885-889.

Chen, Y. P., Jin, X., Xiang, Z., Chen, S. H. & Li, Y. M. (2013) Circulating MicroRNAs as potential biomarkers for alcoholic steatohepatitis. Liver Int., 33, 1257-1265.

Chertkow-Deutsher, Y., Cohen, H., Klein, E. & Ben-Shachar, D. (2010) DNA methylation in vulnerability to post-traumatic stress in rats: evidence for the role of the post-synaptic density protein Dlgap2. Int.J Neuropsychopharmacol., 13, 347-359.

Chhatwal, J. P., Myers, K. M., Ressler, K. J. & Davis, M. (2005) Regulation of gephyrin and GABAA receptor binding within the amygdala after fear acquisition and extinction. J Neurosci., 25, 502-506.

Chhatwal, J. P., Stanek-Rattiner, L., Davis, M. & Ressler, K. J. (2006) Amygdala BDNF signaling is required for consolidation but not encoding of extinction. Nat.Neurosci., 9, 870-872.

Chibnall, J. T. & Duckro, P. N. (1994) Post-traumatic stress disorder in chronic post-traumatic headache patients. Headache, 34, 357-361.

Chiocchetti, A., Comi, C., Indelicato, M., Castelli, L., Mesturini, R., Bensi, T., Mazzarino, M. C., Giordano, M., D'Alfonso, S., Momigliano-Richiardi, P., Liguori, M., Zorzon, M., Amoroso, A., Trojano, M., Monaco, F., Leone, M., Magnani, C. & Dianzani, U. (2005) Osteopontin gene haplotypes correlate with multiple sclerosis development and progression. J Neuroimmunol., 163, 172-178.

Chizh, B. A., Headley, P. M. & Tzschentke, T. M. (2001) NMDA receptor antagonists as analgesics: focus on the NR2B subtype. Trends Pharmacol.Sci., 22, 636-642.

Choi, J. S., Kim, H. Y., Cha, J. H., Choi, J. Y. & Lee, M. Y. (2007) Transient microglial and prolonged astroglial upregulation of osteopontin following transient forebrain ischemia in rats. Brain Res, 1151, 195-202.

Choong, M. L., Yang, H. H. & McNiece, I. (2007) MicroRNA expression profiling during human cord blood-derived CD34 cell erythropoiesis. Exp.Hematol., 35, 551-564.

Chrousos, G. P. & Gold, P. W. (1992) The concepts of stress and stress system disorders. Overview of physical and behavioural homeostasis. JAMA, 267, 1244-1252.

Chrousos, G. P. (1995) The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. N.Engl.J Med., 332, 1351-1362.

Chrousos, G. P. (1998) Stressors, stress, and neuroendocrine integration of the adaptive response. The 1997 Hans Selye Memorial Lecture. Ann.N.Y.Acad.Sci., 851, 311-335.

Chrousos, G. P., Torpy, D. J. & Gold, P. W. (1998) Interactions between the hypothalamic-pituitary-adrenal axis and the female reproductive system: clinical implications. Ann.Intern.Med., 129, 229-240.

Chrousos, G. P. (2000) The stress response and immune function: clinical implications. The 1999 Novera H. Spector Lecture. Ann.N.Y.Acad.Sci., 917, 38-67.

Chrousos, G. P. (2007) Organization and Integration of the Endocrine System. Sleep Med.Clin., 2, 125-145.

Chrousos, G. P. (2009) Stress and disorders of the stress system. Nat.Rev.Endocrinol., 5, 374-381.

Chuah, M. I. & Getchell, M. L. (1999) Metallothionein in olfactory mucosa of Alzheimer's disease patients and apoE-deficient mice. Neuroreport, 10, 1919-1924.

Chwang, W. B., O'Riordan, K. J., Levenson, J. M. & Sweatt, J. D. (2006) ERK/MAPK regulates hippocampal histone phosphorylation following contextual fear conditioning. Learn.Mem., 13, 322-328.

Claes, S. J. (2004) Corticotropin-releasing hormone (CRH) in psychiatry: from stress to psychopathology. Ann.Med., 36, 50-61.

Cline, M. S., Smoot, M., Cerami, E., Kuchinsky, A., Landys, N., Workman, C., Christmas, R., vila-Campilo, I., Creech, M., Gross, B., Hanspers, K., Isserlin, R., Kelley, R., Killcoyne, S., Lotia, S., Maere, S., Morris, J., Ono, K., Pavlovic, V., Pico, A. R., Vailaya, A., Wang, P. L., Adler, A., Conklin, B. R., Hood, L., Kuiper, M., Sander, C., Schmulevich, I.,

Schwikowski, B., Warner, G. J., Ideker, T. & Bader, G. D. (2007) Integration of biological networks and gene expression data using Cytoscape. Nat.Protoc., 2, 2366-2382.

Cohen, H., Zohar, J. & Matar, M. (2003) The relevance of differential response to trauma in an animal model of posttraumatic stress disorder. Biol.Psychiatry, 53, 463-473.

Cohen, H., Zohar, J., Gidron, Y., Matar, M. A., Belkind, D., Loewenthal, U., Kozlovsky, N. & Kaplan, Z. (2006) Blunted HPA axis response to stress influences susceptibility to posttraumatic stress response in rats. Biol.Psychiatry, 59, 1208-1218.

Cohen, H., Kozlovsky, N., Matar, M. A., Zohar, J. & Kaplan, Z. (2011) The characteristic long-term upregulation of hippocampal NF-kappaB complex in PTSD-like behavioural stress response is normalized by high-dose corticosterone and pyrrolidine dithiocarbamate administered immediately after exposure. Neuropsychopharmacology, 36, 2286-2302.

Cohen, J. A., Deblinger, E., Mannarino, A. P. & Steer, R. A. (2004) A multisite, randomized controlled trial for children with sexual abuse-related PTSD symptoms. J Am Acad.Child Adolesc.Psychiatry, 43, 393-402.

Coleman, R. M., Lombard, M.F., Sicard, R. E. & Rencricca N. J. (1989) Fundamental Immunology, Wm. C. Brown Publishers, Dubuque, IA, 207–230.

Colmers, W. F. & Bleakman, D. (1994) Effects of neuropeptide Y on the electrical properties of neurons. Trends Neurosci., 17, 373-379.

Comings, D. E., Comings, B. G., Muhleman, D., Dietz, G., Shahbahrami, B., Tast, D., Knell, E., Kocsis, P., Baumgarten, R., Kovacs, B. W. & . (1991) The dopamine D2 receptor locus as a modifying gene in neuropsychiatric disorders. JAMA, 266, 1793-1800.

Comings, D. E., Muhleman, D. & Gysin, R. (1996) Dopamine D2 receptor (DRD2) gene and susceptibility to posttraumatic stress disorder: a study and replication. Biol.Psychiatry, 40, 368-372.

Conaco, C., Otto, S., Han, J. J. & Mandel, G. (2006) Reciprocal actions of REST and a microRNA promote neuronal identity. Proc.Natl.Acad.Sci.U.S.A, 103, 2422-2427.

Connor, K. M., Sutherland, S. M., Tupler, L. A., Malik, M. L. & Davidson, J. R. (1999) Fluoxetine in post-traumatic stress disorder. Randomised, double-blind study. Br.J Psychiatry, 175, 17-22.

Connor, K. M., Hidalgo, R. B., Crockett, B., Malik, M., Katz, R. J. & Davidson, J. R. (2001) Predictors of treatment response in patients with posttraumatic stress disorder. Prog.Neuropsychopharmacol.Biol.Psychiatry, 25, 337-345.

Connor, T. J., Song, C., Leonard, B. E., Merali, Z. & Anisman, H. (1998) An assessment of the effects of central interleukin-1beta, -2, -6, and tumor necrosis factor-alpha administration on some behavioural, neurochemical, endocrine and immune parameters in the rat. Neuroscience, 84, 923-933.

Conti, L. H. (2012) Interactions between corticotropin-releasing factor and the serotonin 1A receptor system on acoustic startle amplitude and prepulse inhibition of the startle response in two rat strains. Neuropharmacology, 62, 256-263.

Cook, S. C. & Wellman, C. L. (2004) Chronic stress alters dendritic morphology in rat medial prefrontal cortex. J Neurobiol., 60, 236-248.

Cordero, M. I., Venero, C., Kruyt, N. D. & Sandi, C. (2003) Prior exposure to a single stress session facilitates subsequent contextual fear conditioning in rats. Evidence for a role of corticosterone. Horm.Behav., 44, 338-345.

Cordes, K. R. & Srivastava, D. (2009) MicroRNA regulation of cardiovascular development. Circ.Res, 104, 724-732.

Cornelis, M. C., Nugent, N. R., Amstadter, A. B. & Koenen, K. C. (2010) Genetics of post-traumatic stress disorder: review and recommendations for genome-wide association studies. Curr.Psychiatry Rep., 12, 313-326.

Corney, D. C., Flesken-Nikitin, A., Godwin, A. K., Wang, W. & Nikitin, A. Y. (2007) MicroRNA-34b and MicroRNA-34c are targets of p53 and cooperate in control of cell proliferation and adhesion-independent growth. Cancer Res, 67, 8433-8438.

Correale, J. & Bassani Molinas, M. L. (2003) Temporal variations of adhesion molecules and matrix metalloproteinases in the course of MS. J Neuroimmunol., 140, 198-209.

Costello, E. J., Pine, D. S., Hammen, C., March, J. S., Plotsky, P. M., Weissman, M. M., Biederman, J., Goldsmith, H. H., Kaufman, J., Lewinsohn, P. M., Hellander, M., Hoagwood, K., Koretz, D. S., Nelson, C. A. & Leckman, J. F. (2002) Development and natural history of mood disorders. Biol.Psychiatry, 52, 529-542.

Cowen, P. J. (2002) Cortisol, serotonin and depression: all stressed out? Br.J.Psychiatry, 180, 99-100.

Cox, J. & Westbrook, R. F. (1994) The NMDA receptor antagonist MK-801 blocks acquisition and extinction of conditioned hypoalgesic responses in the rat. Q.J Exp.Psychol.B, 47, 187-210.

Crane, G. E. (1959) Cyloserine as an antidepressant agent. Am J Psychiatry, 115, 1025-1026.

Crane, G. E. (1961). The psychotropic effects of cycloserine: a new use for an antibiotic. Comprehensive Psychiatry, 2, 51–59.

Crawley, J. & Goodwin, F. K. (1980) Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacol.Biochem.Behav., 13, 167-170.

Creamer, M., Burgess, P. & McFarlane, A. C. (2001) Post-traumatic stress disorder: findings from the Australian National Survey of Mental Health and Well-being. Psychol.Med., 31, 1237-1247.

Crews, D. (2011) Epigenetic modifications of brain and behavior: theory and practice. Horm.Behav., 59, 393-398.

Cribbs, D. H., Berchtold, N. C., Perreau, V., Coleman, P. D., Rogers, J., Tenner, A. J. & Cotman, C. W. (2012) Extensive innate immune gene activation accompanies brain aging, increasing vulnerability to cognitive decline and neurodegeneration: a microarray study. J Neuroinflammation., 9, 179.

Critchley, H. D., Wiens, S., Rotshtein, P., Ohman, A. & Dolan, R. J. (2004) Neural systems supporting interoceptive awareness. Nat.Neurosci., 7, 189-195.

Crujeiras, A. B., Campion, J., az-Lagares, A., Milagro, F. I., Goyenechea, E., Abete, I., Casanueva, F. F. & Martinez, J. A. (2013) Association of weight regain with specific methylation levels in the NPY and POMC promoters in leukocytes of obese men: A translational study. Regul.Pept., 186C, 1-6.

Crusio, W. E. (2001) Genetic dissection of mouse exploratory behaviour. Behav.Brain Res, 125, 127-132.

Cryan, J. F. & Mombereau, C. (2004) In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice. Mol.Psychiatry, 9, 326-357.

Cryan, J. F. & Sweeney, F. F. (2011) The age of anxiety: role of animal models of anxiolytic action in drug discovery. Br.J Pharmacol., 164, 1129-1161.

Cyster, J. G., Ansel, K. M., Reif, K., Ekland, E. H., Hyman, P. L., Tang, H. L., Luther, S. A. & Ngo, V. N. (2000) Follicular stromal cells and lymphocyte homing to follicles. Immunol.Rev., 176, 181-193.

D'Aquila, P. S., Brain, P. & Willner, P. (1994) Effects of chronic mild stress on performance in behavioural tests relevant to anxiety and depression. Physiol Behav., 56, 861-867.

D'Hooge, R. & De Deyn, P. P. (2001) Applications of the Morris water maze in the study of learning and memory. Brain Res Brain Res Rev., 36, 60-90.

Dalton, T., Pazdernik, T. L., Wagner, J., Samson, F. & Andrews, G. K. (1995) Temporalspatial patterns of expression of metallothionein-I and -III and other stress related genes in rat brain after kainic acid-induced seizures. Neurochem.Int., 27, 59-71.

Daniels, J. K., McFarlane, A. C., Bluhm, R. L., Moores, K. A., Clark, C. R., Shaw, M. E., Williamson, P. C., Densmore, M. & Lanius, R. A. (2010) Switching between executive and default mode networks in posttraumatic stress disorder: alterations in functional connectivity. J Psychiatry Neurosci., 35, 258-266.

Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W. & Kelley, K. W. (2008) From inflammation to sickness and depression: when the immune system subjugates the brain. Nat.Rev.Neurosci., 9, 46-56.

Dantzer, R. (2009) Cytokine, sickness behavior, and depression. Immunol.Allergy Clin.North Am, 29, 247-264.

Datla, S. R. & Griendling, K. K. (2010) Reactive oxygen species, NADPH oxidases, and hypertension. Hypertension, 56, 325-330.

Davidson, J., Kudler, H., Smith, R., Mahorney, S. L., Lipper, S., Hammett, E., Saunders, W. B. & Cavenar, J. O., Jr. (1990) Treatment of posttraumatic stress disorder with amitriptyline and placebo. Arch.Gen.Psychiatry, 47, 259-266.

Davidson, J., Pearlstein, T., Londborg, P., Brady, K. T., Rothbaum, B., Bell, J., Maddock, R., Hegel, M. T. & Farfel, G. (2001) Efficacy of sertraline in preventing relapse of posttraumatic stress disorder: results of a 28-week double-blind, placebo-controlled study. Am J Psychiatry, 158, 1974-1981.

Davidson, J., Baldwin, D., Stein, D. J., Kuper, E., Benattia, I., Ahmed, S., Pedersen, R. & Musgnung, J. (2006) Treatment of posttraumatic stress disorder with venlafaxine extended release: a 6-month randomized controlled trial. Arch.Gen.Psychiatry, 63, 1158-1165.

Davidson, J., Rothbaum, B. O., Tucker, P., Asnis, G., Benattia, I. & Musgnung, J. J. (2006) Venlafaxine extended release in posttraumatic stress disorder: a sertraline- and placebo-controlled study. J Clin.Psychopharmacol., 26, 259-267.

Davidson, J. R., Brady, K., Mellman, T. A., Stein, M. B. & Pollack, M. H. (2007) The efficacy and tolerability of tiagabine in adult patients with post-traumatic stress disorder. J Clin.Psychopharmacol., 27, 85-88.

Davis, A. P., Murphy, C. G., Johnson, R., Lay, J. M., Lennon-Hopkins, K., Saraceni-Richards, C., Sciaky, D., King, B. L., Rosenstein, M. C., Wiegers, T. C. & Mattingly, C. J. (2013) The Comparative Toxicogenomics Database: update 2013. Nucleic Acids Res, 41, D1104-D1114.

Davis, H. P. & Squire, L. R. (1984) Protein synthesis and memory: a review. Psychol.Bull., 96, 518-559.

Davis, L. L., Suris, A., Lambert, M. T., Heimberg, C. & Petty, F. (1997) Post-traumatic stress disorder and serotonin: new directions for research and treatment. J Psychiatry Neurosci., 22, 318-326.

Davis, L. L., Suris, A., Lambert, M. T., Heimberg, C. & Petty, F. (1997) Post-traumatic stress disorder and serotonin: new directions for research and treatment. J Psychiatry Neurosci., 22, 318-326.

Davis, L. L., Jewell, M. E., Ambrose, S., Farley, J., English, B., Bartolucci, A. & Petty, F. (2004) A placebo-controlled study of nefazodone for the treatment of chronic posttraumatic stress disorder: a preliminary study. J Clin.Psychopharmacol., 24, 291-297.

Davis, L. L., Davidson, J. R., Ward, L. C., Bartolucci, A., Bowden, C. L. & Petty, F. (2008) Divalproex in the treatment of posttraumatic stress disorder: a randomized, double-blind, placebo-controlled trial in a veteran population. J Clin.Psychopharmacol., 28, 84-88.

Davis, M., Gendelman, D. S., Tischler, M. D. & Gendelman, P. M. (1982) A primary acoustic startle circuit: lesion and stimulation studies. J Neurosci., 2, 791-805.

Davis, M. (1992) The role of the amygdala in fear and anxiety. Annu.Rev.Neurosci., 15, 353-375.

Davis, M., Walker, D. L. & Lee, Y. (1997) Amygdala and bed nucleus of the stria terminalis: differential roles in fear and anxiety measured with the acoustic startle reflex. Philos.Trans.R.Soc.Lond B Biol.Sci., 352, 1675-1687.

Davis, M. (1998) Are different parts of the extended amygdala involved in fear versus anxiety? Biol.Psychiatry, 44, 1239-1247.

Davis, T. & Vaisvila, R. (2011) High sensitivity 5-hydroxymethylcytosine detection in Balb/C brain tissue. J Vis.Exp..

Davisson, R. L., Oliverio, M. I., Coffman, T. M. & Sigmund, C. D. (2000) Divergent functions of angiotensin II receptor isoforms in the brain. J Clin.Invest, 106, 103-106.

Day, J. J. & Sweatt, J. D. (2010) DNA methylation and memory formation. Nat.Neurosci., 13, 1319-1323.

de Kleine, R. A., Hendriks, G. J., Kusters, W. J., Broekman, T. G. & van, M. A. (2012) A randomized placebo-controlled trial of D-cycloserine to enhance exposure therapy for posttraumatic stress disorder. Biol.Psychiatry, 71, 962-968.

de Kloet, C. S., Vermetten, E., Geuze, E., Kavelaars, A., Heijnen, C. J. & Westenberg, H. G. (2006) Assessment of HPA-axis function in posttraumatic stress disorder: pharmacological and non-pharmacological challenge tests, a review. J Psychiatr.Res, 40, 550-567.

de Kloet, E. R., Fitzsimons, C. P., Datson, N. A., Meijer, O. C. & Vreugdenhil, E. (2009) Glucocorticoid signaling and stress-related limbic susceptibility pathway: About receptors, transcription machinery and microRNA. Brain Research, 1293, 129-141.

De la, F. M., Bernaez, I., Del, R. M. & Hernanz, A. (1993) Stimulation of murine peritoneal macrophage functions by neuropeptide Y and peptide YY. Involvement of protein kinase C. Immunology, 80, 259-265.

De la, F. M., Del, R. M. & Medina, S. (2001) Changes with aging in the modulation by neuropeptide Y of murine peritoneal macrophage functions. J Neuroimmunol., 116, 156-167.

de Souza Caetano, K. A., de Oliveira, A. R. & Brandao, M. L. (2013) Dopamine D2 receptors modulate the expression of contextual conditioned fear: role of the ventral tegmental area and the basolateral amygdala. Behav.Pharmacol., 24, 264-274.

Dedert, E. A., Calhoun, P. S., Watkins, L. L., Sherwood, A. & Beckham, J. C. (2010) Posttraumatic stress disorder, cardiovascular, and metabolic disease: a review of the evidence. Ann.Behav.Med., 39, 61-78.

Defrin, R., Ginzburg, K., Solomon, Z., Polad, E., Bloch, M., Govezensky, M. & Schreiber, S. (2008) Quantitative testing of pain perception in subjects with PTSD--implications for the mechanism of the coexistence between PTSD and chronic pain. Pain, 138, 450-459.

Denhardt, D. T., Noda, M., O'Regan, A. W., Pavlin, D. & Berman, J. S. (2001) Osteopontin as a means to cope with environmental insults: regulation of inflammation, tissue remodeling, and cell survival. J Clin.Invest, 107, 1055-1061.

Denhardt, D. T., Giachelli, C. M. & Rittling, S. R. (2001) Role of osteopontin in cellular signaling and toxicant injury. Annu.Rev.Pharmacol.Toxicol., 41, 723-749.

Detweiler, M. B., Khachiyants, N., Detweiler, J. G., Ali, R. & Kim, K. Y. (2011) Risperidone for post-traumatic combat nightmares: a report of four cases. Consult Pharm., 26, 920-928.

Diehl, L. A., Silveira, P. P., Leite, M. C., Crema, L. M., Portella, A. K., Billodre, M. N., Nunes, E., Henriques, T. P., Fidelix-da-Silva, L. B., Heis, M. D., Goncalves, C. A., Quillfeldt, J. A. & Dalmaz, C. (2007) Long lasting sex-specific effects upon behavior and S100b levels after maternal separation and exposure to a model of post-traumatic stress disorder in rats. Brain Res, 1144, 107-116.

Diehl, L. A., Alvares, L. O., Noschang, C., Engelke, D., Andreazza, A. C., Goncalves, C. A., Quillfeldt, J. A. & Dalmaz, C. (2012) Long-lasting effects of maternal separation on an animal model of post-traumatic stress disorder: effects on memory and hippocampal oxidative stress. Neurochem.Res, 37, 700-707.

Dielenberg, R. A. & McGregor, I. S. (2001) Defensive behavior in rats towards predatory odors: a review. Neurosci.Biobehav.Rev., 25, 597-609.

Dietrich, J. W., Landgrafe, G. & Fotiadou, E. H. (2012) TSH and Thyrotropic Agonists: Key Actors in Thyroid Homeostasis. J Thyroid Res, 2012, 351864.

Dimitrijevic, M., Stanojevic, S., Vujic, V., Kovacevic-Jovanovic, V., Beck-Sickinger, A., Demuth, H. & von, H. S. (2002) Effect of neuropeptide Y on inflammatory paw edema in the rat: involvement of peripheral NPY Y1 and Y5 receptors and interaction with dipeptidyl-peptidase IV (CD26). J Neuroimmunol., 129, 35-42.

Dinarello, C. A. (1998) Interleukin-1, interleukin-1 receptors and interleukin-1 receptor antagonist. Int.Rev.Immunol., 16, 457-499.

Donato, R. (2001) S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. Int.J Biochem.Cell Biol., 33, 637-668.

Donato, R. (2003) Intracellular and extracellular roles of S100 proteins. Microsc.Res Tech., 60, 540-551.

Donner, J., Pirkola, S., Silander, K., Kananen, L., Terwilliger, J. D., Lonnqvist, J., Peltonen, L. & Hovatta, I. (2008) An association analysis of murine anxiety genes in humans implicates novel candidate genes for anxiety disorders. Biol.Psychiatry, 64, 672-680.

Donner, J., Pirkola, S., Silander, K., Kananen, L., Terwilliger, J. D., Lonnqvist, J., Peltonen, L. & Hovatta, I. (2008) An association analysis of murine anxiety genes in humans implicates novel candidate genes for anxiety disorders. Biol.Psychiatry, 64, 672-680.

Donohue, H. S., Gabbott, P. L., Davies, H. A., Rodriguez, J. J., Cordero, M. I., Sandi, C., Medvedev, N. I., Popov, V. I., Colyer, F. M., Peddie, C. J. & Stewart, M. G. (2006) Chronic restraint stress induces changes in synapse morphology in stratum lacunosum-moleculare CA1 rat hippocampus: a stereological and three-dimensional ultrastructural study. Neuroscience, 140, 597-606.

Dow, B. & Kline, N. (1997) Antidepressant treatment of posttraumatic stress disorder and major depression in veterans. Ann.Clin.Psychiatry, 9, 1-5.

Dragan, W. L. & Oniszczenko, W. (2009) The association between dopamine D4 receptor exon III polymorphism and intensity of PTSD symptoms among flood survivors. Anxiety Stress.Coping., 22, 483-495.

Drai, D. & Golani, I. (2001) SEE: a tool for the visualization and analysis of rodent exploratory behavior. Neurosci.Biobehav.Rev., 25, 409-426.

Drury, S. S., Theall, K. P., Keats, B. J. & Scheeringa, M. (2009) The role of the dopamine transporter (DAT) in the development of PTSD in preschool children. J Trauma Stress., 22, 534-539.

Drury, S. S., Brett, Z. H., Henry, C. & Scheeringa, M. (2013) The association of a novel haplotype in the dopamine transporter with preschool age posttraumatic stress disorder. J Child Adolesc.Psychopharmacol., 23, 236-243.

Duguid, J. R., Bohmont, C. W., Liu, N. G. & Tourtellotte, W. W. (1989) Changes in brain gene expression shared by scrapie and Alzheimer disease. Proc.Natl.Acad.Sci.U.S.A, 86, 7260-7264.

Dunn, A. J. (2006) Effects of cytokines and infections on brain neurochemistry. Clin.Neurosci.Res, 6, 52-68.

Dunn, G. P., Koebel, C. M. & Schreiber, R. D. (2006) Interferons, immunity and cancer immunoediting. Nat.Rev.Immunol., 6, 836-848.

Duursma, A. M., Kedde, M., Schrier, M., Le Sage, C. & Agami, R. (2008) miR-148 targets human DNMT3b protein coding region. Rna-A Publication of the Rna Society, 14, 872-877.

Dzwonek, J., Rylski, M. & Kaczmarek, L. (2004) Matrix metalloproteinases and their endogenous inhibitors in neuronal physiology of the adult brain. FEBS Lett., 567, 129-135.

Easow, G., Teleman, A. A. & Cohen, S. M. (2007) Isolation of microRNA targets by miRNP immunopurification. RNA., 13, 1198-1204.

Egede, L. E. & Dismuke, C. E. (2012) Serious psychological distress and diabetes: a review of the literature. Curr.Psychiatry Rep., 14, 15-22.

Ehrlich, I., Humeau, Y., Grenier, F., Ciocchi, S., Herry, C. & Luthi, A. (2009) Amygdala inhibitory circuits and the control of fear memory. Neuron, 62, 757-771.

Ehrlich, M., Gama-Sosa, M. A., Huang, L. H., Midgett, R. M., Kuo, K. C., McCune, R. A. & Gehrke, C. (1982) Amount and distribution of 5-methylcytosine in human DNA from different types of tissues of cells. Nucleic Acids Res, 10, 2709-2721.

Elenkov, I. J., Kvetnansky, R., Hashiramoto, A., Bakalov, V. K., Link, A. A., Zachman, K., Crane, M., Jezova, D., Rovensky, J., Dimitrov, M. A., Gold, P. W., Bonini, S., Fleisher, T., Chrousos, G. P. & Wilder, R. L. (2008) Low-versus high-baseline epinephrine output shapes opposite innate cytokine profiles: presence of Lewis- and Fischer-like neurohormonal immune phenotypes in humans? J Immunol., 181, 1737-1745.

Ellison, J. A., Velier, J. J., Spera, P., Jonak, Z. L., Wang, X., Barone, F. C. & Feuerstein, G. Z. (1998) Osteopontin and its integrin receptor alpha(v)beta3 are upregulated during formation of the glial scar after focal stroke. Stroke, 29, 1698-1706.

Elzinga, B. M. & Bremner, J. D. (2002) Are the neural substrates of memory the final common pathway in posttraumatic stress disorder (PTSD)? J Affect.Disord., 70, 1-17.

Emdad, R., Bonekamp, D., Sondergaard, H. P., Bjorklund, T., Agartz, I., Ingvar, M. & Theorell, T. (2006) Morphometric and psychometric comparisons between non-substance-abusing patients with posttraumatic stress disorder and normal controls. Psychother.Psychosom., 75, 122-132.

Engelhardt, B. (2010) T cell migration into the central nervous system during health and disease: Different molecular keys allow access to different central nervous system compartments. Clin. Exp. Neuroimmunol, 1, 79–93.

Epstein, O. I. (2003) Regulatory activity of ultralow doses. Bull.Exp.Biol.Med., 135 Suppl 7, 8-13.

Epstein, O. I., Pavlov, I. F. & Shtark, M. B. (2006) Improvement of Memory by Means of Ultra-Low Doses of Antibodies to S-100B Antigen. Evid.Based.Complement Alternat.Med., 3, 541-545.

Esler, M., Alvarenga, M., Lambert, G., Kaye, D., Hastings, J., Jennings, G., Morris, M., Schwarz, R. & Richards, J. (2004) Cardiac sympathetic nerve biology and brain monoamine turnover in panic disorder. Ann.N.Y.Acad.Sci., 1018, 505-514.

Espinosa-Parrilla, Y. & Muiños-Gimeno, M. MicroRNA-Mediated Regulation and the Genetic Susceptibility to Anxiety Disorders. Anxiety Disorders, Prof Vladimir Kalinin (Ed) ISBN: 978-953-307-592-1,[InTech], 281-306. 2011.

Fainardi, E., Castellazzi, M., Bellini, T., Manfrinato, M. C., Baldi, E., Casetta, I., Paolino, E., Granieri, E. & Dallocchio, F. (2006) Cerebrospinal fluid and serum levels and intrathecal production of active matrix metalloproteinase-9 (MMP-9) as markers of disease activity in patients with multiple sclerosis. Mult.Scler., 12, 294-301.

Falls, W. A., Miserendino, M. J. & Davis, M. (1992) Extinction of fear-potentiated startle: blockade by infusion of an NMDA antagonist into the amygdala. J Neurosci., 12, 854-863.

Fang, Y., Shi, C., Manduchi, E., Civelek, M. & Davies, P. F. (2010) MicroRNA-10a regulation of proinflammatory phenotype in athero-susceptible endothelium in vivo and in vitro. Proc.Natl.Acad.Sci.U.S.A , 107, 13450-13455.

Fang, Z., Forslund, N., Takenaga, K., Lukanidin, E. & Kozlova, E. N. (2006) Sensory neurite outgrowth on white matter astrocytes is influenced by intracellular and extracellular S100A4 protein. J Neurosci.Res, 83, 619-626.

Fanselow, M. S. & LeDoux, J. E. (1999) Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. Neuron, 23, 229-232.

Fassbender, K., Walter, S., Kuhl, S., Landmann, R., Ishii, K., Bertsch, T., Stalder, A. K., Muehlhauser, F., Liu, Y., Ulmer, A. J., Rivest, S., Lentschat, A., Gulbins, E., Jucker, M., Staufenbiel, M., Brechtel, K., Walter, J., Multhaup, G., Penke, B., Adachi, Y., Hartmann, T. & Beyreuther, K. (2004) The LPS receptor (CD14) links innate immunity with Alzheimer's disease. FASEB J, 18, 203-205.

Felmingham, K. L., Dobson-Stone, C., Schofield, P. R., Quirk, G. J. & Bryant, R. A. (2013) The brain-derived neurotrophic factor Val66Met polymorphism predicts response to exposure therapy in posttraumatic stress disorder. Biol.Psychiatry, 73, 1059-1063.

Felten, D. L., Felten, S. Y., Carlson, S. L., Olschowka, J. A. & Livnat, S. (1985) Noradrenergic and peptidergic innervation of lymphoid tissue. J Immunol., 135, 755s-765s.

Felten, S. Y. & Olschowka, J. (1987) Noradrenergic sympathetic innervation of the spleen: II. Tyrosine hydroxylase (TH)-positive nerve terminals form synapticlike contacts on lymphocytes in the splenic white pulp. J Neurosci.Res, 18, 37-48.

Feng, J., Fouse, S. & Fan, G. (2007) Epigenetic regulation of neural gene expression and neuronal function. Pediatr.Res, 61, 58R-63R.

Feng, J., Zhou, Y., Campbell, S. L., Le, T., Li, E., Sweatt, J. D., Silva, A. J. & Fan, G. (2010) Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons. Nat.Neurosci., 13, 423-430.

Fetzner, M. G., Abrams, M. P. & Asmundson, G. J. (2013) Symptoms of posttraumatic stress disorder and depression in relation to alcohol-use and alcohol-related problems among Canadian forces veterans. Can.J Psychiatry, 58, 417-425.

Feusner, J., Ritchie, T., Lawford, B., Young, R. M., Kann, B. & Noble, E. P. (2001) GABA(A) receptor beta 3 subunit gene and psychiatric morbidity in a post-traumatic stress disorder population. Psychiatry Res, 104, 109-117.

File, S. E. (2000) NKP608, an NK1 receptor antagonist, has an anxiolytic action in the social interaction test in rats. Psychopharmacology (Berl), 152, 105-109.

File, S. E. (2001) Factors controlling measures of anxiety and responses to novelty in the mouse. Behav.Brain Res, 125, 151-157.

File, S. E. & Seth, P. (2003) A review of 25 years of the social interaction test. Eur.J Pharmacol., 463, 35-53.

Finehout, E. J., Franck, Z. & Lee, K. H. (2005) Complement protein isoforms in CSF as possible biomarkers for neurodegenerative disease. Dis.Markers, 21, 93-101.

Finkbeiner, S., Tavazoie, S. F., Maloratsky, A., Jacobs, K. M., Harris, K. M. & Greenberg, M. E. (1997) CREB: a major mediator of neuronal neurotrophin responses. Neuron, 19, 1031-1047.

Fiore, R. & Schratt, G. (2007) MicroRNAs in vertebrate synapse development. ScientificWorldJournal., 7, 167-177.

Fitzsimons, C. P., van Hooijdonk, L. W., Schouten, M., Zalachoras, I., Brinks, V., Zheng, T., Schouten, T. G., Saaltink, D. J., Dijkmans, T., Steindler, D. A., Verhaagen, J., Verbeek, F. J., Lucassen, P. J., de Kloet, E. R., Meijer, O. C., Karst, H., Joels, M., Oitzl, M. S. & Vreugdenhil, E. (2012) Knockdown of the glucocorticoid receptor alters functional integration of newborn neurons in the adult hippocampus and impairs fear-motivated behavior. Mol.Psychiatry.

Flood, J. F., Hernandez, E. N. & Morley, J. E. (1987) Modulation of memory processing by neuropeptide Y. Brain Res, 421, 280-290.

Flood, J. F., Baker, M. L., Hernandez, E. N. & Morley, J. E. (1989) Modulation of memory processing by neuropeptide Y varies with brain injection site. Brain Res, 503, 73-82.

Foa, E. B. & Kozak, M. J. (1986) Emotional processing of fear: exposure to corrective information. Psychol.Bull., 99, 20-35.

Foa, E. B., Steketee, G. & Rothbaum, B. O. (1989) Behavioural Cognitive Conceptualizations of Post-Traumatic Stress Disorder. Behavior Therapy, 20, 155-176.

Foa, E. B. & Tolin, D. F. (2000) Comparison of the PTSD Symptom Scale-Interview Version and the Clinician-Administered PTSD scale. J Trauma Stress., 13, 181-191.

Foell, D., Wittkowski, H., Vogl, T. & Roth, J. (2007) S100 proteins expressed in phagocytes: a novel group of damage-associated molecular pattern molecules. J Leukoc.Biol., 81, 28-37.

Fonseca, H., Ireland, M. & Resnick, M. D. (2002) Familial correlates of extreme weight control behaviors among adolescents. Int.J Eat.Disord., 32, 441-448.

Fontan-Lozano, A., Saez-Cassanelli, J. L., Inda, M. C., de, l. S.-A., Sierra-Dominguez, S. A., Lopez-Lluch, G., gado-Garcia, J. M. & Carrion, A. M. (2007) Caloric restriction increases learning consolidation and facilitates synaptic plasticity through mechanisms dependent on NR2B subunits of the NMDA receptor. J Neurosci., 27, 10185-10195.

Fontanez-Nuin, D. E., Santini, E., Quirk, G. J. & Porter, J. T. (2011) Memory for fear extinction requires mGluR5-mediated activation of infralimbic neurons. Cereb.Cortex, 21, 727-735.

Frank, J. B., Kosten, T. R., Giller, E. L., Jr. & Dan, E. (1988) A randomized clinical trial of phenelzine and imipramine for posttraumatic stress disorder. Am J Psychiatry, 145, 1289-1291.

Frank, M. M. & Fries, L. F. (1991) The role of complement in inflammation and phagocytosis. Immunol.Today, 12, 322-326.

Franklin, T. B., Russig, H., Weiss, I. C., Graff, J., Linder, N., Michalon, A., Vizi, S. & Mansuy, I. M. (2010) Epigenetic transmission of the impact of early stress across generations. Biol.Psychiatry , 68, 408-415.

Franklin, T. B., Russig, H., Weiss, I. C., Graff, J., Linder, N., Michalon, A., Vizi, S. & Mansuy, I. M. (2010) Epigenetic transmission of the impact of early stress across generations. Biol.Psychiatry , 68, 408-415.

Fransson, E. I., Batty, G. D., Tabak, A. G., Brunner, E. J., Kumari, M., Shipley, M. J., Singh-Manoux, A. & Kivimaki, M. (2010) Association between change in body composition and change in inflammatory markers: an 11-year follow-up in the Whitehall II Study. J Clin.Endocrinol.Metab, 95, 5370-5374.

Fraser, G. A. (2009) The use of a synthetic cannabinoid in the management of treatment-resistant nightmares in posttraumatic stress disorder (PTSD). CNS.Neurosci.Ther., 15, 84-88.

Fredrikson, M., Hugdahl, K. & Ohman, A. (1976) Electrodermal conditioning to potentially phobic stimuli in male and female subjects. Biol.Psychol., 4, 305-314.

Fredrikson M., Matthews K.A. (1990) Cardiovascular responses to behavioural stress and hypertension: a meta-analytic review. Ann Behav Med., 12,30–9.

Freeman, T., Roca, V., Guggenheim, F., Kimbrell, T. & Griffin, W. S. (2005) Neuropsychiatric associations of apolipoprotein E alleles in subjects with combat-related posttraumatic stress disorder. J Neuropsychiatry Clin.Neurosci., 17, 541-543.

Friedman, M. J. (1997) Posttraumatic stress disorder. J.Clin.Psychiatry, 58 Suppl 9, 33-36.

Fuks, F., Burgers, W. A., Brehm, A., Hughes-Davies, L. & Kouzarides, T. (2000) DNA methyltransferase Dnmt1 associates with histone deacetylase activity. Nat.Genet., 24, 88-91.

Gabriele, A. & Packard, M. G. (2007) D-Cycloserine enhances memory consolidation of hippocampus-dependent latent extinction. Learn.Mem., 14, 468-471.

Ganon-Elazar, E. & Akirav, I. (2012) Cannabinoids prevent the development of behavioural and endocrine alterations in a rat model of intense stress. Neuropsychopharmacology, 37, 456-466.

Gantner, B. N., Simmons, R. M., Canavera, S. J., Akira, S. & Underhill, D. M. (2003) Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. J Exp.Med., 197, 1107-1117.

Gao, Y., Bezchlibnyk, Y. B., Sun, X., Wang, J. F., McEwen, B. S. & Young, L. T. (2006) Effects of restraint stress on the expression of proteins involved in synaptic vesicle exocytosis in the hippocampus. Neuroscience, 141, 1139-1148.

Garcia, R. (2001) Stress, hippocampal plasticity, and spatial learning. Synapse, 40, 180-183.

Gard, P. R. (2004) Angiotensin as a target for the treatment of Alzheimer's disease, anxiety and depression. Expert.Opin.Ther.Targets., 8, 7-14.

Gardoni, F., Mauceri, D., Malinverno, M., Polli, F., Costa, C., Tozzi, A., Siliquini, S., Picconi, B., Cattabeni, F., Calabresi, P. & Di, L. M. (2009) Decreased NR2B subunit synaptic levels cause impaired long-term potentiation but not long-term depression. J Neurosci., 29, 669-677.

Garrido, A. M. & Griendling, K. K. (2009) NADPH oxidases and angiotensin II receptor signaling. Mol.Cell Endocrinol., 302, 148-158.

Gary, K. A., Sevarino, K. A., Yarbrough, G. G., Prange, A. J., Jr. & Winokur, A. (2003) The thyrotropin-releasing hormone (TRH) hypothesis of homeostatic regulation: implications for TRH-based therapeutics. J Pharmacol.Exp.Ther., 305, 410-416.

Gasque, P., Dean, Y. D., McGreal, E. P., VanBeek, J. & Morgan, B. P. (2000) Complement components of the innate immune system in health and disease in the CNS. Immunopharmacology, 49, 171-186.

Gebhardt, C., Nemeth, J., Angel, P. & Hess, J. (2006) S100A8 and S100A9 in inflammation and cancer. Biochem.Pharmacol., 72, 1622-1631.

Gelernter, J., Southwick, S., Goodson, S., Morgan, A., Nagy, L. & Charney, D. S. (1999) No association between D2 dopamine receptor (DRD2) "A" system alleles, or DRD2 haplotypes, and posttraumatic stress disorder. Biol.Psychiatry, 45, 620-625.

Georgiades A., Fredrikson M., Hyperreactivity (cardiovascular) (2000). In: Fink G, editor. Encyclopedia of stress. Vol. II. San Diego: Academic Press; 421–5.

Geracioti, T. D., Jr., Baker, D. G., Ekhator, N. N., West, S. A., Hill, K. K., Bruce, A. B., Schmidt, D., Rounds-Kugler, B., Yehuda, R., Keck, P. E., Jr. & Kasckow, J. W. (2001) CSF norepinephrine concentrations in posttraumatic stress disorder. Am J Psychiatry, 158, 1227-1230.

Geracioti, T. D., Jr., Carpenter, L. L., Owens, M. J., Baker, D. G., Ekhator, N. N., Horn, P. S., Strawn, J. R., Sanacora, G., Kinkead, B., Price, L. H. & Nemeroff, C. B. (2006) Elevated cerebrospinal fluid substance p concentrations in posttraumatic stress disorder and major depression. Am J Psychiatry, 163, 637-643.

Geracioti, T. D., Jr., Jefferson-Wilson, L., Strawn, J. R., Baker, D. G., Dashevsky, B. A., Horn, P. S. & Ekhator, N. N. (2013) Effect of traumatic imagery on cerebrospinal fluid dopamine and serotonin metabolites in posttraumatic stress disorder. J Psychiatr.Res, 47, 995-998.

Gerlai, R. (2001) Behavioural tests of hippocampal function: simple paradigms complex problems. Behav.Brain Res, 125, 269-277.

Gianaros, P. J. & Manuck, S. B. (2010) Neurobiological pathways linking socioeconomic position and health. Psychosom.Med. , 72, 450-461.

Gibbs, J. R., van der Brug, M. P., Hernandez, D. G., Traynor, B. J., Nalls, M. A., Lai, S. L., Arepalli, S., Dillman, A., Rafferty, I. P., Troncoso, J., Johnson, R., Zielke, H. R., Ferrucci, L., Longo, D. L., Cookson, M. R. & Singleton, A. B.

(2010) Abundant quantitative trait loci exist for DNA methylation and gene expression in human brain. PLoS.Genet., 6, e1000952.

Gijbels, K., Proost, P., Masure, S., Carton, H., Billiau, A. & Opdenakker, G. (1993) Gelatinase B is present in the cerebrospinal fluid during experimental autoimmune encephalomyelitis and cleaves myelin basic protein. J Neurosci.Res, 36, 432-440.

Gilbertson, M. W., Paulus, L. A., Williston, S. K., Gurvits, T. V., Lasko, N. B., Pitman, R. K. & Orr, S. P. (2006) Neurocognitive function in monozygotic twins discordant for combat exposure: relationship to posttraumatic stress disorder. J Abnorm.Psychol., 115, 484-495.

Gill, J. M., Saligan, L., Woods, S. & Page, G. (2009) PTSD is associated with an excess of inflammatory immune activities. Perspect.Psychiatr.Care, 45, 262-277.

Giralt, M., Penkowa, M., Lago, N., Molinero, A. & Hidalgo, J. (2002) Metallothionein-1+2 protect the CNS after a focal brain injury. Exp.Neurol., 173, 114-128.

Git, A., Dvinge, H., Salmon-Divon, M., Osborne, M., Kutter, C., Hadfield, J., Bertone, P. & Caldas, C. (2010) Systematic comparison of microarray profiling, real-time PCR, and next-generation sequencing technologies for measuring differential microRNA expression. RNA, 16, 991-1006.

Givalois, L., Marmigere, F., Rage, F., Ixart, G., Arancibia, S. & Tapia-Arancibia, L. (2001) Immobilization stress rapidly and differentially modulates BDNF and TrkB mRNA expression in the pituitary gland of adult male rats. Neuroendocrinology, 74, 148-159.

Glass, C. K., Saijo, K., Winner, B., Marchetto, M. C. & Gage, F. H. (2010) Mechanisms underlying inflammation in neurodegeneration. Cell, 140, 918-934.

Glatt, S. J., Tylee, D. S., Chandler, S. D., Pazol, J., Nievergelt, C. M., Woelk, C. H., Baker, D. G., Lohr, J. B., Kremen, W. S., Litz, B. T. & Tsuang, M. T. (2013) Blood-based gene-expression predictors of PTSD risk and resilience among deployed marines: a pilot study. Am J Med.Genet.B Neuropsychiatr.Genet., 162B, 313-326.

Glatz, K., Mossner, R., Heils, A. & Lesch, K. P. (2003) Glucocorticoid-regulated human serotonin transporter (5-HTT) expression is modulated by the 5-HTT gene-promotor-linked polymorphic region. J Neurochem., 86, 1072-1078.

Goenjian, A. K., Yehuda, R., Pynoos, R. S., Steinberg, A. M., Tashjian, M., Yang, R. K., Najarian, L. M. & Fairbanks, L. A. (1996) Basal cortisol, dexamethasone suppression of cortisol, and MHPG in adolescents after the 1988 earthquake in Armenia. Am J Psychiatry, 153, 929-934.

Goenjian, A. K., Pynoos, R. S., Steinberg, A. M., Endres, D., Abraham, K., Geffner, M. E. & Fairbanks, L. A. (2003) Hypothalamic-pituitary-adrenal activity among Armenian adolescents with PTSD symptoms. J Trauma Stress., 16, 319-323.

Goldfeld, A. E., Mollica, R. F., Pesavento, B. H. & Faraone, S. V. (1988) The physical and psychological sequelae of torture. Symptomatology and diagnosis. JAMA, 259, 2725-2729.

Goldstein, G., van, K. W., Shelly, C., Miller, D. J. & van Kammen, D. P. (1987) Survivors of imprisonment in the Pacific theater during World War II. Am J Psychiatry, 144, 1210-1213.

Goldstein, L. E., Rasmusson, A. M., Bunney, B. S. & Roth, R. H. (1996) Role of the amygdala in the coordination of behavioural, neuroendocrine, and prefrontal cortical monoamine responses to psychological stress in the rat. J Neurosci., 16, 4787-4798.

Goodwin, R. D. & Davidson, J. R. (2005) Self-reported diabetes and posttraumatic stress disorder among adults in the community. Prev.Med., 40, 570-574.

Goshen, I., Kreisel, T., Ounallah-Saad, H., Renbaum, P., Zalzstein, Y., Ben-Hur, T., Levy-Lahad, E. & Yirmiya, R. (2007) A dual role for interleukin-1 in hippocampal-dependent memory processes. Psychoneuroendocrinology, 32, 1106-1115.

Goshen, I., Kreisel, T., Ben-Menachem-Zidon, O., Licht, T., Weidenfeld, J., Ben-Hur, T. & Yirmiya, R. (2008) Brain interleukin-1 mediates chronic stress-induced depression in mice via adrenocortical activation and hippocampal neurogenesis suppression. Mol.Psychiatry, 13, 717-728.

Goshen, I. & Yirmiya, R. (2009) Interleukin-1 (IL-1): a central regulator of stress responses. Front Neuroendocrinol., 30, 30-45.

Goswami, S., Samuel, S., Sierra, O. R., Cascardi, M. & Pare, D. (2012) A rat model of post-traumatic stress disorder reproduces the hippocampal deficits seen in the human syndrome. Front Behav.Neurosci., 6, 26.

Goswami, S., Rodriguez-Sierra, O., Cascardi, M. & Pare, D. (2013) Animal models of post-traumatic stress disorder: face validity. Front Neurosci., 7, 89.

Gottschalk, W. A., Jiang, H., Tartaglia, N., Feng, L., Figurov, A. & Lu, B. (1999) Signaling mechanisms mediating BDNF modulation of synaptic plasticity in the hippocampus. Learn.Mem., 6, 243-256.

Grabe, H. J., Spitzer, C., Schwahn, C., Marcinek, A., Frahnow, A., Barnow, S., Lucht, M., Freyberger, H. J., John, U., Wallaschofski, H., Volzke, H. & Rosskopf, D. (2009) Serotonin transporter gene (SLC6A4) promoter polymorphisms and the susceptibility to posttraumatic stress disorder in the general population. Am J Psychiatry, 166, 926-933.

Grace, A. A. & Rosenkranz, J. A. (2002) Regulation of conditioned responses of basolateral amygdala neurons. Physiol Behav., 77, 489-493.

Graeff, F. G., Guimaraes, F. S., De Andrade, T. G. & Deakin, J. F. (1996) Role of 5-HT in stress, anxiety, and depression. Pharmacol.Biochem.Behav., 54, 129-141.

Graeff, F. G., Netto, C. F. & Zangrossi, H., Jr. (1998) The elevated T-maze as an experimental model of anxiety. Neurosci.Biobehav.Rev., 23, 237-246.

Grafstrom, R. H., Yuan, R. & Hamilton, D. L. (1985) The characteristics of DNA methylation in an in vitro DNA synthesizing system from mouse fibroblasts. Nucleic Acids Res, 13, 2827-2842.

Graham, D. & Langer, S. Z. (1992) Advances in sodium-ion coupled biogenic amine transporters. Life Sci., 51, 631-645.

Gravallese, E. M. (2003) Osteopontin: a bridge between bone and the immune system. J Clin.Invest, 112, 147-149.

Gravius, A., Pietraszek, M., Dekundy, A. & Danysz, W. (2010) Metabotropic glutamate receptors as therapeutic targets for cognitive disorders. Curr.Top.Med.Chem., 10, 187-206.

Greber, S., Schwarzer, C. & Sperk, G. (1994) Neuropeptide Y inhibits potassium-stimulated glutamate release through Y2 receptors in rat hippocampal slices in vitro. Br.J Pharmacol., 113, 737-740.

Greicius, M. D., Krasnow, B., Boyett-Anderson, J. M., Eliez, S., Schatzberg, A. F., Reiss, A. L. & Menon, V. (2003) Regional analysis of hippocampal activation during memory encoding and retrieval: fMRI study. Hippocampus, 13, 164-174.

Greisen, M. H., Sheikh, S. P., Bolwig, T. G. & Mikkelsen, J. D. (1997) Reduction of neuropeptide Y binding sites in the rat hippocampus after electroconvulsive stimulations. Brain Res, 776, 105-110.

Griebel, G., Blanchard, D. C., Agnes, R. S. & Blanchard, R. J. (1995) Differential modulation of antipredator defensive behavior in Swiss-Webster mice following acute or chronic administration of imipramine and fluoxetine. Psychopharmacology (Berl), 120, 57-66.

Griebel, G., Simiand, J., Serradeil-Le, G. C., Wagnon, J., Pascal, M., Scatton, B., Maffrand, J. P. & Soubrie, P. (2002) Anxiolytic- and antidepressant-like effects of the non-peptide vasopressin V1b receptor antagonist, SSR149415, suggest an innovative approach for the treatment of stress-related disorders. Proc.Natl.Acad.Sci.U.S.A, 99, 6370-6375.

Grillon, C., Morgan, C. A., III, Davis, M. & Southwick, S. M. (1998) Effects of experimental context and explicit threat cues on acoustic startle in Vietnam veterans with posttraumatic stress disorder. Biol.Psychiatry, 44, 1027-1036.

Groblewski, P. A., Lattal, K. M. & Cunningham, C. L. (2009) Effects of D-cycloserine on extinction and reconditioning of ethanol-seeking behavior in mice. Alcohol Clin.Exp.Res, 33, 772-782.

Guastella, A. J., Richardson, R., Lovibond, P. F., Rapee, R. M., Gaston, J. E., Mitchell, P. & Dadds, M. R. (2008) A randomized controlled trial of D-cycloserine enhancement of exposure therapy for social anxiety disorder. Biol.Psychiatry, 63, 544-549.

guilar-Valles, A., Sanchez, E., de, G. P., Garcia-Vazquez, A. I., Ramirez-Amaya, V., Bermudez-Rattoni, F. & Joseph-Bravo, P. (2007) The expression of TRH, its receptors and degrading enzyme is differentially modulated in the rat limbic system during training in the Morris water maze. Neurochem.Int., 50, 404-417.

Gully, D., Geslin, M., Serva, L., Fontaine, E., Roger, P., Lair, C., Darre, V., Marcy, C., Rouby, P. E., Simiand, J., Guitard, J., Gout, G., Steinberg, R., Rodier, D., Griebel, G., Soubrie, P., Pascal, M., Pruss, R., Scatton, B., Maffrand, J. P. & Le, F. G. (2002) 4-(2-Chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylp henyl)ethyl]5-methyl-N-(2-propynyl)-1,3-thiazol-2-amine hydrochloride (SSR125543A): a potent and selective corticotrophin-releasing factor(1) receptor antagonist. I. Biochemical and pharmacological characterization. J Pharmacol.Exp.Ther., 301, 322-332.

Guo, J. U., Su, Y., Zhong, C., Ming, G. L. & Song, H. (2011) Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain. Cell, 145, 423-434.

Gutman, D. A., Owens, M. J., Thrivikraman, K. V. & Nemeroff, C. B. (2011) Persistent anxiolytic affects after chronic administration of the CRF(1) receptor antagonist R121919 in rats. Neuropharmacology , 60, 1135-1141.

Guzowski, J. F. (2002) Insights into immediate-early gene function in hippocampal memory consolidation using antisense oligonucleotide and fluorescent imaging approaches. Hippocampus, 12, 86-104.

Habib, K. E., Weld, K. P., Rice, K. C., Pushkas, J., Champoux, M., Listwak, S., Webster, E. L., Atkinson, A. J., Schulkin, J., Contoreggi, C., Chrousos, G. P., McCann, S. M., Suomi, S. J., Higley, J. D. & Gold, P. W. (2000) Oral administration of a corticotropin-releasing hormone receptor antagonist significantly attenuates behavioural, neuroendocrine, and autonomic responses to stress in primates. Proc.Natl.Acad.Sci.U.S.A, 97, 6079-6084.

Hageman, I., Andersen, H. S. & Jorgensen, M. B. (2001) Post-traumatic stress disorder: a review of psychobiology and pharmacotherapy. Acta Psychiatr.Scand., 104, 411-422.

Halbreich, U., Olympia, J., Carson, S., Glogowski, J., Yeh, C. M., Axelrod, S. & Desu, M. M. (1989) Hypothalamo-pituitary-adrenal activity in endogenously depressed post-traumatic stress disorder patients. Psychoneuroendocrinology, 14, 365-370.

Hall, E., Saxe, G., Stoddard, F., Kaplow, J., Koenen, K., Chawla, N., Lopez, C., King, L. & King, D. (2006) Posttraumatic stress symptoms in parents of children with acute burns. J.Pediatr.Psychol., 31, 403-412.

Hamner, M. B. & Diamond, B. I. (1993) Elevated plasma dopamine in posttraumatic stress disorder: a preliminary report. Biol.Psychiatry, 33, 304-306.

Hamner, M. B., Faldowski, R. A., Ulmer, H. G., Frueh, B. C., Huber, M. G. & Arana, G. W. (2003) Adjunctive risperidone treatment in post-traumatic stress disorder: a preliminary controlled trial of effects on comorbid psychotic symptoms. Int.Clin.Psychopharmacol., 18, 1-8.

Han, K. & Kim, E. (2008) Synaptic adhesion molecules and PSD-95. Prog.Neurobiol., 84, 263-283.

Hanisch, U. K. & Kettenmann, H. (2007) Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat.Neurosci., 10, 1387-1394.

Hansen, T., Olsen, L., Lindow, M., Jakobsen, K. D., Ullum, H., Jonsson, E., Andreassen, O. A., Djurovic, S., Melle, I., Agartz, I., Hall, H., Timm, S., Wang, A. G. & Werge, T. (2007) Brain expressed microRNAs implicated in schizophrenia etiology. PLoS One, 2, e873.

Haqq, A. M., Lien, L. F., Boan, J., Arlotto, M., Slentz, C. A., Muehlbauer, M. J., Rochon, J., Gallup, D., McMahon, R. L., Bain, J. R., Stevens, R., Millington, D., Butler, M. D., Newgard, C. B. & Svetkey, L. P. (2005) The Study of the Effects of Diet on Metabolism and Nutrition (STEDMAN) weight loss project: Rationale and design. Contemp.Clin.Trials, 26, 616-625.

Harada, K., Yamaji, T. & Matsuoka, N. (2008) Activation of the serotonin 5-HT2C receptor is involved in the enhanced anxiety in rats after single-prolonged stress. Pharmacol.Biochem.Behav., 89, 11-16.

Haramati, S., Navon, I., Issler, O., Ezra-Nevo, G., Gil, S., Zwang, R., Hornstein, E. & Chen, A. (2011) MicroRNA as repressors of stress-induced anxiety: the case of amygdalar miR-34. J.Neurosci., 31, 14191-14203.

Harris, J. A. & Westbrook, R. F. (1998) Evidence that GABA transmission mediates context-specific extinction of learned fear. Psychopharmacology (Berl), 140, 105-115.

Hascoet, M., Bourin, M. & Nic Dhonnchadha, B. A. (2001) The mouse light-dark paradigm: a review. Prog.Neuropsychopharmacol.Biol.Psychiatry, 25, 141-166.

Hashimoto, M., Sun, D., Rittling, S. R., Denhardt, D. T. & Young, W. (2007) Osteopontin-deficient mice exhibit less inflammation, greater tissue damage, and impaired locomotor recovery from spinal cord injury compared with wild-type controls. J Neurosci., 27, 3603-3611.

Hashimshony, T., Zhang, J., Keshet, I., Bustin, M. & Cedar, H. (2003) The role of DNA methylation in setting up chromatin structure during development. Nat.Genet., 34, 187-192.

Hashimshony, T., Zhang, J., Keshet, I., Bustin, M. & Cedar, H. (2003) The role of DNA methylation in setting up chromatin structure during development. Nat.Genet., 34, 187-192.

Hauer, D., Weis, F., Papassotiropoulos, A., Schmoeckel, M., Beiras-Fernandez, A., Lieke, J., Kaufmann, I., Kirchhoff, F., Vogeser, M., Roozendaal, B., Briegel, J., de, Q. D. & Schelling, G. (2011) Relationship of a common polymorphism of the glucocorticoid receptor gene to traumatic memories and posttraumatic stress disorder in patients after intensive care therapy. Crit Care Med., 39, 643-650.

He, L., He, X., Lim, L. P., de, S. E., Xuan, Z., Liang, Y., Xue, W., Zender, L., Magnus, J., Ridzon, D., Jackson, A. L., Linsley, P. S., Chen, C., Lowe, S. W., Cleary, M. A. & Hannon, G. J. (2007) A microRNA component of the p53 tumour suppressor network. Nature, 447, 1130-1134.

Hebert, S. S., Horre, K., Nicolai, L., Bergmans, B., Papadopoulou, A. S., Delacourte, A. & De, S. B. (2009) MicroRNA regulation of Alzheimer's Amyloid precursor protein expression. Neurobiol.Dis., 33, 422-428.

Heifets, L. B. & Iseman, M. D. (1991) Individualized therapy versus standard regimens in the treatment of Mycobacterium avium infections. Am Rev.Respir.Dis., 144, 1-2.

Heilig, M., Soderpalm, B., Engel, J. A. & Widerlov, E. (1989) Centrally administered neuropeptide Y (NPY) produces anxiolytic-like effects in animal anxiety models. Psychopharmacology (Berl), 98, 524-529.

Heilig, M., McLeod, S., Brot, M., Heinrichs, S. C., Menzaghi, F., Koob, G. F. & Britton, K. T. (1993) Anxiolytic-like action of neuropeptide Y: mediation by Y1 receptors in amygdala, and dissociation from food intake effects. Neuropsychopharmacology, 8, 357-363.

Heilig, M. & Widerlov, E. (1995) Neurobiology and clinical aspects of neuropeptide Y. Crit Rev.Neurobiol., 9, 115-136.

Heilig, M., Zachrisson, O., Thorsell, A., Ehnvall, A., Mottagui-Tabar, S., Sjogren, M., Asberg, M., Ekman, R., Wahlestedt, C. & Agren, H. (2004) Decreased cerebrospinal fluid neuropeptide Y (NPY) in patients with treatment refractory unipolar major depression: preliminary evidence for association with preproNPY gene polymorphism. J Psychiatr.Res, 38, 113-121.

Heils, A., Teufel, A., Petri, S., Stober, G., Riederer, P., Bengel, D. & Lesch, K. P. (1996) Allelic variation of human serotonin transporter gene expression. J Neurochem., 66, 2621-2624.

Heim, C. & Nemeroff, C. B. (2001) The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. Biol.Psychiatry, 49, 1023-1039.

Heinrichs, S. C., De Souza, E. B., Schulteis, G., Lapsansky, J. L. & Grigoriadis, D. E. (2002) Brain penetrance, receptor occupancy and antistress in vivo efficacy of a small molecule corticotropin releasing factor type I receptor selective antagonist. Neuropsychopharmacology, 27, 194-202.

Heldt, S. A. & Ressler, K. J. (2007) Training-induced changes in the expression of GABAA-associated genes in the amygdala after the acquisition and extinction of Pavlovian fear. Eur.J Neurosci., 26, 3631-3644.

Hemmings, S. M., Martin, L. I., Klopper, M., van der, M. L., Aitken, L., de, W. E., Black, G. F., Hoal, E. G., Walzl, G. & Seedat, S. (2013) BDNF Val66Met and DRD2 Taq1A polymorphisms interact to influence PTSD symptom severity: a preliminary investigation in a South African population. Prog.Neuropsychopharmacol.Biol.Psychiatry, 40, 273-280.

Henninger, G. (1995) Neurobiological and clinical consequences of stress from normal adaptation to PTSD, in Neuroimmunology of Stress, edited by Friedman, M. J., Charney, D. S. & Deutsch, A. Y. Philadelphia, PA, Lippincott-Raven, 381–401

Heresco-Levy, U., Kremer, I., Javitt, D. C., Goichman, R., Reshef, A., Blanaru, M. & Cohen, T. (2002) Pilot-controlled trial of D-cycloserine for the treatment of post-traumatic stress disorder. Int.J Neuropsychopharmacol., 5, 301-307.

Heresco-Levy, U., Gelfin, G., Bloch, B., Levin, R., Edelman, S., Javitt, D. C. & Kremer, I. (2013) A randomized add-on trial of high-dose D-cycloserine for treatment-resistant depression. Int.J Neuropsychopharmacol., 16, 501-506.

Hermos, J. A., Young, M. M., Lawler, E. V., Rosenbloom, D. & Fiore, L. D. (2007) Long-term, high-dose benzodiazepine prescriptions in veteran patients with PTSD: influence of preexisting alcoholism and drug-abuse diagnoses. J Trauma Stress., 20, 909-914.

Herry, C. & Garcia, R. (2002) Prefrontal cortex long-term potentiation, but not long-term depression, is associated with the maintenance of extinction of learned fear in mice. J Neurosci., 22, 577-583.

Herry, C. & Mons, N. (2004) Resistance to extinction is associated with impaired immediate early gene induction in medial prefrontal cortex and amygdala. Eur.J Neurosci., 20, 781-790.

Hertzberg, M. A., Butterfield, M. I., Feldman, M. E., Beckham, J. C., Sutherland, S. M., Connor, K. M. & Davidson, J. R. (1999) A preliminary study of lamotrigine for the treatment of posttraumatic stress disorder. Biol.Psychiatry, 45, 1226-1229.

Hickling, E. J., Blanchard, E. B., Silverman, D. J. & Schwarz, S. P. (1992) Motor vehicle accidents, headaches and post-traumatic stress disorder: assessment findings in a consecutive series. Headache, 32, 147-151.

Hidalgo, J., Borras, M., Garvey, J. S. & Armario, A. (1990) Liver, brain, and heart metallothionein induction by stress. J Neurochem., 55, 651-654.

Higashimoto, M., Isoyama, N., Ishibashi, S., Inoue, M., Takiguchi, M., Suzuki, S., Ohnishi, Y. & Sato, M. (2009) Tissue-dependent preventive effect of metallothionein against DNA damage in dyslipidemic mice under repeated stresses of fasting or restraint. Life Sci., 84, 569-575.

Hilakivi, I. & Leppavuori, A. (1984) Effects of methoxamine, and alpha-1 adrenoceptor agonist, and prazosin, an alpha-1 antagonist, on the stages of the sleep-waking cycle in the cat. Acta Physiol Scand., 120, 363-372.

Hill, M. N., Hunter, R. G. & McEwen, B. S. (2009) Chronic stress differentially regulates cannabinoid CB1 receptor binding in distinct hippocampal subfields. Eur.J Pharmacol., 614, 66-69.

Hirooka, Y., Kishi, T., Sakai, K., Takeshita, A. & Sunagawa, K. (2011) Imbalance of central nitric oxide and reactive oxygen species in the regulation of sympathetic activity and neural mechanisms of hypertension. Am J Physiol Regul.Integr.Comp Physiol, 300, R818-R826.

Hirschhorn, J. N. & Daly, M. J. (2005) Genome-wide association studies for common diseases and complex traits. Nat.Rev.Genet., 6, 95-108.

Hoehn-Saric, R., Schlund, M. W. & Wong, S. H. (2004) Effects of citalopram on worry and brain activation in patients with generalized anxiety disorder. Psychiatry Res, 131, 11-21.

Hofmann, M. A., Drury, S., Fu, C., Qu, W., Taguchi, A., Lu, Y., Avila, C., Kambham, N., Bierhaus, A., Nawroth, P., Neurath, M. F., Slattery, T., Beach, D., McClary, J., Nagashima, M., Morser, J., Stern, D. & Schmidt, A. M. (1999) RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. Cell, 97, 889-901.

Hofmann, S. G., Meuret, A. E., Smits, J. A., Simon, N. M., Pollack, M. H., Eisenmenger, K., Shiekh, M. & Otto, M. W. (2006) Augmentation of exposure therapy with D-cycloserine for social anxiety disorder. Arch.Gen.Psychiatry, 63, 298-304.

Hokfelt, T., Broberger, C., Xu, Z. Q., Sergeyev, V., Ubink, R. & Diez, M. (2000) Neuropeptides--an overview. Neuropharmacology, 39, 1337-1356.

Holahan, M. R. & White, N. M. (2002) Conditioned memory modulation, freezing, and avoidance as measures of amygdala-mediated conditioned fear. Neurobiol.Learn.Mem., 77, 250-275.

Hollinger, S. & Hepler, J. R. (2002) Cellular regulation of RGS proteins: modulators and integrators of G protein signaling. Pharmacol.Rev., 54, 527-559.

Hollmann, M. & Heinemann, S. (1994) Cloned glutamate receptors. Annu.Rev.Neurosci., 17, 31-108.

Hollmann, M. W., Strumper, D., Herroeder, S. & Durieux, M. E. (2005) Receptors, G proteins, and their interactions. Anesthesiology, 103, 1066-1078.

Holmes, A., Wrenn, C. C., Harris, A. P., Thayer, K. E. & Crawley, J. N. (2002) Behavioural profiles of inbred strains on novel olfactory, spatial and emotional tests for reference memory in mice. Genes Brain Behav., 1, 55-69.

Holmes, A., Heilig, M., Rupniak, N. M., Steckler, T. & Griebel, G. (2003) Neuropeptide systems as novel therapeutic targets for depression and anxiety disorders. Trends Pharmacol.Sci., 24, 580-588.

Holsboer, F. & Barden, N. (1996) Antidepressants and hypothalamic-pituitary-adrenocortical regulation. Endocr.Rev., 17, 187-205.

Holsboer, F. (2003) Corticotropin-releasing hormone modulators and depression. Curr.Opin.Investig.Drugs, 4, 46-50.

Holsboer, F. (2008) How can we realize the promise of personalized antidepressant medicines? Nat.Rev.Neurosci., 9, 638-646.

Holscher, C. (1999) Synaptic plasticity and learning and memory: LTP and beyond. J Neurosci.Res, 58, 62-75.

Homayoun, H. & Moghaddam, B. (2006) Bursting of prefrontal cortex neurons in awake rats is regulated by metabotropic glutamate 5 (mGlu5) receptors: rate-dependent influence and interaction with NMDA receptors. Cereb.Cortex, 16, 93-105.

Hope, S., Melle, I., Aukrust, P., Steen, N. E., Birkenaes, A. B., Lorentzen, S., Agartz, I., Ueland, T. & Andreassen, O. A. (2009) Similar immune profile in bipolar disorder and schizophrenia: selective increase in soluble tumor necrosis factor receptor I and von Willebrand factor. Bipolar.Disord., 11, 726-734.

Horita, A. (1998) An update on the CNS actions of TRH and its analogs. Life Sci., 62, 1443-1448.

Horowitz, M. J. (1986). Stress response syndromes (2nd ed.). Northvale, NJ: Jason Aronson

Horowitz, M. J., & Reidbord, S. P. (1992). Memory, emotion, and response to trauma. In S.-A. Christianson (Ed.), The handbook of emotion and memory. Research and theory, 343–357.

Hovatta, I., Tennant, R. S., Helton, R., Marr, R. A., Singer, O., Redwine, J. M., Ellison, J. A., Schadt, E. E., Verma, I. M., Lockhart, D. J. & Barlow, C. (2005) Glyoxalase 1 and glutathione reductase 1 regulate anxiety in mice. Nature, 438, 662-666.

Hovatta, I., Tennant, R. S., Helton, R., Marr, R. A., Singer, O., Redwine, J. M., Ellison, J. A., Schadt, E. E., Verma, I. M., Lockhart, D. J. & Barlow, C. (2005) Glyoxalase 1 and glutathione reductase 1 regulate anxiety in mice. Nature, 438, 662-666.

Hovatta, I., Juhila, J. & Donner, J. (2010) Oxidative stress in anxiety and comorbid disorders. Neurosci.Res, 68, 261-275.

Hrabovszky, E. & Liposits, Z. (2008) Novel aspects of glutamatergic signalling in the neuroendocrine system. J Neuroendocrinol., 20, 743-751.

Huang, C., Snider, F. & Cross, J. C. (2009) Prolactin receptor is required for normal glucose homeostasis and modulation of beta-cell mass during pregnancy. Endocrinology, 150, 1618-1626.

Huang, H. S., Matevossian, A., Whittle, C., Kim, S. Y., Schumacher, A., Baker, S. P. & Akbarian, S. (2007) Prefrontal dysfunction in schizophrenia involves mixed-lineage leukemia 1-regulated histone methylation at GABAergic gene promoters. J Neurosci., 27, 11254-11262.

Hubler, T. R. & Scammell, J. G. (2004) Intronic hormone response elements mediate regulation of FKBP5 by progestins and glucocorticoids. Cell Stress.Chaperones., 9, 243-252.

Huhman, K. L., Moore, T. O., Mougey, E. H. & Meyerhoff, J. L. (1992) Hormonal responses to fighting in hamsters: separation of physical and psychological causes. Physiol Behav., 51, 1083-1086.

Huhman, K. L. (2006) Social conflict models: can they inform us about human psychopathology? Horm.Behav., 50, 640-646.

Hunsberger, J. G., Austin, D. R., Chen, G. & Manji, H. K. (2009) MicroRNAs in mental health: from biological underpinnings to potential therapies. Neuromolecular.Med., 11, 173-182.

Husum, H., Mikkelsen, J. D., Hogg, S., Mathe, A. A. & Mork, A. (2000) Involvement of hippocampal neuropeptide Y in mediating the chronic actions of lithium, electroconvulsive stimulation and citalopram. Neuropharmacology, 39, 1463-1473.

Husum, H., Gruber, S. H., Bolwig, T. G. & Mathe, A. A. (2002) Extracellular levels of NPY in the dorsal hippocampus of freely moving rats are markedly elevated following a single electroconvulsive stimulation, irrespective of anticonvulsive Y1 receptor blockade. Neuropeptides, 36, 363-369.

Hutson, P. H., Semark, J. E. & Middlemiss, D. N. (1990) The TRH analogue MK-771, increases acetylcholine release in hippocampus but not striatum of the conscious rat. Neurosci.Lett., 116, 149-155.

Hyman, C., Hofer, M., Barde, Y. A., Juhasz, M., Yancopoulos, G. D., Squinto, S. P. & Lindsay, R. M. (1991) BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. Nature, 350, 230-232.

Ichiyanagi, T., Ichiyanagi, K., Miyake, M. & Sasaki, H. (2013) Accumulation and loss of asymmetric non-CpG methylation during male germ-cell development. Nucleic Acids Res, 41, 738-745.

Illingworth, R. S., Gruenewald-Schneider, U., Webb, S., Kerr, A. R., James, K. D., Turner, D. J., Smith, C., Harrison, D. J., Andrews, R. & Bird, A. P. (2010) Orphan CpG islands identify numerous conserved promoters in the mammalian genome. PLoS Genet., 6.

Imanaka, A., Morinobu, S., Toki, S. & Yamawaki, S. (2006) Importance of early environment in the development of post-traumatic stress disorder-like behaviors. Behav.Brain Res, 173, 129-137.

Inaba, S., Iwai, M., Furuno, M., Tomono, Y., Kanno, H., Senba, I., Okayama, H., Mogi, M., Higaki, J. & Horiuchi, M. (2009) Continuous activation of renin-angiotensin system impairs cognitive function in renin/angiotensinogen transgenic mice. Hypertension, 53, 356-362.

Inglis, F. M. & Moghaddam, B. (1999) Dopaminergic innervation of the amygdala is highly responsive to stress. J Neurochem., 72, 1088-1094.

Inoue, A. & Zhang, Y. (2011) Replication-dependent loss of 5-hydroxymethylcytosine in mouse preimplantation embryos. Science, 334, 194.

Iqbal, K., Jin, S. G., Pfeifer, G. P. & Szabo, P. E. (2011) Reprogramming of the paternal genome upon fertilization involves genome-wide oxidation of 5-methylcytosine. Proc.Natl.Acad.Sci.U.S.A, 108, 3642-3647.

Ishihara, K., Katsuki, H., Sugimura, M. & Satoh, M. (1992) YM-14673, a new thyrotropin-releasing hormone analog, augments long-term potentiation in the mossy fiber-CA3 system of guinea pig hippocampal slices. J Pharmacobiodyn., 15, 75-78.

Ishikawa, H., Itota, T., Nishitani, Y., Torii, Y., Inoue, K., Sugimoto, T. (2000) Osteopontin-immunoreactive primary sensory neurons in the rat spinal and trigeminal nervous system. Brain Res., 863, 276–281

Israelsson, C., Wang, Y., Kylberg, A., Pick, C. G., Hoffer, B. J. & Ebendal, T. (2009) Closed head injury in a mouse model results in molecular changes indicating inflammatory responses. J Neurotrauma, 26, 1307-1314.

Israelsson, C., Bengtsson, H., Lobell, A., Nilsson, L. N., Kylberg, A., Isaksson, M., Wootz, H., Lannfelt, L., Kullander, K., Hillered, L. & Ebendal, T. (2010) Appearance of Cxcl10-expressing cell clusters is common for traumatic brain injury and neurodegenerative disorders. Eur.J Neurosci., 31, 852-863.

Issa, J. P. (2000) CpG-island methylation in aging and cancer. Curr.Top.Microbiol.Immunol., 249, 101-118.

Itoh, Y., Ogasawara, T., Mushiroi, T., yamazaki, A., Ukai, Y. & Kimura, K. (1994) Effect of NS-3, a thyrotropin-releasing hormone analog, on in vivo acetylcholine release in rat brain: regional differences and its sites of action. J Pharmacol.Exp.Ther., 271, 884-890.

Iwamoto, Y., Morinobu, S., Takahashi, T. & Yamawaki, S. (2007) Single prolonged stress increases contextual freezing and the expression of glycine transporter 1 and vesicle-associated membrane protein 2 mRNA in the hippocampus of rats. Prog.Neuropsychopharmacol.Biol.Psychiatry, 31, 642-651.

Izquierdo, I. & Medina, J. H. (1997) Memory formation: the sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. Neurobiol.Learn.Mem., 68, 285-316.

Jaenisch, R. & Bird, A. (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat.Genet., 33 Suppl, 245-254.

Jakupcak, M., Tull, M. T., McDermott, M. J., Kaysen, D., Hunt, S. & Simpson, T. (2010) PTSD symptom clusters in relationship to alcohol misuse among Iraq and Afghanistan war veterans seeking post-deployment VA health care. Addict.Behav., 35, 840-843.

Johnston, R. J. & Hobert, O. (2003) A microRNA controlling left/right neuronal asymmetry in Caenorhabditis elegans. Nature, 426, 845-849.

Johnston, R. J., Jr., Chang, S., Etchberger, J. F., Ortiz, C. O. & Hobert, O. (2005) MicroRNAs acting in a double-negative feedback loop to control a neuronal cell fate decision. Proc.Natl.Acad.Sci.U.S.A, 102, 12449-12454.

Jonas, D. E., Cusack, K., Forneris, C. A., Wilkins, T.M., Sonis, J., Middleton, J. C., Feltner, C., Meredith, D., Cavanaugh, J., Brownley, K. A., Olmsted, K. R., Greenblatt, A., Weil, A. & Gaynes, B.N. (2013) Psychological and Pharmacological Treatments for Adults With Posttraumatic Stress Disorder (PTSD) [Internet]. Editors Source Rockville (MD): Agency for Healthcare Research and Quality (US); Report No.: 13-EHC011-EF. AHRQ Comparative Effectiveness Reviews.

Jones, K. A. & Thomsen, C. (2013) The role of the innate immune system in psychiatric disorders. Mol.Cell Neurosci., 53, 52-62.

Jones, P. A. (1999) The DNA methylation paradox. Trends Genet., 15, 34-37.

Jones, P. A. (2012) Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat.Rev.Genet., 13, 484-492.

Jones, P. L., Veenstra, G. J., Wade, P. A., Vermaak, D., Kass, S. U., Landsberger, N., Strouboulis, J. & Wolffe, A. P. (1998) Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. Nat.Genet., 19, 187-191.

Jovanovic, T. & Ressler, K. J. (2010) How the neurocircuitry and genetics of fear inhibition may inform our understanding of PTSD. Am J Psychiatry, 167, 648-662.

Junien, J. L., Gue, M. & Bueno, L. (1991) Neuropeptide Y and sigma ligand (JO 1784) act through a Gi protein to block the psychological stress and corticotropin-releasing factor-induced colonic motor activation in rats. Neuropharmacology, 30, 1119-1124.

Juszczak, G. R., Sliwa, A. T., Wolak, P., Tymosiak-Zielinska, A., Lisowski, P. & Swiergiel, A. H. (2006) The usage of video analysis system for detection of immobility in the tail suspension test in mice. Pharmacol.Biochem.Behav., 85, 332-338.

Juszczak, G. R., Blaszczyk, J., Sadowski, B., Sliwa, A. T., Wolak, P., Tymosiak-Zielinska, A., Lisowski, P. & Swiergiel, A. H. (2008) Lipopolysaccharide does not affect acoustic startle reflex in mice. Brain Behav.Immun., 22, 74-79.

Juszczak, G. R., Lisowski, P., Sliwa, A. T. & Swiergiel, A. H. (2008) Computer assisted video analysis of swimming performance in a forced swim test: simultaneous assessment of duration of immobility and swimming style in mice selected for high and low swim-stress induced analgesia. Physiol Behav., 95, 400-407.

Kaczmarek, L., Lapinska-Dzwonek, J. & Szymczak, S. (2002) Matrix metalloproteinases in the adult brain physiology: a link between c-Fos, AP-1 and remodeling of neuronal connections? EMBO J, 21, 6643-6648.

Kajantie, E. (2006) Fetal origins of stress-related adult disease. Ann.N.Y.Acad.Sci., 1083, 11-27.

Kaminer, D., Grimsrud, A., Myer, L., Stein, D. J. & Williams, D. R. (2008) Risk for post-traumatic stress disorder associated with different forms of interpersonal violence in South Africa. Soc.Sci.Med., 67, 1589-1595.

Kamprath, K. & Wotjak, C. T. (2004) Nonassociative learning processes determine expression and extinction of conditioned fear in mice. Learn.Mem., 11, 770-786.

Kang, H. K., Bullman, T. A. & Taylor, J. W. (2006) Risk of selected cardiovascular diseases and posttraumatic stress disorder among former World War II prisoners of war. Ann.Epidemiol., 16, 381-386.

Kang, Y. J., Chen, Y., Yu, A., Voss-McCowan, M. & Epstein, P. N. (1997) Overexpression of metallothionein in the heart of transgenic mice suppresses doxorubicin cardiotoxicity. J Clin.Invest, 100, 1501-1506.

Karalis, K., Sano, H., Redwine, J., Listwak, S., Wilder, R. L. & Chrousos, G. P. (1991) Autocrine or paracrine inflammatory actions of corticotropin-releasing hormone in vivo. Science, 254, 421-423.

Karamyan, V. T. & Speth, R. C. (2008) Distribution of the non-AT1, non-AT2 angiotensin-binding site in the rat brain: preliminary characterization. Neuroendocrinology, 88, 256-265.

Karin, M., Haslinger, A., Holtgreve, H., Richards, R. I., Krauter, P., Westphal, H. M. & Beato, M. (1984) Characterization of DNA sequences through which cadmium and glucocorticoid hormones induce human metallothionein-IIA gene. Nature, 308, 513-519.

Karlovic, D., Martinac, M., Buljan, D. & Zoricic, Z. (2004) Relationship between serum lipid concentrations and posttraumatic stress disorder symptoms in soldiers with combat experiences. Acta Med.Okayama, 58, 23-27.

Karres, J. S., Hilgers, V., Carrera, I., Treisman, J. & Cohen, S. M. (2007) The conserved microRNA miR-8 tunes atrophin levels to prevent neurodegeneration in Drosophila. Cell, 131, 136-145.

Kask, A., Harro, J., von, H. S., Redrobe, J. P., Dumont, Y. & Quirion, R. (2002) The neurocircuitry and receptor subtypes mediating anxiolytic-like effects of neuropeptide Y. Neurosci.Biobehav.Rev., 26, 259-283.

Kasper, S., Hajak, G., Wulff, K., Hoogendijk, W. J., Montejo, A. L., Smeraldi, E., Rybakowski, J. K., Quera-Salva, M. A., Wirz-Justice, A. M., Picarel-Blanchot, F. & Bayle, F. J. (2010) Efficacy of the novel antidepressant agomelatine on the circadian rest-activity cycle and depressive and anxiety symptoms in patients with major depressive disorder: a randomized, double-blind comparison with sertraline. J Clin.Psychiatry, 71, 109-120.

Kass, S. U., Landsberger, N. & Wolffe, A. P. (1997) DNA methylation directs a time-dependent repression of transcription initiation. Curr.Biol., 7, 157-165.

Kawabata, H., Ryomoto, T. & Ishikawa, K. (2000) Effect of angiotensin converting enzyme inhibitor and angiotensin II type 1 receptor antagonist on metabolism and contraction in ischemia-reperfused rabbit heart. Jpn.Circ.J, 64, 276-282.

Kayser, V., Latremoliere, A., Hamon, M. & Bourgoin, S. (2011) N-methyl-D-aspartate receptor-mediated modulations of the anti-allodynic effects of 5-HT1B/1D receptor stimulation in a rat model of trigeminal neuropathic pain. Eur.J Pain, 15, 451-458.

Keane, T. M., Caddell, J. M., Martin, B. W., Zimering, R. T., & Fairbank, J. A. (1983). Substance abuse among Vietnam veterans with posttraumatic stress disorders. Bulletin of the Society of Psychologists in Addictive Behaviors, 2, 117–122.

Keane, T. M., & Kaloupek, D. G. (1997). Comorbid psychiatric disorders in PTSD: Implications for research. In R. Yehuda & A. C. McFarlane (Eds.), Psychobiology of posttraumatic stress disorder (Vol. 821, pp. 24–34). New York: New York Academy of Sciences.

Keane, T. M., Zimmerling, R. T., & Caddeu, J. M. (1985) A behavioural formulation of post-traumatic stress disorder in Vietnam veterans, the Behavior Therapist, 8, 9-12.

Keller, S., Sarchiapone, M., Zarrilli, F., Videtic, A., Ferraro, A., Carli, V., Sacchetti, S., Lembo, F., Angiolillo, A., Jovanovic, N., Pisanti, F., Tomaiuolo, R., Monticelli, A., Balazic, J., Roy, A., Marusic, A., Cocozza, S., Fusco, A.,

Bruni, C. B., Castaldo, G. & Chiariotti, L. (2010) Increased BDNF promoter methylation in the Wernicke area of suicide subjects. Arch.Gen.Psychiatry, 67, 258-267.

Kellner, M., Baker, D. G. & Yehuda, R. (1997) Salivary cortisol and PTSD symptoms in Persian Gulf War combatants. Ann.N.Y.Acad.Sci., 821, 442-443.

Kelly, E. J., Sandgren, E. P., Brinster, R. L. & Palmiter, R. D. (1997) A pair of adjacent glucocorticoid response elements regulate expression of two mouse metallothionein genes. Proc.Natl.Acad.Sci.U.S.A, 94, 10045-10050.

Kemp, J. A. & McKernan, R. M. (2002) NMDA receptor pathways as drug targets. Nat.Neurosci., 5 Suppl, 1039-1042.

Kesner, Y., Zohar, J., Merenlender, A., Gispan, I., Shalit, F. & Yadid, G. (2009) WFS1 gene as a putative biomarker for development of post-traumatic syndrome in an animal model. Mol.Psychiatry, 14, 86-94.

Kessler, R. C. (2000) Posttraumatic stress disorder: the burden to the individual and to society. J Clin.Psychiatry, 61 Suppl 5, 4-12.

Kessler, R. C., Chiu, W. T., Demler, O., Merikangas, K. R. & Walters, E. E. (2005) Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. Arch.Gen.Psychiatry, 62, 617-627.

Kettenmann, H., Hanisch, U. K., Noda, M. & Verkhratsky, A. (2011) Physiology of microglia. Physiol Rev., 91, 461-553.

Khademi, M., Kockum, I., Andersson, M. L., Iacobaeus, E., Brundin, L., Sellebjerg, F., Hillert, J., Piehl, F. & Olsson, T. (2011) Cerebrospinal fluid CXCL13 in multiple sclerosis: a suggestive prognostic marker for the disease course. Mult.Scler., 17, 335-343.

Khairova, R. A., hado-Vieira, R., Du, J. & Manji, H. K. (2009) A potential role for pro-inflammatory cytokines in regulating synaptic plasticity in major depressive disorder. Int.J Neuropsychopharmacol., 12, 561-578.

Kilpatrick, D. G., Koenen, K. C., Ruggiero, K. J., Acierno, R., Galea, S., Resnick, H. S., Roitzsch, J., Boyle, J. & Gelernter, J. (2007) The serotonin transporter genotype and social support and moderation of posttraumatic stress disorder and depression in hurricane-exposed adults. Am J Psychiatry, 164, 1693-1699.

Kim, S. Y., Choi, Y. S., Choi, J. S., Cha, J. H., Kim, O. N., Lee, S. B., Chung, J. W., Chun, M. H. & Lee, M. Y. (2002) Osteopontin in kainic acid-induced microglial reactions in the rat brain. Mol.Cells, 13, 429-435.

Kim, T. Y., Chung, H. G., Shin, H. S., Kim, S. J., Choi, J. H., Chung, M. Y., An, S. K., Choi, T. K., So, H. S. & Cho, H. S. (2013) APOLIPOPROTEIN E GENE POLYMORPHISM, ALCOHOL USE, AND THEIR INTERACTIONS IN COMBAT-RELATED POSTTRAUMATIC STRESS DISORDER. Depress.Anxiety.

Kioussis, D. & Pachnis, V. (2009) Immune and nervous systems: more than just a superficial similarity? Immunity., 31, 705-710.

Kjelstrup, K. G., Tuvnes, F. A., Steffenach, H. A., Murison, R., Moser, E. I. & Moser, M. B. (2002) Reduced fear expression after lesions of the ventral hippocampus. Proc.Natl.Acad.Sci.U.S.A, 99, 10825-10830.

Klapstein, G. J. & Colmers, W. F. (1997) Neuropeptide Y suppresses epileptiform activity in rat hippocampus in vitro. J Neurophysiol., 78, 1651-1661.

Klengel, T., Mehta, D., Anacker, C., Rex-Haffner, M., Pruessner, J. C., Pariante, C. M., Pace, T. W., Mercer, K. B., Mayberg, H. S., Bradley, B., Nemeroff, C. B., Holsboer, F., Heim, C. M., Ressler, K. J., Rein, T. & Binder, E. B. (2013) Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. Nat.Neurosci., 16, 33-41.

Knight, D. C., Nguyen, H. T. & Bandettini, P. A. (2005) The role of the human amygdala in the production of conditioned fear responses. Neuroimage., 26, 1193-1200.

Koba, T., Kodama, Y., Shimizu, K., Nomura, S., Sugawara, M., Kobayashi, Y. & Ogasawara, T. (2001) Persistent behavioural changes in rats following inescapable shock stress: a potential model of posttraumatic stress disorder. World J.Biol.Psychiatry, 2, 34-37.

Kobayashi, H., Sakurai, T., Imai, M., Takahashi, N., Fukuda, A., Yayoi, O., Sato, S., Nakabayashi, K., Hata, K., Sotomaru, Y., Suzuki, Y. & Kono, T. (2012) Contribution of intragenic DNA methylation in mouse gametic DNA methylomes to establish oocyte-specific heritable marks. PLoS Genet., 8, e1002440.

Koch, M. (1999) The neurobiology of startle. Prog.Neurobiol., 59, 107-128.

Koenen, K. C., Saxe, G., Purcell, S., Smoller, J. W., Bartholomew, D., Miller, A., Hall, E., Kaplow, J., Bosquet, M., Moulton, S. & Baldwin, C. (2005) Polymorphisms in FKBP5 are associated with peritraumatic dissociation in medically injured children. Mol.Psychiatry, 10, 1058-1059.

Koenen, K. C. (2007) Genetics of posttraumatic stress disorder: Review and recommendations for future studies. J.Trauma Stress., 20, 737-750.

Koenen, K. C., Aiello, A. E., Bakshis, E., Amstadter, A. B., Ruggiero, K. J., Acierno, R., Kilpatrick, D. G., Gelernter, J. & Galea, S. (2009) Modification of the association between serotonin transporter genotype and risk of posttraumatic stress disorder in adults by county-level social environment. Am J Epidemiol., 169, 704-711.

Koenen, K. C., Uddin, M., Chang, S. C., Aiello, A. E., Wildman, D. E., Goldmann, E. & Galea, S. (2011) SLC6A4 methylation modifies the effect of the number of traumatic events on risk for posttraumatic stress disorder. Depress.Anxiety, 28, 639-647.

Kogan, J. H., Frankland, P. W. & Silva, A. J. (2000) Long-term memory underlying hippocampus-dependent social recognition in mice. Hippocampus, 10, 47-56.

Kolassa, I. T., Ertl, V., Eckart, C., Glockner, F., Kolassa, S., Papassotiropoulos, A., de Quervain, D. J. & Elbert, T. (2010) Association study of trauma load and SLC6A4 promoter polymorphism in posttraumatic stress disorder: evidence from survivors of the Rwandan genocide. J Clin.Psychiatry, 71, 543-547.

Kondo, Y., Woo, E. S., Michalska, A. E., Choo, K. H. & Lazo, J. S. (1995) Metallothionein null cells have increased sensitivity to anticancer drugs. Cancer Res, 55, 2021-2023.

Koo, J. W. & Duman, R. S. (2008) IL-1beta is an essential mediator of the antineurogenic and anhedonic effects of stress. Proc.Natl.Acad.Sci.U.S.A, 105, 751-756.

Koob, G. F. (2008) A role for brain stress systems in addiction. Neuron, 59, 11-34.

Korfias, S., Stranjalis, G., Papadimitriou, A., Psachoulia, C., Daskalakis, G., Antsaklis, A. & Sakas, D. E. (2006) Serum S-100B protein as a biochemical marker of brain injury: a review of current concepts. Curr.Med.Chem., 13, 3719-3731.

Kornek, B. & Lassmann, H. (2003) Neuropathology of multiple sclerosis-new concepts. Brain Res Bull., 61, 321-326.

Korosi, A., Naninck, E. F., Oomen, C. A., Schouten, M., Krugers, H., Fitzsimons, C. & Lucassen, P. J. (2012) Early-life stress mediated modulation of adult neurogenesis and behavior. Behav.Brain Res, 227, 400-409.

Korte, S. M. & De Boer, S. F. (2003) A robust animal model of state anxiety: fear-potentiated behaviour in the elevated plus-maze. Eur.J.Pharmacol., 463, 163-175.

Kosik, K. S. (2006) The neuronal microRNA system. Nat.Rev.Neurosci., 7, 911-920.

Kosten, T. R., Frank, J. B., Dan, E., McDougle, C. J. & Giller, E. L., Jr. (1991) Pharmacotherapy for posttraumatic stress disorder using phenelzine or imipramine. J Nerv.Ment.Dis., 179, 366-370.

Kowarik, M. C., Cepok, S., Sellner, J., Grummel, V., Weber, M. S., Korn, T., Berthele, A. & Hemmer, B. (2012) CXCL13 is the major determinant for B cell recruitment to the CSF during neuroinflammation. J Neuroinflammation., 9, 93.

Kozaric-Kovacic, D. & Eterovic, M. (2013) Lamotrigine abolished aggression in a patient with treatment-resistant posttraumatic stress disorder. Clin.Neuropharmacol., 36, 94-95.

Kozlovsky, N., Matar, M. A., Kaplan, Z., Kotler, M., Zohar, J. & Cohen, H. (2007) Long-term down-regulation of BDNF mRNA in rat hippocampal CA1 subregion correlates with PTSD-like behavioural stress response. Int.J.Neuropsychopharmacol., 10, 741-758.

Kozomara, A. & Griffiths-Jones, S. (2011) miRBase: integrating microRNA annotation and deep-sequencing data. Nucleic Acids Res, 39, D152-D157.

Kramer, M. S., Cutler, N., Feighner, J., Shrivastava, R., Carman, J., Sramek, J. J., Reines, S. A., Liu, G., Snavely, D., Wyatt-Knowles, E., Hale, J. J., Mills, S. G., MacCoss, M., Swain, C. J., Harrison, T., Hill, R. G., Hefti, F., Scolnick, E. M., Cascieri, M. A., Chicchi, G. G., Sadowski, S., Williams, A. R., Hewson, L., Smith, D., Carlson, E. J., Hargreaves, R. J. & Rupniak, N. M. (1998) Distinct mechanism for antidepressant activity by blockade of central substance P receptors. Science, 281, 1640-1645.

Krek, A., Grun, D., Poy, M. N., Wolf, R., Rosenberg, L., Epstein, E. J., MacMenamin, P., da, P., I, Gunsalus, K. C., Stoffel, M. & Rajewsky, N. (2005) Combinatorial microRNA target predictions. Nat.Genet., 37, 495-500.

Krichevsky, A. M., King, K. S., Donahue, C. P., Khrapko, K. & Kosik, K. S. (2003) A microRNA array reveals extensive regulation of microRNAs during brain development. Rna-A Publication of the Rna Society, 9, 1274-1281.

Krishnamurthy, S., Garabadu, D. & Joy, K. P. (2013) Risperidone ameliorates post-traumatic stress disorder-like symptoms in modified stress re-stress model. Neuropharmacology.

Krumbholz, M., Theil, D., Cepok, S., Hemmer, B., Kivisakk, P., Ransohoff, R. M., Hofbauer, M., Farina, C., Derfuss, T., Hartle, C., Newcombe, J., Hohlfeld, R. & Meinl, E. (2006) Chemokines in multiple sclerosis: CXCL12 and CXCL13 up-regulation is differentially linked to CNS immune cell recruitment. Brain, 129, 200-211.

Krysiak, R., Obuchowicz, E. & Herman, Z. S. (2000) Conditioned fear-induced changes in neuropeptide Y-like immunoreactivity in rats: the effect of diazepam and buspirone. Neuropeptides, 34, 148-157.

Kubek, M. J., Low, W. C., Sattin, A., Morzorati, S. L., Meyerhoff, J. L. & Larsen, S. H. (1989) Role of TRH in seizure modulation. Ann.N.Y.Acad.Sci., 553, 286-303.

Kubzansky, L. D., Koenen, K. C., Spiro, A., III, Vokonas, P. S. & Sparrow, D. (2007) Prospective study of posttraumatic stress disorder symptoms and coronary heart disease in the Normative Aging Study. Arch.Gen.Psychiatry, 64, 109-116.

Kubzansky, L. D., Koenen, K. C., Jones, C. & Eaton, W. W. (2009) A prospective study of posttraumatic stress disorder symptoms and coronary heart disease in women. Health Psychol., 28, 125-130.

Kulkarni, A. P., Govender, D. A., Kotwal, G. J. & Kellaway, L. A. (2011) Modulation of anxiety behavior by intranasally administered vaccinia virus complement control protein and curcumin in a mouse model of Alzheimer's disease. Curr.Alzheimer Res., 8, 95-113.

Kumar, A., Takada, Y., Boriek, A. M. & Aggarwal, B. B. (2004) Nuclear factor-kappaB: its role in health and disease. J Mol.Med.(Berl), 82, 434-448.

Kumari, M., Head, J. & Marmot, M. (2004) Prospective study of social and other risk factors for incidence of type 2 diabetes in the Whitehall II study. Arch.Intern.Med., 164, 1873-1880.

Kung, J. C., Chen, T. C., Shyu, B. C., Hsiao, S. & Huang, A. C. (2010) Anxiety- and depressive-like responses and c-fos activity in preproenkephalin knockout mice: oversensitivity hypothesis of enkephalin deficit-induced posttraumatic stress disorder. J Biomed.Sci., 17, 29.

Kuriyama, K., Honma, M., Koyama, S. & Kim, Y. (2011) D-cycloserine facilitates procedural learning but not declarative learning in healthy humans: a randomized controlled trial of the effect of D-cycloserine and valproic acid on overnight properties in the performance of non-emotional memory tasks. Neurobiol.Learn.Mem., 95, 505-509.

Kushner, M. G., Kim, S. W., Donahue, C., Thuras, P., Adson, D., Kotlyar, M., McCabe, J., Peterson, J. & Foa, E. B. (2007) D-cycloserine augmented exposure therapy for obsessive-compulsive disorder. Biol.Psychiatry, 62, 835-838.

Kuss, A. W. & Chen, W. (2008) MicroRNAs in brain function and disease. Current Neurology and Neuroscience Reports, 8, 190-197.

LaBar, K. S., Gatenby, J. C., Gore, J. C., LeDoux, J. E. & Phelps, E. A. (1998) Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fMRI study. Neuron, 20, 937-945.

Labonte, B., Suderman, M., Maussion, G., Navaro, L., Yerko, V., Mahar, I., Bureau, A., Mechawar, N., Szyf, M., Meaney, M. J. & Turecki, G. (2012) Genome-wide epigenetic regulation by early-life trauma. Arch.Gen.Psychiatry, 69, 722-731.

Ladd-Acosta, C., Pevsner, J., Sabunciyan, S., Yolken, R. H., Webster, M. J., Dinkins, T., Callinan, P. A., Fan, J. B., Potash, J. B. & Feinberg, A. P. (2007) DNA methylation signatures within the human brain. Am.J.Hum.Genet., 81, 1304-1315.

Lagos-Quintana, M., Rauhut, R., Lendeckel, W. & Tuschl, T. (2001) Identification of novel genes coding for small expressed RNAs. Science, 294, 853-858.

Lambeth, J. D., Krause, K. H. & Clark, R. A. (2008) NOX enzymes as novel targets for drug development. Semin.Immunopathol., 30, 339-363.

Lamour, Y., Dutar, P. & Jobert, A. (1985) Effects of TRH, cyclo-(His-Pro) and (3-Me-His2)TRH on identified septohippocampal neurons in the rat. Brain Res, 331, 343-347.

Langmann, T., Ebert, S., Walczak, Y., Weigelt, K., Ehrengruber, M. U., Stiewe, T. & Weber, B. H. (2009) Induction of early growth response-1 mediates microglia activation in vitro but is dispensable in vivo. Neuromolecular.Med., 11, 87-96.

Langnaese, K., John, R., Schweizer, H., Ebmeyer, U. & Keilhoff, G. (2008) Selection of reference genes for quantitative real-time PCR in a rat asphyxial cardiac arrest model. BMC Mol.Biol., 9, 53.

Langton, J. M. & Richardson, R. (2008) D-cycloserine facilitates extinction the first time but not the second time: an examination of the role of NMDA across the course of repeated extinction sessions. Neuropsychopharmacology, 33, 3096-3102.

Langton, J. M. & Richardson, R. (2010) The temporal specificity of the switch from NMDAr-dependent extinction to NMDAr-independent re-extinction. Behav.Brain Res, 208, 646-649.

Lappalainen, J., Kranzler, H. R., Malison, R., Price, L. H., Van, D. C., Rosenheck, R. A., Cramer, J., Southwick, S., Charney, D., Krystal, J. & Gelernter, J. (2002) A functional neuropeptide Y Leu7Pro polymorphism associated with alcohol dependence in a large population sample from the United States. Arch.Gen.Psychiatry, 59, 825-831.

Larhammar, D. (1996) Evolution of neuropeptide Y, peptide YY and pancreatic polypeptide. Regul.Pept., 62, 1-11.

Larhammar, D. (1996) Structural diversity of receptors for neuropeptide Y, peptide YY and pancreatic polypeptide. Regul.Pept., 65, 165-174.

Larson, E., Zollman, F., Kondiles, B. & Starr, C. (2013) Memory deficits, postconcussive complaints, and posttraumatic stress disorder in a volunteer sample of veterans. Rehabil.Psychol., 58, 245-252.

Latronico, M. V. & Condorelli, G. (2009) MicroRNAs and cardiac pathology. Nat.Rev.Cardiol., 6, 419-429.

Lau, C. G. & Zukin, R. S. (2007) NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. Nat.Rev.Neurosci., 8, 413-426.

Lau, N. C., Lim, L. P., Weinstein, E. G. & Bartel, D. P. (2001) An abundant class of tiny RNAs with probable regulatory roles in Caenorhabditis elegans. Science, 294, 858-862.

Laurent, L., Wong, E., Li, G., Huynh, T., Tsirigos, A., Ong, C. T., Low, H. M., Kin Sung, K. W., Rigoutsos, I., Loring, J. & Wei, C. L. (2010) Dynamic changes in the human methylome during differentiation. Genome Res, 20, 320-331.

Lauterbach, D., Vora, R. & Rakow, M. (2005) The relationship between posttraumatic stress disorder and self-reported health problems. Psychosom.Med., 67, 939-947.

Lavie, P. (2001) Sleep disturbances in the wake of traumatic events. N.Engl.J Med., 345, 1825-1832.

Lawford, B. R., McD, Y. R., Noble, E. P., Kann, B., Arnold, L., Rowell, J. & Ritchie, T. L. (2003) D2 dopamine receptor gene polymorphism: paroxetine and social functioning in posttraumatic stress disorder. Eur.Neuropsychopharmacol., 13, 313-320.

Layton, B. & Krikorian, R. (2002) Memory mechanisms in posttraumatic stress disorder. J Neuropsychiatry Clin.Neurosci., 14, 254-261.

Lazo, J. S., Kondo, Y., Dellapiazza, D., Michalska, A. E., Choo, K. H. & Pitt, B. R. (1995) Enhanced sensitivity to oxidative stress in cultured embryonic cells from transgenic mice deficient in metallothionein I and II genes. J Biol.Chem., 270, 5506-5510.

Leach, P. T., Poplawski, S. G., Kenney, J. W., Hoffman, B., Liebermann, D. A., Abel, T. & Gould, T. J. (2012) Gadd45b knockout mice exhibit selective deficits in hippocampus-dependent long-term memory. Learn.Mem., 19, 319-324.

Ledgerwood, L., Richardson, R. & Cranney, J. (2003) Effects of D-cycloserine on extinction of conditioned freezing. Behav.Neurosci., 117, 341-349.

Ledgerwood, L., Richardson, R. & Cranney, J. (2005) D-cycloserine facilitates extinction of learned fear: effects on reacquisition and generalized extinction. Biol.Psychiatry, 57, 841-847.

LeDoux, J. E., Iwata, J., Cicchetti, P. & Reis, D. J. (1988) Different projections of the central amygdaloid nucleus mediate autonomic and behavioural correlates of conditioned fear. J Neurosci., 8, 2517-2529.

LeDoux, J. E. (1992) Brain mechanisms of emotion and emotional learning. Curr.Opin.Neurobiol., 2, 191-197.

LeDoux, J. E. (2000) Emotion circuits in the brain. Annu.Rev.Neurosci., 23, 155-184.

Lee, H. & Kim, J. J. (1998) Amygdalar NMDA receptors are critical for new fear learning in previously fear-conditioned rats. J Neurosci., 18, 8444-8454.

Lee, H. J., Lee, M. S., Kang, R. H., Kim, H., Kim, S. D., Kee, B. S., Kim, Y. H., Kim, Y. K., Kim, J. B., Yeon, B. K., Oh, K. S., Oh, B. H., et al. (2005) Influence of the serotonin transporter promoter gene polymorphism on susceptibility to posttraumatic stress disorder. Depress.Anxiety, 21, 135-139.

Lee, I. & Kesner, R. P. (2002) Differential contribution of NMDA receptors in hippocampal subregions to spatial working memory. Nat.Neurosci., 5, 162-168.

Lee, K., Kim, J. H., Kwon, O. B., An, K., Ryu, J., Cho, K., Suh, Y. H. & Kim, H. S. (2012) An activity-regulated microRNA, miR-188, controls dendritic plasticity and synaptic transmission by downregulating neuropilin-2. J Neurosci., 32, 5678-5687.

Lee, M., Hyun, D. H., Halliwell, B. & Jenner, P. (2001) Effect of overexpression of wild-type and mutant Cu/Zn-superoxide dismutases on oxidative stress and cell death induced by hydrogen peroxide, 4-hydroxynonenal or serum deprivation: potentiation of injury by ALS-related mutant superoxide dismutases and protection by Bcl-2. J Neurochem., 78, 209-220.

Lee, M. A., Palace, J., Stabler, G., Ford, J., Gearing, A. & Miller, K. (1999) Serum gelatinase B, TIMP-1 and TIMP-2 levels in multiple sclerosis. A longitudinal clinical and MRI study. Brain, 122 (Pt 2), 191-197.

Lee, M. Y., Shin, S. L., Choi, Y. S., Kim, E. J., Cha, J. H., Chun, M. H., Lee, S. B. & Kim, S. Y. (1999) Transient upregulation of osteopontin mRNA in hippocampus and striatum following global forebrain ischemia in rats. Neurosci.Lett., 271, 81-84.

Lee, R. C., Feinbaum, R. L. & Ambros, V. (1993) The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell, 75, 843-854.

Lee, R. C. & Ambros, V. (2001) An extensive class of small RNAs in Caenorhabditis elegans. Science, 294, 862-864.

Lee, R. S., Tamashiro, K. L., Yang, X., Purcell, R. H., Harvey, A., Willour, V. L., Huo, Y., Rongione, M., Wand, G. S. & Potash, J. B. (2010) Chronic corticosterone exposure increases expression and decreases deoxyribonucleic acid methylation of Fkbp5 in mice. Endocrinology, 151, 4332-4343.

Lee, H., Kwak, S., Paik J, et al. (2007) Association between serotonin 2A receptor gene polymorphism and posttraumatic stress disorder. Psychiatry Investig., 4, 104–108.

Lee, H., Kang, R., Lim, S., et al. (2006) No association between the brain-derived neurotrophic factor gene Val66Met polymorphism and post-traumatic stress disorder. Stress Health., 22, 115–119.

Leeies, M., Pagura, J., Sareen, J. & Bolton, J. M. (2010) The use of alcohol and drugs to self-medicate symptoms of posttraumatic stress disorder. Depress.Anxiety, 27, 731-736.

Lelas, S., Wong, H., Li, Y. W., Heman, K. L., Ward, K. A., Zeller, K. L., Sieracki, K. K., Polino, J. L., Godonis, H. E., Ren, S. X., Yan, X. X., Arneric, et al.. (2004) Anxiolytic-like effects of the corticotropin-releasing factor1 (CRF1) antagonist DMP904 [4-(3-pentylamino)-2,7-dimethyl-8-(2-methyl-4-methoxyphenyl)-pyrazolo-[1,5-a]-pyr imidine] administered acutely or chronically at doses occupying central CRF1 receptors in rats. J Pharmacol.Exp.Ther., 309, 293-302.

Lemieux, A. M. & Coe, C. L. (1995) Abuse-related posttraumatic stress disorder: evidence for chronic neuroendocrine activation in women. Psychosom.Med., 57, 105-115.

Leppert, D., Ford, J., Stabler, G., Grygar, C., Lienert, C., Huber, S., Miller, K. M., Hauser, S. L. & Kappos, L. (1998) Matrix metalloproteinase-9 (gelatinase B) is selectively elevated in CSF during relapses and stable phases of multiple sclerosis. Brain, 121 (Pt 12), 2327-2334.

Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., Benjamin, J., Muller, C. R., Hamer, D. H. & Murphy, D. L. (1996) Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science, 274, 1527-1531.

Leslie, J. C., Norwood, K., Kennedy, P. J., Begley, M. & Shaw, D. (2012) Facilitation of extinction of operant behaviour in C57Bl/6 mice by chlordiazepoxide and D-cycloserine. Psychopharmacology (Berl) , 223, 223-235.

Leslie, J. C. & Norwood, K. (2013) Facilitation of extinction and re-extinction of operant behavior in mice by chlordiazepoxide and D-cycloserine. Neurobiol.Learn.Mem., 102, 1-6.

Leto, T. L. & Geiszt, M. (2006) Role of Nox family NADPH oxidases in host defense. Antioxid.Redox.Signal., 8, 1549-1561.

Leussis, M. P., Freund, N., Brenhouse, H. C., Thompson, B. S. & Andersen, S. L. (2012) Depressive-like behavior in adolescents after maternal separation: sex differences, controllability, and GABA. Dev.Neurosci., 34, 210-217.

Levin, E. D., Perraut, C., Pollard, N. & Freedman, J. H. (2006) Metallothionein expression and neurocognitive function in mice. Physiol Behav., 87, 513-518.

LEVINE, S. (1957) Infantile experience and resistance to physiological stress. Science, 126, 405.

Levite, M. (1998) Neuropeptides, by direct interaction with T cells, induce cytokine secretion and break the commitment to a distinct T helper phenotype. Proc.Natl.Acad.Sci.U.S.A, 95, 12544-12549.

Levy, B. S. & Sidel, V. W. (2013) Adverse health consequences of the Iraq War. Lancet, 381, 949-958.

Lewis, B. P., Shih, I. H., Jones-Rhoades, M. W., Bartel, D. P. & Burge, C. B. (2003) Prediction of mammalian microRNA targets. Cell, 115, 787-798.

Lewitus, G. M., Cohen, H. & Schwartz, M. (2008) Reducing post-traumatic anxiety by immunization. Brain Behav.Immun., 22, 1108-1114.

Li, Q. & Verma, I. M. (2002) NF-kappaB regulation in the immune system. Nat.Rev.Immunol., 2, 725-734.

Licinio, J. & Wong, M., (2003) Cytokine pathways in the brain in cytokines and mental health. In: Kronfol, Z. (Ed.), Cytokines and Mental Health. Kluwer Academic Pub., Boston, pp. 39 - 53.

Licinio, J. & Wong, M. L. (1997) Pathways and mechanisms for cytokine signaling of the central nervous system. J Clin.Invest, 100, 2941-2947.

Lijam, N., Paylor, R., McDonald, M. P., Crawley, J. N., Deng, C. X., Herrup, K., Stevens, K. E., Maccaferri, G., McBain, C. J., Sussman, D. J. & Wynshaw-Boris, A. (1997) Social interaction and sensorimotor gating abnormalities in mice lacking Dvl1. Cell, 90, 895-905.

Lim, L. P., Lau, N. C., Garrett-Engele, P., Grimson, A., Schelter, J. M., Castle, J., Bartel, D. P., Linsley, P. S. & Johnson, J. M. (2005) Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature, 433, 769-773.

Lin, H. C., Mao, S. C., Su, C. L. & Gean, P. W. (2010) Alterations of excitatory transmission in the lateral amygdala during expression and extinction of fear memory. Int.J Neuropsychopharmacol., 13, 335-345.

Lin, Q., Wei, W., Coelho, C. M., Li, X., Baker-Andresen, D., Dudley, K., Ratnu, V. S., Boskovic, Z., Kobor, M. S., Sun, Y. E. & Bredy, T. W. (2011) The brain-specific microRNA miR-128b regulates the formation of fear-extinction memory. Nat.Neurosci., 14, 1115-1117.

Lin, Y. C., Yao, P. L. & Richburg, J. H. (2010) FasL gene-deficient mice display a limited disruption in spermatogenesis and inhibition of mono-(2-ethylhexyl) phthalate-induced germ cell apoptosis. Toxicol.Sci., 114, 335-345.

Ling, H. Y., Ou, H. S., Feng, S. D., Zhang, X. Y., Tuo, Q. H., Chen, L. X., Zhu, B. Y., Gao, Z. P., Tang, C. K., Yin, W. D., Zhang, L. & Liao, D. F. (2009) CHANGES IN microRNA (miR) profile and effects of miR-320 in insulin-resistant 3T3-L1 adipocytes. Clin.Exp.Pharmacol.Physiol, 36, e32-e39.

Lipp, H. P. & Wolfer, D. P. (1998) Genetically modified mice and cognition. Curr.Opin.Neurobiol., 8, 272-280.

Lisowski, P., Juszczak, G. R., Goscik, J., Wieczorek, M., Zwierzchowski, L. & Swiergiel, A. H. (2011) Effect of chronic mild stress on hippocampal transcriptome in mice selected for high and low stress-induced analgesia and displaying different emotional behaviors. Eur.Neuropsychopharmacol., 21, 45-62.

Lisowski, P., Wieczorek, M., Goscik, J., Juszczak, G. R., Stankiewicz, A. M., Zwierzchowski, L. & Swiergiel, A. H. (2013) Effects of chronic stress on prefrontal cortex transcriptome in mice displaying different genetic backgrounds. J Mol.Neurosci., 50, 33-57.

Lister, R., Pelizzola, M., Dowen, R. H., Hawkins, R. D., Hon, G., Tonti-Filippini, J., Nery, J. R., Lee, L., Ye, Z., Ngo, Q. M., Edsall, L., ntosiewicz-Bourget, J., Stewart, R., Ruotti, V., Millar, A. H., Thomson, J. A., Ren, B. & Ecker, J. R. (2009) Human DNA methylomes at base resolution show widespread epigenomic differences. Nature, 462, 315-322.

Litz, B. T., Salters-Pedneault, K., Steenkamp, M. M., Hermos, J. A., Bryant, R. A., Otto, M. W. & Hofmann, S. G. (2012) A randomized placebo-controlled trial of D-cycloserine and exposure therapy for posttraumatic stress disorder. J Psychiatr.Res, 46, 1184-1190.

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., Sharma, S., Pearson, D., Plotsky, P. M. & Meaney, M. J. (1997) Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. Science, 277, 1659-1662.

Liu, F., Havens, J., Yu, Q., Wang, G., Davisson, R. L., Pickel, V. M. & Iadecola, C. (2012) The link between angiotensin II-mediated anxiety and mood disorders with NADPH oxidase-induced oxidative stress. Int.J Physiol Pathophysiol.Pharmacol., 4, 28-35.

Liuzzi, G. M., Trojano, M., Fanelli, M., Avolio, C., Fasano, A., Livrea, P. & Riccio, P. (2002) Intrathecal synthesis of matrix metalloproteinase-9 in patients with multiple sclerosis: implication for pathogenesis. Mult.Scler., 8, 222-228.

Lock, C., Hermans, G., Pedotti, R., Brendolan, A., Schadt, E., Garren, H., Langer-Gould, A., Strober, S., Cannella, B., Allard, J., Klonowski, P., Austin, A., Lad, N., Kaminski, N., Galli, S. J., Oksenberg, J. R., Raine, C. S., Heller, R. &

Steinman, L. (2002) Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. Nat.Med., 8, 500-508.

Lock, L. F., Takagi, N. & Martin, G. R. (1987) Methylation of the Hprt gene on the inactive X occurs after chromosome inactivation. Cell, 48, 39-46.

Loez, C. A., Olsen, E. S., Adams, J. C., Mou, K., Denhardt, D. T. & Davis, R.L. (1995) Osteopontin expression detected in adult cochleae and inner ear fluid. Hear Res., 85, 210–220

Logue, M. W., Baldwin, C., Guffanti, G., Melista, E., Wolf, E. J., Reardon, A. F., Uddin, M., Wildman, D., Galea, S., Koenen, K. C. & Miller, M. W. (2012) A genome-wide association study of post-traumatic stress disorder identifies the retinoid-related orphan receptor alpha (RORA) gene as a significant risk locus. Mol.Psychiatry.

Lolait, S. J., O'Carroll, A. M., Mahan, L. C., Felder, C. C., Button, D. C., Young, W. S., III, Mezey, E. & Brownstein, M. J. (1995) Extrapituitary expression of the rat V1b vasopressin receptor gene. Proc.Natl.Acad.Sci.U.S.A, 92, 6783-6787.

Lorberbaum, J. P., Kose, S., Johnson, M. R., Arana, G. W., Sullivan, L. K., Hamner, M. B., Ballenger, J. C., Lydiard, R. B., Brodrick, P. S., Bohning, D. E. & George, M. S. (2004) Neural correlates of speech anticipatory anxiety in generalized social phobia. Neuroreport, 15, 2701-2705.

Loscher, C. E., Mills, K. H. & Lynch, M. A. (2003) Interleukin-1 receptor antagonist exerts agonist activity in the hippocampus independent of the interleukin-1 type I receptor. J Neuroimmunol., 137, 117-124.

Lotarski, S. M., Donevan, S., El-Kattan, A., Osgood, S., Poe, J., Taylor, C. P. & Offord, J. (2011) Anxiolytic-like activity of pregabalin in the Vogel conflict test in alpha2delta-1 (R217A) and alpha2delta-2 (R279A) mouse mutants. J Pharmacol.Exp.Ther., 338, 615-621.

Lotze, M. T. & Tracey, K. J. (2005) High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. Nat.Rev.Immunol., 5, 331-342.

Low, W. C., Roepke, J., Farber, S. D., Hill, T. G., Sattin, A. & Kubek, M. J. (1989) Distribution of thyrotropin-releasing hormone (TRH) in the hippocampal formation as determined by radioimmunoassay. Neurosci.Lett., 103, 314-319.

Lu, A. T., Ogdie, M. N., Jarvelin, M. R., Moilanen, I. K., Loo, S. K., McCracken, J. T., McGough, J. J., Yang, M. H., Peltonen, L., Nelson, S. F., Cantor, R. M. & Smalley, S. L. (2008) Association of the cannabinoid receptor gene (CNR1) with ADHD and post-traumatic stress disorder. Am J Med.Genet.B Neuropsychiatr.Genet., 147B, 1488-1494.

Luco, R. F., Pan, Q., Tominaga, K., Blencowe, B. J., Pereira-Smith, O. M. & Misteli, T. (2010) Regulation of alternative splicing by histone modifications. Science, 327, 996-1000.

Lugli, G., Larson, J., Martone, M. E., Jones, Y. & Smalheiser, N. R. (2005) Dicer and eIF2c are enriched at postsynaptic densities in adult mouse brain and are modified by neuronal activity in a calpain-dependent manner. J Neurochem., 94, 896-905.

Lugli, G., Larson, J., Demars, M. P. & Smalheiser, N. R. (2012) Primary microRNA precursor transcripts are localized at post-synaptic densities in adult mouse forebrain. J Neurochem., 123, 459-466.

Lukaschek, K., Baumert, J., Kruse, J., Emeny, R. T., Lacruz, M. E., Huth, C., Thorand, B., Holle, R., Rathmann, W., Meisinger, C. & Ladwig, K. H. (2013) Relationship between posttraumatic stress disorder and type 2 diabetes in a population-based cross-sectional study with 2970 participants. J Psychosom.Res, 74, 340-345.

Lundberg, J. M., Rudehill, A., Sollevi, A., Fried, G. & Wallin, G. (1989) Co-release of neuropeptide Y and noradrenaline from pig spleen in vivo: importance of subcellular storage, nerve impulse frequency and pattern, feedback regulation and resupply by axonal transport. Neuroscience, 28, 475-486.

Lutz, B. (2009) Endocannabinoid signals in the control of emotion. Curr.Opin.Pharmacol., 9, 46-52.

Ma, D. K., Chiang, C. H., Ponnusamy, K., Ming, G. L. & Song, H. (2008) G9a and Jhdm2a regulate embryonic stem cell fusion-induced reprogramming of adult neural stem cells. Stem Cells, 26, 2131-2141.

MacMillan, H. L., Fleming, J. E., Streiner, D. L., Lin, E., Boyle, M. H., Jamieson, E., Duku, E. K., Walsh, C. A., Wong, M. Y. & Beardslee, W. R. (2001) Childhood abuse and lifetime psychopathology in a community sample. Am J Psychiatry, 158, 1878-1883.

Madsen, T. M., Greisen, M. H., Nielsen, S. M., Bolwig, T. G. & Mikkelsen, J. D. (2000) Electroconvulsive stimuli enhance both neuropeptide Y receptor Y1 and Y2 messenger RNA expression and levels of binding in the rat hippocampus. Neuroscience, 98, 33-39.

Magarinos, A. M. & McEwen, B. S. (1995) Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: involvement of glucocorticoid secretion and excitatory amino acid receptors. Neuroscience , 69, 89-98.

Magliozzi, R., Columba-Cabezas, S., Serafini, B. & Aloisi, F. (2004) Intracerebral expression of CXCL13 and BAFF is accompanied by formation of lymphoid follicle-like structures in the meninges of mice with relapsing experimental autoimmune encephalomyelitis. J Neuroimmunol., 148, 11-23.

Maia, D. B., Marmar, C. R., Mendlowicz, M. V., Metzler, T., Nobrega, A., Peres, M. C., Coutinho, E. S., Volchan, E. & Figueira, I. (2008) Abnormal serum lipid profile in Brazilian police officers with post-traumatic stress disorder. J Affect.Disord., 107, 259-263.

Makeyev, E. V., Zhang, J., Carrasco, M. A. & Maniatis, T. (2007) The MicroRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. Mol.Cell, 27, 435-448.

Makino, S., Smith, M. A. & Gold, P. W. (1995) Increased expression of corticotropin-releasing hormone and vasopressin messenger ribonucleic acid (mRNA) in the hypothalamic paraventricular nucleus during repeated stress: association with reduction in glucocorticoid receptor mRNA levels. Endocrinology, 136, 3299-3309.

Malan-Muller, S., Hemmings, S. M. & Seedat, S. (2013) Big effects of small RNAs: a review of microRNAs in anxiety. Mol.Neurobiol., 47, 726-739.

Malizia, A. L., Cunningham, V. J., Bell, C. J., Liddle, P. F., Jones, T. & Nutt, D. J. (1998) Decreased brain GABA(A)-benzodiazepine receptor binding in panic disorder: preliminary results from a quantitative PET study. Arch.Gen.Psychiatry, 55, 715-720.

Mannaioni, G., Marino, M. J., Valenti, O., Traynelis, S. F. & Conn, P. J. (2001) Metabotropic glutamate receptors 1 and 5 differentially regulate CA1 pyramidal cell function. J Neurosci., 21, 5925-5934.

Maren, S. (2001) Neurobiology of Pavlovian fear conditioning. Annu.Rev.Neurosci., 24, 897-931.

Marenholz, I. & Heizmann, C. W. (2004) S100A16, a ubiquitously expressed EF-hand protein which is up-regulated in tumors. Biochem.Biophys.Res Commun., 313, 237-244.

Margis, R., Zanatto, V. C., Tramontina, F., Vinade, E., Lhullier, F., Portela, L. V., Souza, D. O., Dalmaz, C., Kapczinski, F. & Goncalves, C. A. (2004) Changes in S100B cerebrospinal fluid levels of rats subjected to predator stress. Brain Res, 1028, 213-218.

Marques, C. A., Keil, U., Bonert, A., Steiner, B., Haass, C., Muller, W. E. & Eckert, A. (2003) Neurotoxic mechanisms caused by the Alzheimer's disease-linked Swedish amyloid precursor protein mutation: oxidative stress, caspases, and the JNK pathway. J Biol.Chem., 278, 28294-28302.

Marshall, R. D., Beebe, K. L., Oldham, M. & Zaninelli, R. (2001) Efficacy and safety of paroxetine treatment for chronic PTSD: a fixed-dose, placebo-controlled study. Am J Psychiatry, 158, 1982-1988.

Marsicano, G., Wotjak, C. T., Azad, S. C., Bisogno, T., Rammes, G., Cascio, M. G., Hermann, H., Tang, J., Hofmann, C., Zieglgansberger, W., Di, M., V & Lutz, B. (2002) The endogenous cannabinoid system controls extinction of aversive memories. Nature, 418, 530-534.

Martenyi, F., Brown, E. B., Zhang, H., Koke, S. C. & Prakash, A. (2002) Fluoxetine v. placebo in prevention of relapse in post-traumatic stress disorder. Br.J Psychiatry, 181, 315-320.

Martinez-Beamonte, R., Navarro, M. A., Larraga, A., Strunk, M., Barranquero, C., Acin, S., Guzman, M. A., Inigo, P. & Osada, J. (2011) Selection of reference genes for gene expression studies in rats. J Biotechnol., 151, 325-334.

Martinowich, K., Hattori, D., Wu, H., Fouse, S., He, F., Hu, Y., Fan, G. & Sun, Y. E. (2003) DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. Science, 302, 890-893.

Mason, J. W., Wang, S., Yehuda, R., Lubin, H., Johnson, D., Bremner, J. D., Charney, D. & Southwick, S. (2002) Marked lability in urinary cortisol levels in subgroups of combat veterans with posttraumatic stress disorder during an intensive exposure treatment program. Psychosom.Med., 64, 238-246.

Mason, J. W., Giller, E. L., Kosten, T. R., Ostroff, R. B. & Podd, L. (1986) Urinary free-cortisol levels in posttraumatic stress disorder patients. J. Nerv. Ment. Dis., 174, 145–149.

Masood, A., Nadeem, A., Mustafa, S. J. & O'Donnell, J. M. (2008) Reversal of oxidative stress-induced anxiety by inhibition of phosphodiesterase-2 in mice. J Pharmacol.Exp.Ther., 326, 369-379.

Mathe, A. A., Jimenez, P. A., Theodorsson, E. & Stenfors, C. (1998) Neuropeptide Y, neurokinin A and neurotensin in brain regions of Fawn Hooded "depressed", Wistar, and Sprague Dawley rats. Effects of electroconvulsive stimuli. Prog.Neuropsychopharmacol.Biol.Psychiatry, 22, 529-546.

Mathew, S. J., Vythilingam, M., Murrough, J. W., Zarate, C. A., Jr., Feder, A., Luckenbaugh, D. A., Kinkead, B., Parides, M. K., Trist, D. G., Bani, M. S., Bettica, P. U., Ratti, E. M. & Charney, D. S. (2011) A selective neurokinin-1 receptor antagonist in chronic PTSD: a randomized, double-blind, placebo-controlled, proof-of-concept trial. Eur.Neuropsychopharmacol., 21, 221-229.

Matsumoto, K., Uzunova, V., Pinna, G., Taki, K., Uzunov, D. P., Watanabe, H., Mienville, J. M., Guidotti, A. & Costa, E. (1999) Permissive role of brain allopregnanolone content in the regulation of pentobarbital-induced righting reflex loss. Neuropharmacology, 38, 955-963.

Mattson, M. P. (2003) Methylation and acetylation in nervous system development and neurodegenerative disorders. Ageing Res Rev., 2, 329-342.

Maunakea, A. K., Nagarajan, R. P., Bilenky, M., Ballinger, T. J., D'Souza, C., Fouse, S. D., Johnson, B. E., Hong, C., Nielsen, C., Zhao, Y., Turecki, G., Delaney, A., et al. (2010) Conserved role of intragenic DNA methylation in regulating alternative promoters. Nature, 466, 253-257.

May, P. C., Johnson, S. A., Poirier, J., Lampert-Etchells, M. & Finch, C. E. (1989) Altered gene expression in Alzheimer's disease brain tissue. Can.J Neurol.Sci., 16, 473-476.

May, P. C., Lampert-Etchells, M., Johnson, S. A., Poirier, J., Masters, J. N. & Finch, C. E. (1990) Dynamics of gene expression for a hippocampal glycoprotein elevated in Alzheimer's disease and in response to experimental lesions in rat. Neuron, 5, 831-839.

McAfoose, J. & Baune, B. T. (2009) Evidence for a cytokine model of cognitive function. Neurosci.Biobehav.Rev., 33, 355-366.

McBain, C. J. & Mayer, M. L. (1994) N-methyl-D-aspartic acid receptor structure and function. Physiol Rev., 74, 723-760.

McEwen, B. S. & Stellar, E. (1993) Stress and the individual. Mechanisms leading to disease. Arch.Intern.Med., 153, 2093-2101.

McEwen, B. S. (2002) Sex, stress and the hippocampus: allostasis, allostatic load and the aging process. Neurobiol.Aging, 23, 921-939.

McEwen, B. S. (2008) Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. Eur.J Pharmacol., 583, 174-185.

McGeer, P. L. & McGeer, E. G. (2004) Inflammation and neurodegeneration in Parkinson's disease. Parkinsonism.Relat Disord., 10 Suppl 1, S3-S7.

McGhee, J. D. & Felsenfeld, G. (1980) Nucleosome structure. Annu.Rev.Biochem., 49, 1115-1156.

McGhee, L. L., Maani, C. V., Garza, T. H., Gaylord, K. M. & Black, I. H. (2008) The correlation between ketamine and posttraumatic stress disorder in burned service members. J Trauma, 64, S195-S198.

McGowan, P. O., Sasaki, A., D'Alessio, A. C., Dymov, S., Labonte, B., Szyf, M., Turecki, G. & Meaney, M. J. (2009) Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nat.Neurosci., 12, 342-348.

McGuire, J., Herman, J. P., Horn, P. S., Sallee, F. R. & Sah, R. (2010) Enhanced fear recall and emotional arousal in rats recovering from chronic variable stress. Physiol Behav., 101, 474-482.

McHugh, S. B., Deacon, R. M., Rawlins, J. N. & Bannerman, D. M. (2004) Amygdala and ventral hippocampus contribute differentially to mechanisms of fear and anxiety. Behav.Neurosci., 118, 63-78.

McMahon, F. J., Stine, O. C., Meyers, D. A., Simpson, S. G. & DePaulo, J. R. (1995) Patterns of maternal transmission in bipolar affective disorder. Am J Hum.Genet., 56, 1277-1286.

McNally, R. J. (2007) Mechanisms of exposure therapy: how neuroscience can improve psychological treatments for anxiety disorders. Clin.Psychol.Rev., 27, 750-759.

Meaney, M. J. (2001) Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Annu.Rev.Neurosci., 24, 1161-1192.

Medina, J. F., Repa, J. C., Mauk, M. D. & LeDoux, J. E. (2002) Parallels between cerebellum- and amygdala-dependent conditioning. Nat.Rev.Neurosci., 3, 122-131.

Meerson, A., Cacheaux, L., Goosens, K. A., Sapolsky, R. M., Soreq, H. & Kaufer, D. (2010) Changes in brain MicroRNAs contribute to cholinergic stress reactions. J Mol.Neurosci., 40, 47-55.

Meffert, M. K. & Baltimore, D. (2005) Physiological functions for brain NF-kappaB. Trends Neurosci., 28, 37-43.

Mehta, D., Gonik, M., Klengel, T., Rex-Haffner, M., Menke, A., Rubel, J., Mercer, K. B., Putz, B., Bradley, B., Holsboer, F., Ressler, K. J., Muller-Myhsok, B. & Binder, E. B. (2011) Using polymorphisms in FKBP5 to define biologically distinct subtypes of posttraumatic stress disorder: evidence from endocrine and gene expression studies. Arch.Gen.Psychiatry, 68, 901-910.

Mehta, D. & Binder, E. B. (2012) Gene x environment vulnerability factors for PTSD: the HPA-axis. Neuropharmacology, 62, 654-662.

Meighan, P. C., Meighan, S. E., Davis, C. J., Wright, J. W. & Harding, J. W. (2007) Effects of matrix metalloproteinase inhibition on short- and long-term plasticity of schaffer collateral/CA1 synapses. J Neurochem., 102, 2085-2096.

Meighan, S. E., Meighan, P. C., Choudhury, P., Davis, C. J., Olson, M. L., Zornes, P. A., Wright, J. W. & Harding, J. W. (2006) Effects of extracellular matrix-degrading proteases matrix metalloproteinases 3 and 9 on spatial learning and synaptic plasticity. J Neurochem., 96, 1227-1241.

Meller, R., Stevens, S. L., Minami, M., Cameron, J. A., King, S., Rosenzweig, H., Doyle, K., Lessov, N. S., Simon, R. P. & Stenzel-Poore, M. P. (2005) Neuroprotection by osteopontin in stroke. J Cereb.Blood Flow Metab, 25, 217-225.

Mellman, T. A., Bustamante, V., David, D. & Fins, A. I. (2002) Hypnotic medication in the aftermath of trauma. J Clin.Psychiatry, 63, 1183-1184.

Mellman, T. A., Alim, T., Brown, D. D., Gorodetsky, E., Buzas, B., Lawson, W. B., Goldman, D. & Charney, D. S. (2009) Serotonin polymorphisms and posttraumatic stress disorder in a trauma exposed African American population. Depress.Anxiety, 26, 993-997.

Meloni, E. G. & Davis, M. (1999) Enhancement of the acoustic startle response in rats by the dopamine D1 receptor agonist SKF 82958. Psychopharmacology (Berl), 144, 373-380.

Mendlewicz, J., Massat, I., Souery, D., Del-Favero, J., Oruc, L., Nothen, M. M., Blackwood, D., Muir, W., Battersby, S., Lerer, B., Segman, R. H., Kaneva, R., et al. (2004) Serotonin transporter 5HTTLPR polymorphism and affective disorders: no evidence of association in a large European multicenter study. Eur.J Hum.Genet., 12, 377-382.

Mendlowicz, M. V. & Stein, M. B. (2000) Quality of life in individuals with anxiety disorders. Am J Psychiatry, 157, 669-682.

Menza, M. A., Robertson-Hoffman, D. E. & Bonapace, A. S. (1993) Parkinson's disease and anxiety: comorbidity with depression. Biol.Psychiatry, 34, 465-470.

Mercer, K. B., Orcutt, H. K., Quinn, J. F., Fitzgerald, C. A., Conneely, K. N., Barfield, R. T., Gillespie, C. F. & Ressler, K. J. (2012) Acute and posttraumatic stress symptoms in a prospective gene x environment study of a university campus shooting. Arch.Gen.Psychiatry, 69, 89-97.

Metzker, M. L. (2010) Sequencing technologies - the next generation. Nat.Rev.Genet., 11, 31-46.

Mezuk, B., Eaton, W. W., Albrecht, S. & Golden, S. H. (2008) Depression and type 2 diabetes over the lifespan: a meta-analysis. Diabetes Care, 31, 2383-2390.

Miczek, K. A., Maxson, S. C., Fish, E. W. & Faccidomo, S. (2001) Aggressive behavioural phenotypes in mice. Behav.Brain Res, 125, 167-181.

Migaud, M., Roques, B. P. & Durieux, C. (1995) Evidence for a high-affinity uptake system for cholecystokinin octapeptide (CCK8) in rat cortical synaptosomes. Eur.J Neurosci., 7, 1074-1079.

Migaud, M., Charlesworth, P., Dempster, M., Webster, L. C., Watabe, A. M., Makhinson, M., He, Y., Ramsay, M. F., Morris, R. G., Morrison, J. H., O'Dell, T. J. & Grant, S. G. (1998) Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. Nature, 396, 433-439.

Mikkelsen, J. D., Woldbye, D., Kragh, J., Larsen, P. J. & Bolwig, T. G. (1994) Electroconvulsive shocks increase the expression of neuropeptide Y (NPY) mRNA in the piriform cortex and the dentate gyrus. Brain Res Mol.Brain Res, 23, 317-322.

Milad, M. R. & Quirk, G. J. (2002) Neurons in medial prefrontal cortex signal memory for fear extinction. Nature, 420, 70-74.

Milad, M. R., Pitman, R. K., Ellis, C. B., Gold, A. L., Shin, L. M., Lasko, N. B., Zeidan, M. A., Handwerger, K., Orr, S. P. & Rauch, S. L. (2009) Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. Biol.Psychiatry, 66, 1075-1082.

Millecamps, M., Centeno, M. V., Berra, H. H., Rudick, C. N., Lavarello, S., Tkatch, T. & Apkarian, A. V. (2007) D-cycloserine reduces neuropathic pain behavior through limbic NMDA-mediated circuitry. Pain, 132, 108-123.

Miller, A. H., Maletic, V. & Raison, C. L. (2009) Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol.Psychiatry, 65, 732-741.

Miller, C. A. & Sweatt, J. D. (2007) Covalent modification of DNA regulates memory formation. Neuron, 53, 857-869.

Miller, G. E., Chen, E. & Zhou, E. S. (2007) If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. Psychol.Bull., 133, 25-45.

Milward, E. A., Fitzsimmons, C., Szklarczyk, A. & Conant, K. (2007) The matrix metalloproteinases and CNS plasticity: an overview. J Neuroimmunol., 187, 9-19.

Missig, G., Ayers, L. W., Schulkin, J. & Rosen, J. B. (2010) Oxytocin reduces background anxiety in a fear-potentiated startle paradigm. Neuropsychopharmacology, 35, 2607-2616.

Moarefi, A. H. & Chedin, F. (2011) ICF syndrome mutations cause a broad spectrum of biochemical defects in DNMT3B-mediated de novo DNA methylation. J Mol.Biol., 409, 758-772.

Moarefi, A. H. & Chedin, F. (2011) ICF syndrome mutations cause a broad spectrum of biochemical defects in DNMT3B-mediated de novo DNA methylation. J Mol.Biol., 409, 758-772.

Molina-Holgado, E. & Molina-Holgado, F. (2010) Mending the broken brain: neuroimmune interactions in neurogenesis. J Neurochem., 114, 1277-1290.

Molina-Holgado, F., Pinteaux, E., Moore, J. D., Molina-Holgado, E., Guaza, C., Gibson, R. M. & Rothwell, N. J. (2003) Endogenous interleukin-1 receptor antagonist mediates anti-inflammatory and neuroprotective actions of cannabinoids in neurons and glia. J Neurosci., 23, 6470-6474.

Molina, V. A., Volosin, M., Cancela, L., Keller, E., Murua, V. S. & Basso, A. M. (1990) Effect of chronic variable stress on monoamine receptors: influence of imipramine administration. Pharmacol.Biochem.Behav., 35, 335-340.

Molitoris, J. K., McColl, K. S. & Distelhorst, C. W. (2011) Glucocorticoid-mediated repression of the oncogenic microRNA cluster miR-17~92 contributes to the induction of Bim and initiation of apoptosis. Mol.Endocrinol., 25, 409-420.

Monje, M. L., Toda, H. & Palmer, T. D. (2003) Inflammatory blockade restores adult hippocampal neurogenesis. Science, 302, 1760-1765.

Monroe, S. M., Simons, A. D. & Thase, M. E. (1991) Onset of depression and time to treatment entry: roles of life stress. J Consult Clin.Psychol., 59, 566-573.

Montkowski, A., Landgraf, R., Yassouridis, A., Holsboer, F. & Schobitz, B. (1997) Central administration of IL-1 reduces anxiety and induces sickness behaviour in rats. Pharmacol.Biochem.Behav., 58, 329-336.

Moore, N. L., Gauchan, S. & Genovese, R. F. (2012) Differential severity of anxiogenic effects resulting from a brief swim or underwater trauma in adolescent male rats. Pharmacol.Biochem.Behav., 102, 264-268.

Mooy, J. M., de, V. H., Grootenhuis, P. A., Bouter, L. M. & Heine, R. J. (2000) Major stressful life events in relation to prevalence of undetected type 2 diabetes: the Hoorn Study. Diabetes Care, 23, 197-201.

Morgan, B. P. & Gasque, P. (1996) Expression of complement in the brain: role in health and disease. Immunol.Today, 17, 461-466.

Morgan, B. P. & Gasque, P. (1997) Extrahepatic complement biosynthesis: where, when and why? Clin.Exp.Immunol., 107, 1-7.

Morgan, C. A., III, Rasmusson, A. M., Winters, B., Hauger, R. L., Morgan, J., Hazlett, G. & Southwick, S. (2003) Trauma exposure rather than posttraumatic stress disorder is associated with reduced baseline plasma neuropeptide-Y levels. Biol.Psychiatry, 54, 1087-1091.

Morgan, M. A., Romanski, L. M. & LeDoux, J. E. (1993) Extinction of emotional learning: contribution of medial prefrontal cortex. Neurosci.Lett., 163, 109-113.

Morgan, D. G., Johnson, S. A. (1987). Differences in gene-expression in the cerebral-cortex of Alzheimers-disease and agematched control brains. Journal of Cellular, 196.

Moroz, O. V., Antson, A. A., Dodson, E. J., Burrell, H. J., Grist, S. J., Lloyd, R. M., Maitland, N. J., Dodson, G. G., Wilson, K. S., Lukanidin, E. & Bronstein, I. B. (2002) The structure of S100A12 in a hexameric form and its proposed role in receptor signalling. Acta Crystallogr.D.Biol.Crystallogr., 58, 407-413.

Morris, R. G., Garrud, P., Rawlins, J. N. & O'Keefe, J. (1982) Place navigation impaired in rats with hippocampal lesions. Nature, 297, 681-683.

Morris, R. G. M. (1981) Spatial localization does not require the presence of local cues. Learn. Motiv. 12, 239–260

Mourlon, V., Baudin, A., Blanc, O., Lauber, A., Giros, B., Naudon, L. & Dauge, V. (2010) Maternal deprivation induces depressive-like behaviours only in female rats. Behav.Brain Res, 213, 278-287.

Muinos-Gimeno, M., Guidi, M., Kagerbauer, B., Martin-Santos, R., Navines, R., Alonso, P., Menchon, J. M., Gratacos, M., Estivill, X. & Espinosa-Parrilla, Y. (2009) Allele variants in functional MicroRNA target sites of the neurotrophin-3 receptor gene (NTRK3) as susceptibility factors for anxiety disorders. Hum.Mutat., 30, 1062-1071.

Muinos-Gimeno, M., Espinosa-Parrilla, Y., Guidi, M., Kagerbauer, B., Sipila, T., Maron, E., Pettai, K., Kananen, L., Navines, R., Martin-Santos, R., Gratacos, M., Metspalu, A., Hovatta, I. & Estivill, X. (2011) Human microRNAs miR-22, miR-138-2, miR-148a, and miR-488 are associated with panic disorder and regulate several anxiety candidate genes and related pathways. Biol.Psychiatry, 69, 526-533.

Mukherjee, P. & Pasinetti, G. M. (2001) Complement anaphylatoxin C5a neuroprotects through mitogen-activated protein kinase-dependent inhibition of caspase 3. J Neurochem., 77, 43-49.

Mukherjee, P., Thomas, S. & Pasinetti, G. M. (2008) Complement anaphylatoxin C5a neuroprotects through regulation of glutamate receptor subunit 2 in vitro and in vivo. J Neuroinflammation., 5, 5.

Mullen, P. E., Martin, J. L., Anderson, J. C., Romans, S. E. & Herbison, G. P. (1996) The long-term impact of the physical, emotional, and sexual abuse of children: a community study. Child Abuse Negl. , 20, 7-21.

Munglani, R., Hudspith, M. J. & Hunt, S. P. (1996) The therapeutic potential of neuropeptide Y. Analgesic, anxiolytic and antihypertensive. Drugs, 52, 371-389.

Murgatroyd, C., Patchev, A. V., Wu, Y., Micale, V., Bockmuhl, Y., Fischer, D., Holsboer, F., Wotjak, C. T., Almeida, O. F. & Spengler, D. (2009) Dynamic DNA methylation programs persistent adverse effects of early-life stress. Nat.Neurosci., 12, 1559-1566.

Murphy, D. L., Li, Q., Engel, S., Wichems, C., Andrews, A., Lesch, K. P. & Uhl, G. (2001) Genetic perspectives on the serotonin transporter. Brain Res Bull., 56, 487-494.

Mustapic, M., Pivac, N., Kozaric-Kovacic, D., Dezeljin, M., Cubells, J. F. & Muck-Seler, D. (2007) Dopamine beta-hydroxylase (DBH) activity and -1021C/T polymorphism of DBH gene in combat-related post-traumatic stress disorder. Am J Med.Genet.B Neuropsychiatr.Genet., 144B, 1087-1089.

Myers, K. M. & Davis, M. (2002) Behavioural and neural analysis of extinction. Neuron, 36, 567-584.

Myers, K. M., Carlezon, W. A., Jr. & Davis, M. (2011) Glutamate receptors in extinction and extinction-based therapies for psychiatric illness. Neuropsychopharmacology, 36, 274-293.

Nagalakshmi, U., Wang, Z., Waern, K., Shou, C., Raha, D., Gerstein, M. & Snyder, M. (2008) The transcriptional landscape of the yeast genome defined by RNA sequencing. Science, 320, 1344-1349.

Nagy, V., Bozdagi, O., Matynia, A., Balcerzyk, M., Okulski, P., Dzwonek, J., Costa, R. M., Silva, A. J., Kaczmarek, L. & Huntley, G. W. (2006) Matrix metalloproteinase-9 is required for hippocampal late-phase long-term potentiation and memory. J Neurosci., 26, 1923-1934.

Nagy, V., Bozdagi, O. & Huntley, G. W. (2007) The extracellular protease matrix metalloproteinase-9 is activated by inhibitory avoidance learning and required for long-term memory. Learn.Mem., 14, 655-664.

Nair, A. & Bonneau, R. H. (2006) Stress-induced elevation of glucocorticoids increases microglia proliferation through NMDA receptor activation. J Neuroimmunol., 171, 72-85.

Nair, D., Dayyat, E. A., Zhang, S. X., Wang, Y. & Gozal, D. (2011) Intermittent hypoxia-induced cognitive deficits are mediated by NADPH oxidase activity in a murine model of sleep apnea. PLoS One, 6, e19847.

Nakamura, M., Ueno, S., Sano, A. & Tanabe, H. (2000) The human serotonin transporter gene linked polymorphism (5-HTTLPR) shows ten novel allelic variants. Mol.Psychiatry, 5, 32-38.

Nakazawa, T., Komai, S., Watabe, A. M., Kiyama, Y., Fukaya, M., rima-Yoshida, F., Horai, R., Sudo, K., Ebine, K., Delawary, M., Goto, J., Umemori, H., Tezuka, T., Iwakura, Y., Watanabe, M., Yamamoto, T. & Manabe, T. (2006) NR2B tyrosine phosphorylation modulates fear learning as well as amygdaloid synaptic plasticity. EMBO J, 25, 2867-2877.

Nan, X., Ng, H. H., Johnson, C. A., Laherty, C. D., Turner, B. M., Eisenman, R. N. & Bird, A. (1998) Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. Nature, 393, 386-389.

Nanda, S. A., Qi, C., Roseboom, P. H. & Kalin, N. H. (2008) Predator stress induces behavioural inhibition and amygdala somatostatin receptor 2 gene expression. Genes Brain Behav., 7, 639-648.

Nankova, B., Kvetnansky, R., Hiremagalur, B., Sabban, B., Rusnak, M. & Sabban, E. L. (1996) Immobilization stress elevates gene expression for catecholamine biosynthetic enzymes and some neuropeptides in rat sympathetic ganglia: effects of adrenocorticotropin and glucocorticoids. Endocrinology, 137, 5597-5604.

Naviaux, R. K. (2013) Metabolic features of the cell danger response. Mitochondrion..

Neal, J. W., Singhrao, S. K., Jasani, B. & Newman, G. R. (1996) Immunocytochemically detectable metallothionein is expressed by astrocytes in the ischaemic human brain. Neuropathol.Appl.Neurobiol., 22, 243-247.

Neeper, M., Schmidt, A. M., Brett, J., Yan, S. D., Wang, F., Pan, Y. C., Elliston, K., Stern, D. & Shaw, A. (1992) Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. J Biol.Chem., 267, 14998-15004.

Nelson, A., Genthe, J. A., Gall, K. M. & Edwards, E. (2009) Patterns of youths' glycemic control with insulin pump therapy. Pediatr.Nurs., 35, 234-239.

Nelson, E. C., Agrawal, A., Pergadia, M. L., Lynskey, M. T., Todorov, A. A., Wang, J. C., Todd, R. D., Martin, N. G., Heath, A. C., Goate, A. M., Montgomery, G. W. & Madden, P. A. (2009) Association of childhood trauma exposure and GABRA2 polymorphisms with risk of posttraumatic stress disorder in adults. Mol.Psychiatry, 14, 234-235.

Newport, D. J., Heim, C., Bonsall, R., Miller, A. H. & Nemeroff, C. B. (2004) Pituitary-adrenal responses to standard and low-dose dexamethasone suppression tests in adult survivors of child abuse. Biol.Psychiatry, 55, 10-20.

Newton, R. A. & Hogg, N. (1998) The human S100 protein MRP-14 is a novel activator of the beta 2 integrin Mac-1 on neutrophils. J Immunol., 160, 1427-1435.

Neylan, T. C., Sun, B., Rempel, H., Ross, J., Lenoci, M., O'Donovan, A. & Pulliam, L. (2011) Suppressed monocyte gene expression profile in men versus women with PTSD. Brain Behav.Immun., 25, 524-531.

Ng, F., Berk, M., Dean, O. & Bush, A. I. (2008) Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. Int.J Neuropsychopharmacol., 11, 851-876.

Nguyen Dinh, C. A. & Touyz, R. M. (2011) Cell signaling of angiotensin II on vascular tone: novel mechanisms. Curr.Hypertens.Rep., 13, 122-128.

Nguyen, K. T., Deak, T., Will, M. J., Hansen, M. K., Hunsaker, B. N., Fleshner, M., Watkins, L. R. & Maier, S. F. (2000) Timecourse and corticosterone sensitivity of the brain, pituitary, and serum interleukin-1beta protein response to acute stress. Brain Res, 859, 193-201.

Nibuya, M., Takahashi, M., Russell, D. S. & Duman, R. S. (1999) Repeated stress increases catalytic TrkB mRNA in rat hippocampus. Neurosci.Lett., 267, 81-84.

Nic Dhonnchadha, B. A., Szalay, J. J., chat-Mendes, C., Platt, D. M., Otto, M. W., Spealman, R. D. & Kantak, K. M. (2010) D-cycloserine deters reacquisition of cocaine self-administration by augmenting extinction learning. Neuropsychopharmacology, 35, 357-367.

Nicolas, F. E., Pais, H., Schwach, F., Lindow, M., Kauppinen, S., Moulton, V. & Dalmay, T. (2008) Experimental identification of microRNA-140 targets by silencing and overexpressing miR-140. RNA, 14, 2513-2520.

Nillni, E. A. & Sevarino, K. A. (1999) The biology of pro-thyrotropin-releasing hormone-derived peptides. Endocr.Rev., 20, 599-648.

Ninan, I., Bath, K. G., Dagar, K., Perez-Castro, R., Plummer, M. R., Lee, F. S. & Chao, M. V. (2010) The BDNF Val66Met polymorphism impairs NMDA receptor-dependent synaptic plasticity in the hippocampus. J Neurosci., 30, 8866-8870.

Noble, E. P. (2000) The DRD2 gene in psychiatric and neurological disorders and its phenotypes. Pharmacogenomics., 1, 309-333.

Noiri, E., Dickman, K., Miller, F., Romanov, G., Romanov, V. I., Shaw, R., Chambers, A. F., Rittling, S. R., Denhardt, D. T. & Goligorsky, M. S. (1999) Reduced tolerance to acute renal ischemia in mice with a targeted disruption of the osteopontin gene. Kidney Int., 56, 74-82.

Nomiyama, T., Perez-Tilve, D., Ogawa, D., Gizard, F., Zhao, Y., Heywood, E. B., Jones, K. L., Kawamori, R., Cassis, L. A., Tschop, M. H. & Bruemmer, D. (2007) Osteopontin mediates obesity-induced adipose tissue macrophage infiltration and insulin resistance in mice. J Clin.Invest, 117, 2877-2888.

Norberg, M. M., Krystal, J. H. & Tolin, D. F. (2008) A meta-analysis of D-cycloserine and the facilitation of fear extinction and exposure therapy. Biol.Psychiatry, 63, 1118-1126.

Norrholm, S. D., Jovanovic, T., Smith, A. K., Binder, E., Klengel, T., Conneely, K., Mercer, K. B., Davis, J. S., Kerley, K., Winkler, J., Gillespie, C. F., Bradley, B. & Ressler, K. J. (2013) Differential Genetic and Epigenetic Regulation of catechol-O-methyltransferase is Associated with Impaired Fear Inhibition in Posttraumatic Stress Disorder. Front Behav.Neurosci., 7, 30.

Norris, F. H., Murphy, A. D., Baker, C. K., Perilla, J. L., Rodriguez, F. G. & Rodriguez, J. J. (2003) Epidemiology of trauma and posttraumatic stress disorder in Mexico. J Abnorm.Psychol., 112, 646-656.

Norton, P. J. & Price, E. C. (2007) A meta-analytic review of adult cognitive-behavioural treatment outcome across the anxiety disorders. J Nerv.Ment.Dis., 195, 521-531.

Novitskaya, V., Grigorian, M., Kriajevska, M., Tarabykina, S., Bronstein, I., Berezin, V., Bock, E. & Lukanidin, E. (2000) Oligomeric forms of the metastasis-related Mts1 (S100A4) protein stimulate neuronal differentiation in cultures of rat hippocampal neurons. J Biol.Chem., 275, 41278-41286.

Nuti, A., Ceravolo, R., Piccinni, A., Dell'Agnello, G., Bellini, G., Gambaccini, G., Rossi, C., Logi, C., Dell'Osso, L. & Bonuccelli, U. (2004) Psychiatric comorbidity in a population of Parkinson's disease patients. Eur.J Neurol., 11, 315-320.

O'Carroll, R. E., Drysdale, E., Cahill, L., Shajahan, P. & Ebmeier, K. P. (1999) Stimulation of the noradrenergic system enhances and blockade reduces memory for emotional material in man. Psychol.Med. , 29, 1083-1088.

O'Connor, R. M., Grenham, S., Dinan, T. G. & Cryan, J. F. (2013) microRNAs as novel antidepressant targets: converging effects of ketamine and electroconvulsive shock therapy in the rat hippocampus. Int.J Neuropsychopharmacol., 16, 1885-1892.

O'Donovan, A., Neylan, T. C., Metzler, T. & Cohen, B. E. (2012) Lifetime exposure to traumatic psychological stress is associated with elevated inflammation in the Heart and Soul Study. Brain Behav.Immun., 26, 642-649.

O'Dowd, B. S., Zhao, W. Q., Ng, K. T. & Robinson, S. R. (1997) Chicks injected with antisera to either S-100 alpha or S-100 beta protein develop amnesia for a passive avoidance task. Neurobiol.Learn.Mem., 67, 197-206.

O'Regan, A. W., Nau, G. J., Chupp, G. L. & Berman, J. S. (2000) Osteopontin (Eta-1) in cell-mediated immunity: teaching an old dog new tricks. Immunol.Today, 21, 475-478.

Oberdoerffer, S. (2012) A conserved role for intragenic DNA methylation in alternative pre-mRNA splicing. Transcription., 3, 106-109.

Odegaard, J. I., Ricardo-Gonzalez, R. R., Red, E. A., Vats, D., Morel, C. R., Goforth, M. H., Subramanian, V., Mukundan, L., Ferrante, A. W. & Chawla, A. (2008) Alternative M2 activation of Kupffer cells by PPARdelta ameliorates obesity-induced insulin resistance. Cell Metab, 7, 496-507.

Oflaz, F., Hatipoglu, S. & Aydin, H. (2008) Effectiveness of psychoeducation intervention on post-traumatic stress disorder and coping styles of earthquake survivors. J Clin.Nurs., 17, 677-687.

Ohashi, K., Burkart, V., Flohe, S. & Kolb, H. (2000) Cutting edge: heat shock protein 60 is a putative endogenous ligand of the toll-like receptor-4 complex. J Immunol., 164, 558-561.

Okamura, Y., Watari, M., Jerud, E. S., Young, D. W., Ishizaka, S. T., Rose, J., Chow, J. C. & Strauss, J. F., III (2001) The extra domain A of fibronectin activates Toll-like receptor 4. J Biol.Chem., 276, 10229-10233.

Olson, M. L., Meighan, P. C., Brown, T. E., Asay, A. L., Benoist, C. C., Harding, J. W. & Wright, J. W. (2008) Hippocampal MMP-3 elevation is associated with passive avoidance conditioning. Regul.Pept. , 146, 19-25.

Onder, E., Tural, U. & Aker, T. (2006) A comparative study of fluoxetine, moclobemide, and tianeptine in the treatment of posttraumatic stress disorder following an earthquake. Eur.Psychiatry, 21, 174-179.

Ooi, S. K., Qiu, C., Bernstein, E., Li, K., Jia, D., Yang, Z., Erdjument-Bromage, H., Tempst, P., Lin, S. P., Allis, C. D., Cheng, X. & Bestor, T. H. (2007) DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. Nature, 448, 714-717.

Oomen, C. A., Soeters, H., Audureau, N., Vermunt, L., van Hasselt, F. N., Manders, E. M., Joels, M., Lucassen, P. J. & Krugers, H. (2010) Severe early life stress hampers spatial learning and neurogenesis, but improves hippocampal synaptic plasticity and emotional learning under high-stress conditions in adulthood. J Neurosci., 30, 6635-6645.

Oomen, C. A., Soeters, H., Audureau, N., Vermunt, L., van Hasselt, F. N., Manders, E. M., Joels, M., Krugers, H. & Lucassen, P. J. (2011) Early maternal deprivation affects dentate gyrus structure and emotional learning in adult female rats. Psychopharmacology (Berl), 214, 249-260.

Oprica, M., Zhu, S., Goiny, M., Pham, T. M., Mohammed, A. H., Winblad, B., Bartfai, T. & Schultzberg, M. (2005) Transgenic overexpression of interleukin-1 receptor antagonist in the CNS influences behaviour, serum corticosterone and brain monoamines. Brain Behav.Immun., 19, 223-234.

Orr, S. P., Metzger, L. J., Lasko, N. B., Macklin, M. L., Peri, T. & Pitman, R. K. (2000) De novo conditioning in trauma-exposed individuals with and without posttraumatic stress disorder. J Abnorm.Psychol., 109, 290-298.

Osaka, H., Mukherjee, P., Aisen, P. S. & Pasinetti, G. M. (1999) Complement-derived anaphylatoxin C5a protects against glutamate-mediated neurotoxicity. J Cell Biochem., 73, 303-311.

Otto, M. W., Basden, S. L., Leyro, T. M., McHugh, R. K. & Hofmann, S. G. (2007) Clinical perspectives on the combination of D-cycloserine and cognitive-behavioural therapy for the treatment of anxiety disorders. CNS.Spectr., 12, 51-61.

Otto, M. W., Tolin, D. F., Simon, N. M., Pearlson, G. D., Basden, S., Meunier, S. A., Hofmann, S. G., Eisenmenger, K., Krystal, J. H. & Pollack, M. H. (2010) Efficacy of d-cycloserine for enhancing response to cognitive-behavior therapy for panic disorder. Biol.Psychiatry, 67, 365-370.

Otto, S. J., McCorkle, S. R., Hover, J., Conaco, C., Han, J. J., Impey, S., Yochum, G. S., Dunn, J. J., Goodman, R. H. & Mandel, G. (2007) A new binding motif for the transcriptional repressor REST uncovers large gene networks devoted to neuronal functions. J Neurosci., 27, 6729-6739.

Ozanne, S. E. & Constancia, M. (2007) Mechanisms of disease: the developmental origins of disease and the role of the epigenotype. Nat.Clin.Pract.Endocrinol.Metab, 3, 539-546.

Padala, P. R., Madison, J., Monnahan, M., Marcil, W., Price, P., Ramaswamy, S., Din, A. U., Wilson, D. R. & Petty, F. (2006) Risperidone monotherapy for post-traumatic stress disorder related to sexual assault and domestic abuse in women. Int.Clin.Psychopharmacol., 21, 275-280.

Padilla, E., Shumake, J., Barrett, D. W., Holmes, G., Sheridan, E. C. & Gonzalez-Lima, F. (2010) Novelty-evoked activity in open field predicts susceptibility to helpless behavior. Physiol Behav., 101, 746-754.

Page, A. J., Young, R. L., Martin, C. M., Umaerus, M., O'Donnell, T. A., Cooper, N. J., Coldwell, J. R., Hulander, M., Mattsson, J. P., Lehmann, A. & Blackshaw, L. A. (2005) Metabotropic glutamate receptors inhibit mechanosensitivity in vagal sensory neurons. Gastroenterology, 128, 402-410.

Palin, K., Bluthe, R. M., Verrier, D., Tridon, V., Dantzer, R. & Lestage, J. (2004) Interleukin-1beta mediates the memory impairment associated with a delayed type hypersensitivity response to bacillus Calmette-Guerin in the rat hippocampus. Brain Behav.Immun., 18, 223-230.

Panahi, Y., Moghaddam, B. R., Sahebkar, A., Nazari, M. A., Beiraghdar, F., Karami, G. & Saadat, A. R. (2011) A randomized, double-blind, placebo-controlled trial on the efficacy and tolerability of sertraline in Iranian veterans with post-traumatic stress disorder. Psychol.Med., 41, 2159-2166.

Panocka, I., Massi, M., Lapo, I., Swiderski, T., Kowalczyk, M. & Sadowski, B. (2001) Antidepressant-type effect of the NK3 tachykinin receptor agonist aminosenktide in mouse lines differing in endogenous opioid system activity. Peptides, 22, 1037-1042.

Paolone, G., Botreau, F. & Stewart, J. (2009) The facilitative effects of D-cycloserine on extinction of a cocaine-induced conditioned place preference can be long lasting and resistant to reinstatement. Psychopharmacology (Berl), 202, 403-409.

Park, C. R., Campbell, A. M. & Diamond, D. M. (2001) Chronic psychosocial stress impairs learning and memory and increases sensitivity to yohimbine in adult rats. Biol.Psychiatry, 50, 994-1004.

Park, L., Raman, K. G., Lee, K. J., Lu, Y., Ferran, L. J., Jr., Chow, W. S., Stern, D. & Schmidt, A. M. (1998) Suppression of accelerated diabetic atherosclerosis by the soluble receptor for advanced glycation endproducts. Nat.Med., 4, 1025-1031.

Park, L., Zhou, P., Pitstick, R., Capone, C., Anrather, J., Norris, E. H., Younkin, L., Younkin, S., Carlson, G., McEwen, B. S. & Iadecola, C. (2008) Nox2-derived radicals contribute to neurovascular and behavioural dysfunction in mice overexpressing the amyloid precursor protein. Proc.Natl.Acad.Sci.U.S.A, 105, 1347-1352.

Parker, B. J. & Wen, J. (2009) Predicting microRNA targets in time-series microarray experiments via functional data analysis. BMC.Bioinformatics., 10 Suppl 1, S32.

Parslow, R. A. & Jorm, A. F. (2007) Pretrauma and posttrauma neurocognitive functioning and PTSD symptoms in a community sample of young adults. Am J Psychiatry, 164, 509-515.

Parsons, C. G. (2001) NMDA receptors as targets for drug action in neuropathic pain. Eur.J Pharmacol., 429, 71-78.

Parsons, M. J., Grimm, C. H., Paya-Cano, J. L., Sugden, K., Nietfeld, W., Lehrach, H. & Schalkwyk, L. C. (2008) Using hippocampal microRNA expression differences between mouse inbred strains to characterise miRNA function. Mamm.Genome, 19, 552-560.

Parsons, M. J., Grimm, C., Paya-Cano, J. L., Fernandes, C., Liu, L., Philip, V. M., Chesler, E. J., Nietfeld, W., Lehrach, H. & Schalkwyk, L. C. (2012) Genetic variation in hippocampal microRNA expression differences in C57BL/6 J X DBA/2 J (BXD) recombinant inbred mouse strains. BMC Genomics, 13, 476.

Pashenkov, M., Soderstrom, M. & Link, H. (2003) Secondary lymphoid organ chemokines are elevated in the cerebrospinal fluid during central nervous system inflammation. J Neuroimmunol., 135, 154-160.

Pasquinelli, A. E. (2012) MicroRNAs and their targets: recognition, regulation and an emerging reciprocal relationship. Nat.Rev.Genet., 13, 271-282.

Pastor, W. A., Pape, U. J., Huang, Y., Henderson, H. R., Lister, R., Ko, M., McLoughlin, E. M., Brudno, Y., Mahapatra, S., Kapranov, P., Tahiliani, M., Daley, G. Q., Liu, X. S., Ecker, J. R., Milos, P. M., Agarwal, S. & Rao, A. (2011) Genome-wide mapping of 5-hydroxymethylcytosine in embryonic stem cells. Nature, 473, 394-397.

Paulus, M. P. & Stein, M. B. (2006) An insular view of anxiety. Biol.Psychiatry, 60, 383-387.

Pavel, J., Benicky, J., Murakami, Y., Sanchez-Lemus, E. & Saavedra, J. M. (2008) Peripherally administered angiotensin II AT1 receptor antagonists are anti-stress compounds in vivo. Ann.N.Y.Acad.Sci., 1148, 360-366.

Penkowa, M., Carrasco, J., Giralt, M., Moos, T. & Hidalgo, J. (1999) CNS wound healing is severely depressed in metallothionein I- and II-deficient mice. J Neurosci., 19, 2535-2545.

Penkowa, M., Moos, T., Carrasco, J., Hadberg, H., Molinero, A., Bluethmann, H. & Hidalgo, J. (1999) Strongly compromised inflammatory response to brain injury in interleukin-6-deficient mice. Glia, 25, 343-357.

Penkowa, M., Giralt, M., Moos, T., Thomsen, P. S., Hernandez, J. & Hidalgo, J. (1999) Impaired inflammatory response to glial cell death in genetically metallothionein-I- and -II-deficient mice. Exp.Neurol., 156, 149-164.

Penkowa, M. & Hidalgo, J. (2000) Metallothionein I+II expression and their role in experimental autoimmune encephalomyelitis. Glia, 32, 247-263.

Penkowa, M., Espejo, C., Martinez-Caceres, E. M., Poulsen, C. B., Montalban, X. & Hidalgo, J. (2001) Altered inflammatory response and increased neurodegeneration in metallothionein I+II deficient mice during experimental autoimmune encephalomyelitis. J Neuroimmunol., 119, 248-260.

Penkowa, M., Espejo, C., Ortega-Aznar, A., Hidalgo, J., Montalban, X. & Martinez Caceres, E. M. (2003) Metallothionein expression in the central nervous system of multiple sclerosis patients. Cell Mol.Life Sci., 60, 1258-1266.

Penner, S. B., Smyth, D. D. & Glavin, G. B. (1993) Effects of neuropeptide Y and [Leu31,Pro34] neuropeptide Y on experimental gastric lesion formation and gastric secretion in the rat. J Pharmacol.Exp.Ther., 266, 339-343.

Pereira, J. P., Kelly, L. M. & Cyster, J. G. (2010) Finding the right niche: B-cell migration in the early phases of T-dependent antibody responses. Int.Immunol., 22, 413-419.

Perkins, D. F. & Luster, T. (1999) The relationship between sexual abuse and purging: findings from community-wide surveys of female adolescents. Child Abuse Negl., 23, 371-382.

Perkins, D. O., Jeffries, C. D., Jarskog, L. F., Thomson, J. M., Woods, K., Newman, M. A., Parker, J. S., Jin, J. & Hammond, S. M. (2007) microRNA expression in the prefrontal cortex of individuals with schizophrenia and schizoaffective disorder. Genome Biol., 8, R27.

Peskind, E. R., Bonner, L. T., Hoff, D. J. & Raskind, M. A. (2003) Prazosin reduces trauma-related nightmares in older men with chronic posttraumatic stress disorder. J Geriatr.Psychiatry Neurol., 16, 165-171.

Peters, J., Kalivas, P. W. & Quirk, G. J. (2009) Extinction circuits for fear and addiction overlap in prefrontal cortex. Learn.Mem., 16, 279-288.

Petrakis, I. L., Ralevski, E., Desai, N., Trevisan, L., Gueorguieva, R., Rounsaville, B. & Krystal, J. H. (2012) Noradrenergic vs serotonergic antidepressant with or without naltrexone for veterans with PTSD and comorbid alcohol dependence. Neuropsychopharmacology, 37, 996-1004.

Petri, V., Shimoyama, M., Hayman, G. T., Smith, J. R., Tutaj, M., de Pons, J., Dwinell, M. R., Munzenmaier, D. H., Twigger, S.N., Jacob, H. J. & RGD Team (2011) The Rat Genome Database pathway portal. Database (Oxford).

Pettersson, E., Lundeberg, J. & Ahmadian, A. (2009) Generations of sequencing technologies. Genomics, 93, 105-111.

Phelps, E. A., Delgado, M. R., Nearing, K. I. & LeDoux, J. E. (2004) Extinction learning in humans: role of the amygdala and vmPFC. Neuron, 43, 897-905.

Philbert, J., Belzung, C. & Griebel, G. (2013) The CRF(1) receptor antagonist SSR125543 prevents stress-induced cognitive deficit associated with hippocampal dysfunction: comparison with paroxetine and D-cycloserine. Psychopharmacology (Berl), 228, 97-107.

Phillips, M. I. & de Oliveira, E. M. (2008) Brain renin angiotensin in disease. J Mol.Med.(Berl), 86, 715-722.

Picciotto, M. R. & Wickman, K. (1998) Using knockout and transgenic mice to study neurophysiology and behavior. Physiol Rev., 78, 1131-1163.

Pickup, J. C. (2004) Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes Care, 27, 813-823.

Pietrzak, R. H., Goldstein, R. B., Southwick, S. M. & Grant, B. F. (2012) Physical health conditions associated with posttraumatic stress disorder in U.S. older adults: results from wave 2 of the National Epidemiologic Survey on Alcohol and Related Conditions. J Am Geriatr.Soc., 60, 296-303.

Pietrzak, R. H., Galea, S., Southwick, S. M. & Gelernter, J. (2013) Examining the relation between the serotonin transporter 5-HTTPLR genotype x trauma exposure interaction on a contemporary phenotypic model of posttraumatic stress symptomatology: a pilot study. J Affect.Disord., 148, 123-128.

Pine, D. S., Grun, J., Zarahn, E., Fyer, A., Koda, V., Li, W., Szeszko, P. R., Ardekani, B. & Bilder, R. M. (2001) Cortical brain regions engaged by masked emotional faces in adolescents and adults: an fMRI study. Emotion., 1, 137-147.

Pineles, B. L., Romero, R., Montenegro, D., Tarca, A. L., Han, Y. M., Kim, Y. M., Draghici, S., Espinoza, J., Kusanovic, J. P., Mittal, P., Hassan, S. S. & Kim, C. J. (2007) Distinct subsets of microRNAs are expressed differentially in the human placentas of patients with preeclampsia. Am J Obstet.Gynecol., 196, 261-266.

Pisani, A., Gubellini, P., Bonsi, P., Conquet, F., Picconi, B., Centonze, D., Bernardi, G. & Calabresi, P. (2001) Metabotropic glutamate receptor 5 mediates the potentiation of N-methyl-D-aspartate responses in medium spiny striatal neurons. Neuroscience, 106, 579-587.

Pitman, R. K. & Orr, S. P. (1990) Twenty-four hour urinary cortisol and catecholamine excretion in combat-related posttraumatic stress disorder. Biol.Psychiatry, 27, 245-247.

Pitman, R. K., Sanders, K. M., Zusman, R. M., Healy, A. R., Cheema, F., Lasko, N. B., Cahill, L. & Orr, S. P. (2002) Pilot study of secondary prevention of posttraumatic stress disorder with propranolol. Biol.Psychiatry, 51, 189-192.

Pizzorusso, T., Ratto, G. M., Putignano, E. & Maffei, L. (2000) Brain-derived neurotrophic factor causes cAMP response element-binding protein phosphorylation in absence of calcium increases in slices and cultured neurons from rat visual cortex. J Neurosci., 20, 2809-2816.

Plotsky, P. M. & Meaney, M. J. (1993) Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. Brain Res Mol.Brain Res, 18, 195-200.

Poleszak, E., Wosko, S., Serefko, A., Szopa, A., Wlaz, A., Szewczyk, B., Nowak, G. & Wlaz, P. (2013) Effects of ifenprodil on the antidepressant-like activity of NMDA ligands in the forced swim test in mice. Prog.Neuropsychopharmacol.Biol.Psychiatry, 46, 29-35.

Pontone, G. M., Williams, J. R., Anderson, K. E., Chase, G., Goldstein, S. A., Grill, S., Hirsch, E. S., Lehmann, S., Little, J. T., Margolis, R. L., Rabins, P. V., Weiss, H. D. & Marsh, L. (2009) Prevalence of anxiety disorders and anxiety subtypes in patients with Parkinson's disease. Mov Disord., 24, 1333-1338.

Popp, C., Dean, W., Feng, S. H., Cokus, S. J., Andrews, S., Pellegrini, M., Jacobsen, S. E. & Reik, W. (2010) Genome-wide erasure of DNA methylation in mouse primordial germ cells is affected by AID deficiency. Nature, 463, 1101-U126.

Poundja, J., Sanche, S., Tremblay, J. & Brunet, A. (2012) Trauma reactivation under the influence of propranolol: an examination of clinical predictors. Eur.J Psychotraumatol., 3.

Prod'homme, T., Weber, M. S., Steinman, L. & Zamvil, S. S. (2006) A neuropeptide in immune-mediated inflammation, Y? Trends Immunol., 27, 164-167.

Prokai, L. (2002) Central nervous system effects of thyrotropin-releasing hormone and its analogues: opportunities and perspectives for drug discovery and development. Prog.Drug Res, 59, 133-169.

Prut, L. & Belzung, C. (2003) The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur.J Pharmacol., 463, 3-33.

Pugh, C. R., Nguyen, K. T., Gonyea, J. L., Fleshner, M., Wakins, L. R., Maier, S. F. & Rudy, J. W. (1999) Role of interleukin-1 beta in impairment of contextual fear conditioning caused by social isolation. Behav.Brain Res, 106, 109-118.

Pynoos, R. S., Ritzmann, R. F., Steinberg, A. M., Goenjian, A. & Prisecaru, I. (1996) A behavioural animal model of posttraumatic stress disorder featuring repeated exposure to situational reminders. Biol.Psychiatry, 39, 129-134.

Qiu, Z. K., Zhang, L. M., Zhao, N., Chen, H. X., Zhang, Y. Z., Liu, Y. Q., Mi, T. Y., Zhou, W. W., Li, Y., Yang, R. F., Xu, J. P. & Li, Y. F. (2013) Repeated administration of AC-5216, a ligand for the 18kDa translocator protein, improves behavioural deficits in a mouse model of post-traumatic stress disorder. Prog.Neuropsychopharmacol.Biol.Psychiatry, 45, 40-46.

Quach, H., Barreiro, L. B., Laval, G., Zidane, N., Patin, E., Kidd, K. K., Kidd, J. R., Bouchier, C., Veuille, M., Antoniewski, C. & Quintana-Murci, L. (2009) Signatures of purifying and local positive selection in human miRNAs. Am.J.Hum.Genet., 84, 316-327.

Quach, H., Barreiro, L. B., Laval, G., Zidane, N., Patin, E., Kidd, K. K., Kidd, J. R., Bouchier, C., Veuille, M., Antoniewski, C. & Quintana-Murci, L. (2009) Signatures of purifying and local positive selection in human miRNAs. Am J Hum.Genet., 84, 316-327.

Quirk, G. J., Russo, G. K., Barron, J. L. & Lebron, K. (2000) The role of ventromedial prefrontal cortex in the recovery of extinguished fear. J Neurosci., 20, 6225-6231.

Quirk, G. J. (2002) Memory for extinction of conditioned fear is long-lasting and persists following spontaneous recovery. Learn.Mem., 9, 402-407.

Quirk, G. J., Garcia, R. & Gonzalez-Lima, F. (2006) Prefrontal mechanisms in extinction of conditioned fear. Biol.Psychiatry, 60, 337-343.

Quirk, G. J. & Mueller, D. (2008) Neural mechanisms of extinction learning and retrieval. Neuropsychopharmacology, 33, 56-72.

Qureshi, S. U., Pyne, J. M., Magruder, K. M., Schulz, P. E. & Kunik, M. E. (2009) The link between post-traumatic stress disorder and physical comorbidities: a systematic review. Psychiatr.Q., 80, 87-97.

Qureshi, S. U., Long, M. E., Bradshaw, M. R., Pyne, J. M., Magruder, K. M., Kimbrell, T., Hudson, T. J., Jawaid, A., Schulz, P. E. & Kunik, M. E. (2011) Does PTSD impair cognition beyond the effect of trauma? J Neuropsychiatry Clin.Neurosci., 23, 16-28.

Raison, C. L., Borisov, A. S., Majer, M., Drake, D. F., Pagnoni, G., Woolwine, B. J., Vogt, G. J., Massung, B. & Miller, A. H. (2009) Activation of central nervous system inflammatory pathways by interferon-alpha: relationship to monoamines and depression. Biol.Psychiatry, 65, 296-303.

Raison, C. L., Dantzer, R., Kelley, K. W., Lawson, M. A., Woolwine, B. J., Vogt, G., Spivey, J. R., Saito, K. & Miller, A. H. (2010) CSF concentrations of brain tryptophan and kynurenines during immune stimulation with IFN-alpha: relationship to CNS immune responses and depression. Mol.Psychiatry, 15, 393-403.

Rakofsky, J. J., Ressler, K. J. & Dunlop, B. W. (2012) BDNF function as a potential mediator of bipolar disorder and post-traumatic stress disorder comorbidity. Mol.Psychiatry, 17, 22-35.

Rammal, H., Bouayed, J., Younos, C. & Soulimani, R. (2008) Evidence that oxidative stress is linked to anxiety-related behaviour in mice. Brain Behav.Immun., 22, 1156-1159.

Ramsahoye, B. H., Biniszkiewicz, D., Lyko, F., Clark, V., Bird, A. P. & Jaenisch, R. (2000) Non-CpG methylation is prevalent in embryonic stem cells and may be mediated by DNA methyltransferase 3a. Proc.Natl.Acad.Sci.U.S.A, 97, 5237-5242.

Ransohoff, R. M. (2009) Chemokines and chemokine receptors: standing at the crossroads of immunobiology and neurobiology. Immunity., 31, 711-721.

Raskind, M. A., Peskind, E. R., Kanter, E. D., Petrie, E. C., Radant, A., Thompson, C. E., Dobie, D. J., Hoff, D., Rein, R. J., Straits-Troster, K., Thomas, R. G. & McFall, M. M. (2003) Reduction of nightmares and other PTSD symptoms in combat veterans by prazosin: a placebo-controlled study. Am J Psychiatry, 160, 371-373.

Raskind, M. A., Peskind, E. R., Hoff, D. J., Hart, K. L., Holmes, H. A., Warren, D., Shofer, J., O'Connell, J., Taylor, F., Gross, C., Rohde, K. & McFall, M. E. (2007) A parallel group placebo controlled study of prazosin for trauma nightmares and sleep disturbance in combat veterans with post-traumatic stress disorder. Biol.Psychiatry, 61, 928-934.

Rasmusson, A. M., Hauger, R. L., Morgan, C. A., Bremner, J. D., Charney, D. S. & Southwick, S. M. (2000) Low baseline and yohimbine-stimulated plasma neuropeptide Y (NPY) levels in combat-related PTSD. Biol.Psychiatry, 47, 526-539.

Rasmusson, A. M., Shi, L. & Duman, R. (2002) Downregulation of BDNF mRNA in the hippocampal dentate gyrus after re-exposure to cues previously associated with footshock. Neuropsychopharmacology, 27, 133-142.

Rattiner, L. M., Davis, M., French, C. T. & Ressler, K. J. (2004) Brain-derived neurotrophic factor and tyrosine kinase receptor B involvement in amygdala-dependent fear conditioning. J Neurosci., 24, 4796-4806.

Rauch, S. L., Savage, C. R., Alpert, N. M., Fischman, A. J. & Jenike, M. A. (1997) The functional neuroanatomy of anxiety: a study of three disorders using positron emission tomography and symptom provocation. Biol.Psychiatry, 42, 446-452.

Razin, A. (1998) CpG methylation, chromatin structure and gene silencing-a three-way connection. EMBO J, 17, 4905-4908.

Reali, C., Scintu, F., Pillai, R., Donato, R., Michetti, F. & Sogos, V. (2005) S100b counteracts effects of the neurotoxicant trimethyltin on astrocytes and microglia. J Neurosci.Res, 81, 677-686.

Rebaudo, R., Melani, R., Balestrino, M., Cupello, A., Haglid, K. & Hyden, H. (2000) Antiserum against S-100 protein prevents long term potentiation through a cAMP-related mechanism. Neurochem.Res, 25, 541-545.

Redrobe, J. P., Dumont, Y., St-Pierre, J. A. & Quirion, R. (1999) Multiple receptors for neuropeptide Y in the hippocampus: putative roles in seizures and cognition. Brain Res, 848, 153-166.

Reich, D. B., Winternitz, S., Hennen, J., Watts, T. & Stanculescu, C. (2004) A preliminary study of risperidone in the treatment of posttraumatic stress disorder related to childhood abuse in women. J Clin.Psychiatry, 65, 1601-1606.

Reik, W. & Walter, J. (2001) Evolution of imprinting mechanisms: the battle of the sexes begins in the zygote. Nat.Genet. , 27, 255-256.

Reisel, D., Bannerman, D. M., Schmitt, W. B., Deacon, R. M., Flint, J., Borchardt, T., Seeburg, P. H. & Rawlins, J. N. (2002) Spatial memory dissociations in mice lacking GluR1. Nat.Neurosci., 5, 868-873.

Rescorla, R. A. (2004) Spontaneous recovery. Learn.Mem., 11, 501-509.

Ressler, K. J., Rothbaum, B. O., Tannenbaum, L., Anderson, P., Graap, K., Zimand, E., Hodges, L. & Davis, M. (2004) Cognitive enhancers as adjuncts to psychotherapy: use of D-cycloserine in phobic individuals to facilitate extinction of fear. Arch.Gen.Psychiatry, 61, 1136-1144.

Ressler, K. J., Mercer, K. B., Bradley, B., Jovanovic, T., Mahan, A., Kerley, K., Norrholm, S. D., Kilaru, V., Smith, A. K., Myers, A. J., Ramirez, M., Engel, A., Hammack, S. E., Toufexis, D., Braas, K. M., Binder, E. B. & May, V. (2011) Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. Nature, 470, 492-497.

Reus, G. Z., Stringari, R. B., Ribeiro, K. F., Cipriano, A. L., Panizzutti, B. S., Stertz, L., Lersch, C., Kapczinski, F. & Quevedo, J. (2011) Maternal deprivation induces depressive-like behaviour and alters neurotrophin levels in the rat brain. Neurochem.Res, 36, 460-466.

Reyes-Herrera, P. H., Ficarra, E., Acquaviva, A. & Macii, E. (2011) miREE: miRNA recognition elements ensemble. BMC.Bioinformatics., 12, 454.

Reyes-Herrera, P. H., Ficarra, E., Acquaviva, A. & Macii, E. (2011) miREE: miRNA recognition elements ensemble. Bmc Bioinformatics, 12.

Richardson, R., Ledgerwood, L. & Cranney, J. (2004) Facilitation of fear extinction by D-cycloserine: theoretical and clinical implications. Learn.Mem., 11, 510-516.

Rideout, W. M., III, Coetzee, G. A., Olumi, A. F. & Jones, P. A. (1990) 5-Methylcytosine as an endogenous mutagen in the human LDL receptor and p53 genes. Science, 249, 1288-1290.

Riggs, D. S., Rukstalis, M., Volpicelli, J. R., Kalmanson, D. & Foa, E. B. (2003) Demographic and social adjustment characteristics of patients with comorbid posttraumatic stress disorder and alcohol dependence: potential pitfalls to PTSD treatment. Addict.Behav., 28, 1717-1730.

Rinaldi, A., Vincenti, S., De, V. F., Bozzoni, I., Oliverio, A., Presutti, C., Fragapane, P. & Mele, A. (2010) Stress induces region specific alterations in microRNAs expression in mice. Behav.Brain Res, 208, 265-269.

Risch, N. & Merikangas, K. (1996) The future of genetic studies of complex human diseases. Science, 273, 1516-1517.

Rivest, S. (2009) Regulation of innate immune responses in the brain. Nat.Rev.Immunol., 9, 429-439.

Rivier, C. & Rivest, S. (1991) Effect of stress on the activity of the hypothalamic-pituitary-gonadal axis: peripheral and central mechanisms. Biol.Reprod., 45, 523-532.

Robertson, K. D. & Wolffe, A. P. (2000) DNA methylation in health and disease. Nat.Rev.Genet., 1, 11-19.

Robison, A. J. & Nestler, E. J. (2011) Transcriptional and epigenetic mechanisms of addiction. Nat.Rev.Neurosci., 12, 623-637.

Rod, N. H., Gronbaek, M., Schnohr, P., Prescott, E. & Kristensen, T. S. (2009) Perceived stress as a risk factor for changes in health behaviour and cardiac risk profile: a longitudinal study. J Intern.Med., 266, 467-475.

Rodgers, R. J. & Dalvi, A. (1997) Anxiety, defence and the elevated plus-maze. Neurosci.Biobehav.Rev., 21, 801-810.

Rogaev, E. I. (2005) Small RNAs in human brain development and disorders. Biochemistry (Mosc.), 70, 1404-1407.

Rollins, C., Glass, N. E., Perrin, N. A., Billhardt, K. A., Clough, A., Barnes, J., Hanson, G. C. & Bloom, T. L. (2012) Housing instability is as strong a predictor of poor health outcomes as level of danger in an abusive relationship: findings from the SHARE Study. J Interpers.Violence, 27, 623-643.

Roth, T. L., Roth, E. D. & Sweatt, J. D. (2010) Epigenetic regulation of genes in learning and memory. Essays Biochem., 48, 263-274.

Roth, T. L., Zoladz, P. R., Sweatt, J. D. & Diamond, D. M. (2011) Epigenetic modification of hippocampal Bdnf DNA in adult rats in an animal model of post-traumatic stress disorder. J.Psychiatr.Res., 45, 919-926.

Rothbaum, B. O. & Foa, E. B. (2002) Post-traumatic stress disorder and sleep. N.Engl.J Med., 346, 1334-1335.

Rothbaum, B. O. & Davis, M. (2003) Applying learning principles to the treatment of post-trauma reactions. Ann.N.Y.Acad.Sci., 1008, 112-121.

Rothbaum, B. O., Killeen, T. K., Davidson, J. R., Brady, K. T., Connor, K. M. & Heekin, M. H. (2008) Placebo-controlled trial of risperidone augmentation for selective serotonin reuptake inhibitor-resistant civilian posttraumatic stress disorder. J Clin.Psychiatry, 69, 520-525.

Rudy, J. W., Huff, N. C. & Matus-Amat, P. (2004) Understanding contextual fear conditioning: insights from a two-process model. Neurosci.Biobehav.Rev., 28, 675-685.

Rupprecht, T. A., Plate, A., Adam, M., Wick, M., Kastenbauer, S., Schmidt, C., Klein, M., Pfister, H. W. & Koedel, U. (2009) The chemokine CXCL13 is a key regulator of B cell recruitment to the cerebrospinal fluid in acute Lyme neuroborreliosis. J Neuroinflammation., 6, 42.

Rusiecki, J. A., Chen, L., Srikantan, V., Zhang, L., Yan, L., Polin, M. L. & Baccarelli, A. (2012) DNA methylation in repetitive elements and post-traumatic stress disorder: a case-control study of US military service members. Epigenomics., 4, 29-40.

Ryckman, C., Vandal, K., Rouleau, P., Talbot, M. & Tessier, P. A. (2003) Proinflammatory activities of S100: proteins S100A8, S100A9, and S100A8/A9 induce neutrophil chemotaxis and adhesion. J Immunol. , 170, 3233-3242.

Saal, S. & Harvey, S. J. (2009) MicroRNAs and the kidney: coming of age. Curr.Opin.Nephrol.Hypertens., 18, 317-323.

Saavedra, J. M., Sanchez-Lemus, E. & Benicky, J. (2011) Blockade of brain angiotensin II AT1 receptors ameliorates stress, anxiety, brain inflammation and ischemia: Therapeutic implications. Psychoneuroendocrinology, 36, 1-18.

Sabbatini, M., Coppi, G., Maggioni, A., Olgiati, V., Panocka, I. & Amenta, F. (1998) Effect of lesions of the nucleus basalis magnocellularis and of treatment with posatirelin on cholinergic neurotransmission enzymes in the rat cerebral cortex. Mech.Ageing Dev., 104, 183-194.

Sack, W. H., Clarke, G. N. & Seeley, J. (1995) Posttraumatic stress disorder across two generations of Cambodian refugees. J.Am.Acad.Child Adolesc.Psychiatry, 34, 1160-1166.

Saetre, P., Emilsson, L., Axelsson, E., Kreuger, J., Lindholm, E. & Jazin, E. (2007) Inflammation-related genes up-regulated in schizophrenia brains. BMC Psychiatry, 7, 46.

Sah, R. & Geracioti, T. D. (2013) Neuropeptide Y and posttraumatic stress disorder. Mol.Psychiatry, 18, 646-655.

Saini, H. K., Griffiths-Jones, S. & Enright, A. J. (2007) Genomic analysis of human microRNA transcripts. Proc.Natl.Acad.Sci.U.S.A, 104, 17719-17724.

Sajdyk, T. J., Vandergriff, M. G. & Gehlert, D. R. (1999) Amygdalar neuropeptide Y Y1 receptors mediate the anxiolytic-like actions of neuropeptide Y in the social interaction test. Eur.J Pharmacol., 368, 143-147.

Sajdyk, T. J., Schober, D. A. & Gehlert, D. R. (2002) Neuropeptide Y receptor subtypes in the basolateral nucleus of the amygdala modulate anxiogenic responses in rats. Neuropharmacology, 43, 1165-1172.

Salim, S., Chugh, G. & Asghar, M. (2012) Inflammation in anxiety. Adv.Protein Chem.Struct.Biol., 88, 1-25.

Salomon, R. & Kaye, A. M. (1970) Methylation of mouse DNA in vivo: di- and tripyrimidine sequences containing 5-methylcytosine. Biochim.Biophys.Acta, 204, 340-351.

Sangokoya, C., Telen, M. J. & Chi, J. T. (2010) microRNA miR-144 modulates oxidative stress tolerance and associates with anemia severity in sickle cell disease. Blood, 116, 4338-4348.

Santamaria-Kisiel, L., Rintala-Dempsey, A. C. & Shaw, G. S. (2006) Calcium-dependent and -independent interactions of the S100 protein family. Biochem.J, 396, 201-214.

Santini, E., Muller, R. U. & Quirk, G. J. (2001) Consolidation of extinction learning involves transfer from NMDA-independent to NMDA-dependent memory. J Neurosci., 21, 9009-9017.

Sarapas, C., Cai, G., Bierer, L. M., Golier, J. A., Galea, S., Ising, M., Rein, T., Schmeidler, J., Muller-Myhsok, B., Uhr, M., Holsboer, F., Buxbaum, J. D. & Yehuda, R. (2011) Genetic markers for PTSD risk and resilience among survivors of the World Trade Center attacks. Dis.Markers, 30, 101-110.

Sartor, C. E., Grant, J. D., Lynskey, M. T., McCutcheon, V. V., Waldron, M., Statham, D. J., Bucholz, K. K., Madden, P. A., Heath, A. C., Martin, N. G. & Nelson, E. C. (2012) Common heritable contributions to low-risk trauma, high-risk trauma, posttraumatic stress disorder, and major depression. Arch.Gen.Psychiatry, 69, 293-299.

Sasaki, H. & Matsui, Y. (2008) Epigenetic events in mammalian germ-cell development: reprogramming and beyond. Nat.Rev.Genet., 9, 129-140.

Sato, M. & Bremner, I. (1993) Oxygen free radicals and metallothionein. Free Radic.Biol.Med., 14, 325-337.

Sato, W., Tomita, A., Ichikawa, D., Lin, Y., Kishida, H., Miyake, S., Ogawa, M., Okamoto, T., Murata, M., Kuroiwa, Y., Aranami, T. & Yamamura, T. (2012) CCR2(+)CCR5(+) T cells produce matrix metalloproteinase-9 and osteopontin in the pathogenesis of multiple sclerosis. J Immunol., 189, 5057-5065.

Satoh, J., Obayashi, S., Misawa, T., Tabunoki, H., Yamamura, T., Arima, K. & Konno, H. (2008) Neuromyelitis optica/Devic's disease: gene expression profiling of brain lesions. Neuropathology., 28, 561-576.

Sattin, A. (1999) The role of TRH and related peptides in the mechanism of action of ECT. J ECT., 15, 76-92.

Sautter, F. J., Bissette, G., Wiley, J., Manguno-Mire, G., Schoenbachler, B., Myers, L., Johnson, J. E., Cerbone, A. & Malaspina, D. (2003) Corticotropin-releasing factor in posttraumatic stress disorder (PTSD) with secondary psychotic symptoms, nonpsychotic PTSD, and healthy control subjects. Biol.Psychiatry, 54, 1382-1388.

Sayin, A., Kucukyildirim, S., Akar, T., Bakkaloglu, Z., Demircan, A., Kurtoglu, G., Demirel, B., Candansayar, S. & Mergen, H. (2010) A prospective study of serotonin transporter gene promoter (5-HTT gene linked polymorphic region) and intron 2 (variable number of tandem repeats) polymorphisms as predictors of trauma response to mild physical injury. DNA Cell Biol., 29, 71-77.

Scaccianoce, S., Del, B. P., Pannitteri, G. & Passarelli, F. (2004) Relationship between stress and circulating levels of S100B protein. Brain Res, 1004, 208-211.

Scapagnini, G., Davinelli, S., Drago, F., De, L. A. & Oriani, G. (2012) Antioxidants as antidepressants: fact or fiction? CNS.Drugs, 26, 477-490.

Scatena, M., Liaw, L. & Giachelli, C. M. (2007) Osteopontin: a multifunctional molecule regulating chronic inflammation and vascular disease. Arterioscler.Thromb.Vasc.Biol., 27, 2302-2309.

Schafe, G. E., Nader, K., Blair, H. T. & LeDoux, J. E. (2001) Memory consolidation of Pavlovian fear conditioning: a cellular and molecular perspective. Trends Neurosci., 24, 540-546.

Scheibner, K. A., Lutz, M. A., Boodoo, S., Fenton, M. J., Powell, J. D. & Horton, M. R. (2006) Hyaluronan fragments act as an endogenous danger signal by engaging TLR2. J Immunol., 177, 1272-1281.

Schelling, G., Briegel, J., Roozendaal, B., Stoll, C., Rothenhausler, H. B. & Kapfhammer, H. P. (2001) The effect of stress doses of hydrocortisone during septic shock on posttraumatic stress disorder in survivors. Biol.Psychiatry, 50, 978-985.

Schiavone, S., Sorce, S., Dubois-Dauphin, M., Jaquet, V., Colaianna, M., Zotti, M., Cuomo, V., Trabace, L. & Krause, K. H. (2009) Involvement of NOX2 in the development of behavioural and pathologic alterations in isolated rats. Biol.Psychiatry, 66, 384-392.

Schloss, P. & Williams, D. C. (1998) The serotonin transporter: a primary target for antidepressant drugs. J Psychopharmacol., 12, 115-121.

Schmidt, A. M., Yan, S. D. & Stern, D. M. (1995) The dark side of glucose. Nat.Med., 1, 1002-1004.

Schmidt, S., Saunders, A. M., De La Paz, M. A., Postel, E. A., Heinis, R. M., Agarwal, A., Scott, W. K., Gilbert, J. R., McDowell, J. G., Bazyk, A., Gass, J. D., Haines, J. L. & Pericak-Vance, M. A. (2000) Association of the apolipoprotein E gene with age-related macular degeneration: possible effect modification by family history, age, and gender. Mol.Vis., 6, 287-293.

Schmidt, U., Herrmann, L., Hagl, K., Novak, B., Huber, C., Holsboer, F., Wotjak, C. T. & Buell, D. R. (2013) Therapeutic Action of Fluoxetine is Associated with a Reduction in Prefrontal Cortical miR-1971 Expression Levels in a Mouse Model of Posttraumatic Stress Disorder. Front Psychiatry, 4, 66.

Schmittgen, T. D. & Zakrajsek, B. A. (2000) Effect of experimental treatment on housekeeping gene expression: validation by real-time, quantitative RT-PCR. J Biochem.Biophys.Methods, 46, 69-81.

Schneider, H., Pitossi, F., Balschun, D., Wagner, A., del, R. A. & Besedovsky, H. O. (1998) A neuromodulatory role of interleukin-1beta in the hippocampus. Proc.Natl.Acad.Sci.U.S.A, 95, 7778-7783.

Schnurr, P. P., Lunney, C. A., Sengupta, A. & Waelde, L. C. (2003) A descriptive analysis of PTSD chronicity in Vietnam veterans. J Trauma Stress., 16, 545-553.

Schouten, M., Aschrafi, A., Bielefeld, P., Doxakis, E. & Fitzsimons, C. P. (2013) microRNAs and the regulation of neuronal plasticity under stress conditions. Neuroscience, 241, 188-205.

Schratt, G. M., Tuebing, F., Nigh, E. A., Kane, C. G., Sabatini, M. E., Kiebler, M. & Greenberg, M. E. (2006) A brain-specific microRNA regulates dendritic spine development. Nature, 439, 283-289.

Schwarcz, R. (2001) Glutamate 2000: old dog, new tricks. J Pharmacol.Exp.Ther., 296, 659-662.

Schwartz, M. & Shechter, R. (2010) Protective autoimmunity functions by intracranial immunosurveillance to support the mind: The missing link between health and disease. Mol.Psychiatry, 15, 342-354.

Schwarzer, C., Kofler, N. & Sperk, G. (1998) Up-regulation of neuropeptide Y-Y2 receptors in an animal model of temporal lobe epilepsy. Mol.Pharmacol., 53, 6-13.

Scott, H., Howarth, J., Lee, Y. B., Wong, L. F., Bantounas, I., Phylactou, L., Verkade, P. & Uney, J. B. (2012) MiR-3120 is a mirror microRNA that targets heat shock cognate protein 70 and auxilin messenger RNAs and regulates clathrin vesicle uncoating. J Biol.Chem., 287, 14726-14733.

Searle, P. F., Davison, B. L., Stuart, G. W., Wilkie, T. M., Norstedt, G. & Palmiter, R. D. (1984) Regulation, linkage, and sequence of mouse metallothionein I and II genes. Mol.Cell Biol., 4, 1221-1230.

Seckl, J. R. (1994) Glucocorticoids and small babies. Q.J Med., 87, 259-262.

Seeman, T., Epel, E., Gruenewald, T., Karlamangla, A. & McEwen, B. S. (2010) Socio-economic differentials in peripheral biology: cumulative allostatic load. Ann.N.Y.Acad.Sci., 1186, 223-239.

Segman, R. H., Cooper-Kazaz, R., Macciardi, F., Goltser, T., Halfon, Y., Dobroborski, T. & Shalev, A. Y. (2002) Association between the dopamine transporter gene and posttraumatic stress disorder. Mol.Psychiatry, 7, 903-907.

Segman, R. H., Shefi, N., Goltser-Dubner, T., Friedman, N., Kaminski, N. & Shalev, A. Y. (2005) Peripheral blood mononuclear cell gene expression profiles identify emergent post-traumatic stress disorder among trauma survivors. Mol.Psychiatry, 10, 500-13, 425.

Selbach, M., Schwanhausser, B., Thierfelder, N., Fang, Z., Khanin, R. & Rajewsky, N. (2008) Widespread changes in protein synthesis induced by microRNAs. Nature, 455, 58-63.

Selbach, M., Schwanhausser, B., Thierfelder, N., Fang, Z., Khanin, R. & Rajewsky, N. (2008) Widespread changes in protein synthesis induced by microRNAs. Nature, 455, 58-63.

Sellebjerg, F., Jaliashvili, I., Christiansen, M. & Garred, P. (1998) Intrathecal activation of the complement system and disability in multiple sclerosis. J Neurol.Sci., 157, 168-174.

Selvaraju, R., Bernasconi, L., Losberger, C., Graber, P., Kadi, L., vellana-Adalid, V., Picard-Riera, N., Baron Van, E. A., Cirillo, R., Kosco-Vilbois, M., Feger, G., Papoian, R. & Boschert, U. (2004) Osteopontin is upregulated during in vivo demyelination and remyelination and enhances myelin formation in vitro. Mol.Cell Neurosci., 25, 707-721.

Senolt, L., Grigorian, M., Lukanidin, E., Simmen, B., Michel, B. A., Pavelka, K., Gay, R. E., Gay, S. & Neidhart, M. (2006) S100A4 is expressed at site of invasion in rheumatoid arthritis synovium and modulates production of matrix metalloproteinases. Ann.Rheum.Dis., 65, 1645-1648.

Serafini, B., Rosicarelli, B., Magliozzi, R., Stigliano, E. & Aloisi, F. (2004) Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. Brain Pathol., 14, 164-174.

Serova, L. I., Tillinger, A., Alaluf, L. G., Laukova, M., Keegan, K. & Sabban, E. L. (2013) Single intranasal neuropeptide Y infusion attenuates development of PTSD-like symptoms to traumatic stress in rats. Neuroscience, 236, 298-312.

Serradeil-Le, G. C., Wagnon, J., Simiand, J., Griebel, G., Lacour, C., Guillon, G., Barberis, C., Brossard, G., Soubrie, P., Nisato, D., Pascal, M., Pruss, R., Scatton, B., Maffrand, J. P. & Le, F. G. (2002) Characterization of (2S,4R)-1-[5-chloro-1-[(2,4-dimethoxyphenyl)sulfonyl]-3-(2-methoxy-phenyl)-2-oxo-   2,3-dihydro-1H-indol-3-yl]-4-hydroxy-N,N-dimethyl-2-pyrrolidine carboxamide (SSR149415), a selective and orally active vasopressin V1b receptor antagonist. J Pharmacol.Exp.Ther., 300, 1122-1130.

Shah, V. B., Huang, Y., Keshwara, R., Ozment-Skelton, T., Williams, D. L. & Keshvara, L. (2008) Beta-glucan activates microglia without inducing cytokine production in Dectin-1-dependent manner. J Immunol., 180, 2777-2785.

Shaw, D., Norwood, K., Sharp, K., Quigley, L., McGovern, S. F. & Leslie, J. C. (2009) Facilitation of extinction of operant behaviour in mice by D-cycloserine. Psychopharmacology (Berl), 202, 397-402.

Shelton, M. D. & Mieyal, J. J. (2008) Regulation by reversible S-glutathionylation: molecular targets implicated in inflammatory diseases. Mol.Cells, 25, 332-346.

Shelton, R. C. (2007) The molecular neurobiology of depression. Psychiatr.Clin.North Am, 30, 1-11.

Shen, Y., Li, R., McGeer, E. G. & McGeer, P. L. (1997) Neuronal expression of mRNAs for complement proteins of the classical pathway in Alzheimer brain. Brain Res, 769, 391-395.

Shendure, J. & Ji, H. (2008) Next-generation DNA sequencing. Nat.Biotechnol., 26, 1135-1145.

Shestatzky, M., Greenberg, D. & Lerer, B. (1988) A controlled trial of phenelzine in posttraumatic stress disorder. Psychiatry Res, 24, 149-155.

Shin, S. L., Cha, J. H., Chun, M. H., Chung, J. W. & Lee, M. Y. (1999) Expression of osteopontin mRNA in the adult rat brain. Neurosci.Lett., 273, 73-76.

Shin, T., Ahn, M., Kim, H., Moon, C., Kang, T. Y., Lee, J. M., Sim, K. B. & Hyun, J. W. (2005) Temporal expression of osteopontin and CD44 in rat brains with experimental cryolesions. Brain Res, 1041, 95-101.

Shipherd, J. C., Keyes, M., Jovanovic, T., Ready, D. J., Baltzell, D., Worley, V., Gordon-Brown, V., Hayslett, C. & Duncan, E. (2007) Veterans seeking treatment for posttraumatic stress disorder: what about comorbid chronic pain? J Rehabil.Res Dev., 44, 153-166.

Shirane, K., Toh, H., Kobayashi, H., Miura, F., Chiba, H., Ito, T., Kono, T. & Sasaki, H. (2013) Mouse oocyte methylomes at base resolution reveal genome-wide accumulation of non-CpG methylation and role of DNA methyltransferases. PLoS Genet., 9, e1003439.

Siegmund, A. & Wotjak, C. T. (2007) A mouse model of posttraumatic stress disorder that distinguishes between conditioned and sensitised fear. J Psychiatr.Res, 41, 848-860.

Siegmund, A., Dahlhoff, M., Habersetzer, U., Mederer, A., Wolf, E., Holsboer, F. & Wotjak, C. T. (2009) Maternal inexperience as a risk factor of innate fear and PTSD-like symptoms in mice. J Psychiatr.Res, 43, 1156-1165.

Siemers, E. R., Shekhar, A., Quaid, K. & Dickson, H. (1993) Anxiety and motor performance in Parkinson's disease. Mov Disord., 8, 501-506.

Silva, A. J., Giese, K. P., Fedorov, N. B., Frankland, P. W. & Kogan, J. H. (1998) Molecular, cellular, and neuroanatomical substrates of place learning. Neurobiol.Learn.Mem., 70, 44-61.

Silverman, M. N., Pearce, B. D., Biron, C. A. & Miller, A. H. (2005) Immune modulation of the hypothalamic-pituitary-adrenal (HPA) axis during viral infection. Viral Immunol., 18, 41-78.

Silverstein, S. C. (1995) Phagocytosis of microbes: insights and prospects. Trends Cell Biol., 5, 141-142.

Simonini, M. V., Camargo, L. M., Dong, E., Maloku, E., Veldic, M., Costa, E. & Guidotti, A. (2006) The benzamide MS-275 is a potent, long-lasting brain region-selective inhibitor of histone deacetylases. Proc.Natl.Acad.Sci.U.S.A, 103, 1587-1592.

Singhrao, S. K., Neal, J. W., Rushmere, N. K., Morgan, B. P. & Gasque, P. (2000) Spontaneous classical pathway activation and deficiency of membrane regulators render human neurons susceptible to complement lysis. Am J Pathol., 157, 905-918.

Skelton, K., Ressler, K. J., Norrholm, S. D., Jovanovic, T. & Bradley-Davino, B. (2012) PTSD and gene variants: new pathways and new thinking. Neuropharmacology, 62, 628-637.

Smith, A. K., Dimulescu, I., Falkenberg, V. R., Narasimhan, S., Heim, C., Vernon, S. D. & Rajeevan, M. S. (2008) Genetic evaluation of the serotonergic system in chronic fatigue syndrome. Psychoneuroendocrinology, 33, 188-197.

Smith, A. K., Conneely, K. N., Kilaru, V., Mercer, K. B., Weiss, T. E., Bradley, B., Tang, Y., Gillespie, C. F., Cubells, J. F. & Ressler, K. J. (2011) Differential immune system DNA methylation and cytokine regulation in post-traumatic stress disorder. Am J Med.Genet.B Neuropsychiatr.Genet., 156B, 700-708.

Smits, J. A., Rosenfield, D., Otto, M. W., Marques, L., Davis, M. L., Meuret, A. E., Simon, N. M., Pollack, M. H. & Hofmann, S. G. (2013) d-cycloserine enhancement of exposure therapy for social anxiety disorder depends on the success of exposure sessions. J Psychiatr.Res.

Solter, V., Thaller, V., Karlovic, D. & Crnkovic, D. (2002) Elevated serum lipids in veterans with combat-related chronic posttraumatic stress disorder. Croat.Med.J, 43, 685-689.

Song, C., Merali, Z. & Anisman, H. (1999) Variations of nucleus accumbens dopamine and serotonin following systemic interleukin-1, interleukin-2 or interleukin-6 treatment. Neuroscience, 88, 823-836.

Sonne, C., Carlsson, J., Elklit, A., Mortensen, E. L. & Ekstrom, M. (2013) Treatment of traumatized refugees with Sertraline versus Venlafaxine in combination with psychotherapy - study protocol for a randomized clinical trial. Trials, 14, 137.

Sorce, S. & Krause, K. H. (2009) NOX enzymes in the central nervous system: from signaling to disease. Antioxid.Redox.Signal., 11, 2481-2504.

Sorg, B. A. & Kalivas, P.W. (1995) Stress and neuronal sensitization. In: Friedman, M.J., Charney, D. S. & Deutch, A.Y., editors. Neurobiological and clinical consequences of stress. From normal adaptation to post-traumatic stress disorder. Philadelphia, New York: Lipponcott-Raven; 1995. p. 83–102.

Sousa, N., Almeida, O. F. & Wotjak, C. T. (2006) A hitchhiker's guide to behavioural analysis in laboratory rodents. Genes Brain Behav., 5 Suppl 2, 5-24.

Southwick, S. M., Bremner, J. D., Rasmusson, A., Morgan, C. A., III, Arnsten, A. & Charney, D. S. (1999) Role of norepinephrine in the pathophysiology and treatment of posttraumatic stress disorder. Biol.Psychiatry, 46, 1192-1204.

Southwick, S. M., Davis, M., Horner, B., Cahill, L., Morgan, C. A., III, Gold, P. E., Bremner, J. D. & Charney, D. C. (2002) Relationship of enhanced norepinephrine activity during memory consolidation to enhanced long-term memory in humans. Am J Psychiatry, 159, 1420-1422.

Spulber, S., Mateos, L., Oprica, M., Cedazo-Minguez, A., Bartfai, T., Winblad, B. & Schultzberg, M. (2009) Impaired long term memory consolidation in transgenic mice overexpressing the human soluble form of IL-1ra in the brain. J Neuroimmunol., 208, 46-53.

Stadler, M. B., Murr, R., Burger, L., Ivanek, R., Lienert, F., Scholer, A., van, N. E., Wirbelauer, C., Oakeley, E. J., Gaidatzis, D., Tiwari, V. K. & Schubeler, D. (2011) DNA-binding factors shape the mouse methylome at distal regulatory regions. Nature, 480, 490-495.

Stahl, S. M. (2005) Is psychopharmacologic "inoculation" effective in preventing posttraumatic stress disorder? J Clin.Psychiatry, 66, 5-6.

Stankiewicz, A. M., Swiergiel, A. H. & Lisowski, P. (2013) Epigenetics of Stress Adaptations in the Brain. Brain Res Bull..

Steckert, A. V., Valvassori, S. S., Moretti, M., Dal-Pizzol, F. & Quevedo, J. (2010) Role of oxidative stress in the pathophysiology of bipolar disorder. Neurochem.Res, 35, 1295-1301.

Steckler, T., Drinkenburg, W. H., Sahgal, A. & Aggleton, J. P. (1998) Recognition memory in rats--I. Concepts and classification. Prog.Neurobiol., 54, 289-311.

Steckler, T., Drinkenburg, W. H., Sahgal, A. & Aggleton, J. P. (1998) Recognition memory in rats--II. Neuroanatomical substrates. Prog.Neurobiol., 54, 313-332.

Steckler, T., Sahgal, A., Aggleton, J. P. & Drinkenburg, W. H. (1998) Recognition memory in rats--III. Neurochemical substrates. Prog.Neurobiol., 54, 333-348.

Steckler, T. & Risbrough, V. (2012) Pharmacological treatment of P. Neuropharmacology, 62, 617-627.

Stein, M. B., Heuser, I. J., Juncos, J. L. & Uhde, T. W. (1990) Anxiety disorders in patients with Parkinson's disease. Am J Psychiatry, 147, 217-220.

Stein, M. B., Yehuda, R., Koverola, C. & Hanna, C. (1997) Enhanced dexamethasone suppression of plasma cortisol in adult women traumatized by childhood sexual abuse. Biol.Psychiatry, 42, 680-686.

Stein, M. B., Jang, K. L., Taylor, S., Vernon, P. A. & Livesley, W. J. (2002) Genetic and environmental influences on trauma exposure and posttraumatic stress disorder symptoms: a twin study. Am.J.Psychiatry, 159, 1675-1681.

Stein, M. B., Kline, N. A. & Matloff, J. L. (2002) Adjunctive olanzapine for SSRI-resistant combat-related PTSD: a double-blind, placebo-controlled study. Am J Psychiatry, 159, 1777-1779.

Stein, M. B., Kerridge, C., Dimsdale, J. E. & Hoyt, D. B. (2007) Pharmacotherapy to prevent PTSD: Results from a randomized controlled proof-of-concept trial in physically injured patients. J Trauma Stress., 20, 923-932.

Steiner, D. F. (1998) The proprotein convertases. Curr.Opin.Chem.Biol., 2, 31-39.

Steinman, L. (2008) Nuanced roles of cytokines in three major human brain disorders. J Clin.Invest, 118, 3557-3563.

Stemmelin, J., Lukovic, L., Salome, N. & Griebel, G. (2005) Evidence that the lateral septum is involved in the antidepressant-like effects of the vasopressin V1b receptor antagonist, SSR149415. Neuropsychopharmacology, 30, 35-42.

Sternberg, E. M. (2006) Neural regulation of innate immunity: a coordinated nonspecific host response to pathogens. Nat.Rev.Immunol., 6, 318-328.

Stocca, G. & Nistri, A. (1995) Enhancement of NMDA receptor mediated synaptic potentials of rat hippocampal neurones in vitro by thyrotropin releasing hormone. Neurosci.Lett., 184, 9-12.

Storch, E. A., Murphy, T. K., Goodman, W. K., Geffken, G. R., Lewin, A. B., Henin, A., Micco, J. A., Sprich, S., Wilhelm, S., Bengtson, M. & Geller, D. A. (2010) A preliminary study of D-cycloserine augmentation of cognitive-behavioural therapy in pediatric obsessive-compulsive disorder. Biol.Psychiatry, 68, 1073-1076.

Strathdee, G. & Brown, R. (2002) Aberrant DNA methylation in cancer: potential clinical interventions. Expert.Rev.Mol.Med., 4, 1-17.

Strekalova, T., Spanagel, R., Bartsch, D., Henn, F. A. & Gass, P. (2004) Stress-induced anhedonia in mice is associated with deficits in forced swimming and exploration. Neuropsychopharmacology, 29, 2007-2017.

Su, T. P., Zhang, L., Chung, M. Y., Chen, Y. S., Bi, Y. M., Chou, Y. H., Barker, J. L., Barrett, J. E., Maric, D., Li, X. X., Li, H., Webster, M. J., Benedek, D., Carlton, J. R. & Ursano, R. (2009) Levels of the potential biomarker p11 in peripheral blood cells distinguish patients with PTSD from those with other major psychiatric disorders. J Psychiatr.Res, 43, 1078-1085.

Su, Y., Zhang, K. & Schluesener, H. J. (2010) Antimicrobial peptides in the brain. Arch.Immunol.Ther.Exp.(Warsz.), 58, 365-377.

Suarez, Y. & Sessa, W. C. (2009) MicroRNAs as novel regulators of angiogenesis. Circ.Res, 104, 442-454.

Sugimori, M., Lang, E. J., Silver, R. B. & Llinas, R. (1994) High-resolution measurement of the time course of calcium-concentration microdomains at squid presynaptic terminals. Biol.Bull., 187, 300-303.

Sultan, F. A., Wang, J., Tront, J., Liebermann, D. A. & Sweatt, J. D. (2012) Genetic deletion of Gadd45b, a regulator of active DNA demethylation, enhances long-term memory and synaptic plasticity. J Neurosci., 32, 17059-17066.

Sun, B. K. & Tsao, H. (2008). Small RNAs in development and disease. Journal of the American Academy of Dermatology 59, 725-37.

Susser, E., Neugebauer, R., Hoek, H. W., Brown, A. S., Lin, S., Labovitz, D. & Gorman, J. M. (1996) Schizophrenia after prenatal famine. Further evidence. Arch.Gen.Psychiatry, 53, 25-31.

Sutker, P. B., Galina, Z. H., West, J. A. & Allain, A. N. (1990) Trauma-induced weight loss and cognitive deficits among former prisoners of war. J Consult Clin.Psychol., 58, 323-328.

Sutker, P. B., Winstead, D. K., Galina, Z. H. & Allain, A. N. (1991) Cognitive deficits and psychopathology among former prisoners of war and combat veterans of the Korean conflict. Am J Psychiatry, 148, 67-72.

Suzuki, K., Nakajima, K., Otaki, N., Kimura, M., Kawaharada, U., Uehara, K., Hara, F., Nakazato, Y. & Takatama, M. (1994) Localization of metallothionein in aged human brain. Pathol.Int., 44, 20-26.

Suzuki, M. M. & Bird, A. (2008) DNA methylation landscapes: provocative insights from epigenomics. Nat.Rev.Genet., 9, 465-476.

Swerdlow, N. R., Martinez, Z. A., Hanlon, F. M., Platten, A., Farid, M., Auerbach, P., Braff, D. L. & Geyer, M. A. (2000) Toward understanding the biology of a complex phenotype: rat strain and substrain differences in the sensorimotor gating-disruptive effects of dopamine agonists. J Neurosci., 20, 4325-4336.

Swinbourne, J. M. & Touyz, S. W. (2007) The co-morbidity of eating disorders and anxiety disorders: a review. Eur.Eat.Disord.Rev., 15, 253-274.

Szapiro, G., Vianna, M. R., McGaugh, J. L., Medina, J. H. & Izquierdo, I. (2003) The role of NMDA glutamate receptors, PKA, MAPK, and CAMKII in the hippocampus in extinction of conditioned fear. Hippocampus, 13, 53-58.

Szyf, M., Weaver, I. C., Champagne, F. A., Diorio, J. & Meaney, M. J. (2005) Maternal programming of steroid receptor expression and phenotype through DNA methylation in the rat. Front Neuroendocrinol. , 26, 139-162.

Szyf, M. (2009) Epigenetics, DNA methylation, and chromatin modifying drugs. Annu.Rev.Pharmacol.Toxicol., 49, 243-263.

Tache, Y. & Bonaz, B. (2007) Corticotropin-releasing factor receptors and stress-related alterations of gut motor function. J Clin.Invest, 117, 33-40.

Takahashi, T., Morinobu, S., Iwamoto, Y. & Yamawaki, S. (2006) Effect of paroxetine on enhanced contextual fear induced by single prolonged stress in rats. Psychopharmacology (Berl), 189, 165-173.

Takamizawa, J., Konishi, H., Yanagisawa, K., Tomida, S., Osada, H., Endoh, H., Harano, T., Yatabe, Y., Nagino, M., Nimura, Y., Mitsudomi, T. & Takahashi, T. (2004) Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. Cancer Res, 64, 3753-3756.

Talebizadeh, Z., Butler, M. G. & Theodoro, M. F. (2008) Feasibility and relevance of examining lymphoblastoid cell lines to study role of microRNAs in autism. Autism Res, 1, 240-250.

Tambuyzer, B. R., Ponsaerts, P. & Nouwen, E. J. (2009) Microglia: gatekeepers of central nervous system immunology. J Leukoc.Biol., 85, 352-370.

Tang, X. Q., Gal, J., Zhuang, X., Wang, W. X., Zhu, H. N. & Tang, G. L. (2007) A simple array platform for microRNA analysis and its application in mouse tissues. Rna-A Publication of the Rna Society, 13, 1803-1822.

Tang, Y., Lu, A., Aronow, B. J., Wagner, K. R. & Sharp, F. R. (2002) Genomic responses of the brain to ischemic stroke, intracerebral haemorrhage, kainate seizures, hypoglycemia, and hypoxia. Eur.J Neurosci., 15, 1937-1952.

Tansey, M. G. & Goldberg, M. S. (2010) Neuroinflammation in Parkinson's disease: its role in neuronal death and implications for therapeutic intervention. Neurobiol.Dis., 37, 510-518.

Taylor, F. B., Martin, P., Thompson, C., Williams, J., Mellman, T. A., Gross, C., Peskind, E. R. & Raskind, M. A. (2008) Prazosin effects on objective sleep measures and clinical symptoms in civilian trauma posttraumatic stress disorder: a placebo-controlled study. Biol.Psychiatry , 63, 629-632.

Taylor, S. M. & Jones, P. A. (1985) Cellular differentiation. Int.J Obes., 9 Suppl 1, 15-21.

Teyler, T. J. (1987) Long-term potentiation and memory. Int.J Neurol., 21-22, 163-171.

Thakur, G. A., Joober, R. & Brunet, A. (2009) Development and persistence of posttraumatic stress disorder and the 5-HTTLPR polymorphism. J Trauma Stress., 22, 240-243.

Thanos, P. K., Bermeo, C., Wang, G. J. & Volkow, N. D. (2009) D-cycloserine accelerates the extinction of cocaine-induced conditioned place preference in C57bL/c mice. Behav.Brain Res, 199, 345-349.

Theeler, B. J. & Erickson, J. C. (2012) Posttraumatic headache in military personnel and veterans of the iraq and afghanistan conflicts. Curr.Treat.Options.Neurol., 14, 36-49.

Thiagarajan, P. S., Akbasli, A. C., Kinter, M. T., Willard, B. & Cathcart, M. K. (2013) Vimentin is a target of PKCbeta phosphorylation in MCP-1-activated primary human monocytes. Inflamm.Res.

Thompson, B. L. & Rosen, J. B. (2000) Effects of TRH on acoustic startle, conditioned fear and active avoidance in rats. Neuropeptides, 34, 38-44.

Thompson, K. L., Pine, P. S., Rosenzweig, B. A., Turpaz, Y. & Retief, J. (2007) Characterization of the effect of sample quality on high density oligonucleotide microarray data using progressively degraded rat liver RNA. BMC Biotechnol., 7, 57.

Thompson, L. T., Moskal, J. R. & Disterhoft, J. F. (1992) Hippocampus-dependent learning facilitated by a monoclonal antibody or D-cycloserine. Nature, 359, 638-641.

Thomson, D. W., Bracken, C. P. & Goodall, G. J. (2011) Experimental strategies for microRNA target identification. Nucleic Acids Res., 39, 6845-6853.

Thorsell, A., Carlsson, K., Ekman, R. & Heilig, M. (1999) Behavioural and endocrine adaptation, and up-regulation of NPY expression in rat amygdala following repeated restraint stress. Neuroreport, 10, 3003-3007.

Thorsell, A., Michalkiewicz, M., Dumont, Y., Quirion, R., Caberlotto, L., Rimondini, R., Mathe, A. A. & Heilig, M. (2000) Behavioural insensitivity to restraint stress, absent fear suppression of behavior and impaired spatial learning in transgenic rats with hippocampal neuropeptide Y overexpression. Proc.Natl.Acad.Sci.U.S.A, 97, 12852-12857.

Thyer, B. A. & Sowers-Hoag, K. M. (1988) Behavior therapy for separation anxiety disorder. Behav.Modif., 12, 205-233.

Timpl, P., Spanagel, R., Sillaber, I., Kresse, A., Reul, J. M., Stalla, G. K., Blanquet, V., Steckler, T., Holsboer, F. & Wurst, W. (1998) Impaired stress response and reduced anxiety in mice lacking a functional corticotropin-releasing hormone receptor 1. Nat.Genet., 19, 162-166.

Torregrossa, M. M., Sanchez, H. & Taylor, J. R. (2010) D-cycloserine reduces the context specificity of pavlovian extinction of cocaine cues through actions in the nucleus accumbens. J Neurosci., 30, 10526-10533.

Torres, G. E., Gainetdinov, R. R. & Caron, M. G. (2003) Plasma membrane monoamine transporters: structure, regulation and function. Nat.Rev.Neurosci., 4, 13-25.

Tremolizzo, L., Carboni, G., Ruzicka, W. B., Mitchell, C. P., Sugaya, I., Tueting, P., Sharma, R., Grayson, D. R., Costa, E. & Guidotti, A. (2002) An epigenetic mouse model for molecular and behavioural neuropathologies related to schizophrenia vulnerability. Proc.Natl.Acad.Sci.U.S.A, 99, 17095-17100.

Trendelenburg, G., Prass, K., Priller, J., Kapinya, K., Polley, A., Muselmann, C., Ruscher, K., Kannbley, U., Schmitt, A. O., Castell, S., Wiegand, F., Meisel, A., Rosenthal, A. & Dirnagl, U. (2002) Serial analysis of gene expression identifies metallothionein-II as major neuroprotective gene in mouse focal cerebral ischemia. J Neurosci., 22, 5879-5888.

Trias, J. & Benz, R. (1994) Permeability of the cell wall of Mycobacterium smegmatis. Mol.Microbiol., 14, 283-290.

Trief, P. M., Ouimette, P., Wade, M., Shanahan, P. & Weinstock, R. S. (2006) Post-traumatic stress disorder and diabetes: co-morbidity and outcomes in a male veterans sample. J Behav.Med., 29, 411-418.

True, W. R., Rice, J., Eisen, S. A., Heath, A. C., Goldberg, J., Lyons, M. J. & Nowak, J. (1993) A twin study of genetic and environmental contributions to liability for posttraumatic stress symptoms. Arch.Gen.Psychiatry, 50, 257-264.

Tsai, G. E., Falk, W. E., Gunther, J. & Coyle, J. T. (1999) Improved cognition in Alzheimer's disease with short-term D-cycloserine treatment. Am J Psychiatry, 156, 467-469.

Tsakiri, N., Kimber, I., Rothwell, N. J. & Pinteaux, E. (2008) Differential effects of interleukin-1 alpha and beta on interleukin-6 and chemokine synthesis in neurones. Mol.Cell Neurosci., 38, 259-265.

Tsaluchidu, S., Cocchi, M., Tonello, L. & Puri, B. K. (2008) Fatty acids and oxidative stress in psychiatric disorders. BMC Psychiatry, 8 Suppl 1, S5.

Tucker, P., Zaninelli, R., Yehuda, R., Ruggiero, L., Dillingham, K. & Pitts, C. D. (2001) Paroxetine in the treatment of chronic posttraumatic stress disorder: results of a placebo-controlled, flexible-dosage trial. J Clin.Psychiatry, 62, 860-868.

Tucker, P., Trautman, R. P., Wyatt, D. B., Thompson, J., Wu, S. C., Capece, J. A. & Rosenthal, N. R. (2007) Efficacy and safety of topiramate monotherapy in civilian posttraumatic stress disorder: a randomized, double-blind, placebo-controlled study. J Clin.Psychiatry, 68, 201-206.

Tucker, T., Marra, M. & Friedman, J. M. (2009) Massively parallel sequencing: the next big thing in genetic medicine. Am J Hum.Genet., 85, 142-154.

Tuppo, E. E. & Arias, H. R. (2005) The role of inflammation in Alzheimer's disease. Int.J Biochem.Cell Biol., 37, 289-305.

Turek-Plewa, J. & Jagodzinski, P. P. (2005) The role of mammalian DNA methyltransferases in the regulation of gene expression. Cell Mol.Biol.Lett., 10, 631-647.

Turner, B. M. (2002) Cellular memory and the histone code. Cell, 111, 285-291.

Turner, B. (2001) Chromatin and Gene Regulation. Blackwell Science Ltd., Oxford.

Turner, J. H., Neylan, T. C., Schiller, N. B., Li, Y. & Cohen, B. E. (2013) Objective Evidence of Myocardial Ischemia in Patients with Posttraumatic Stress Disorder. Biol.Psychiatry.

Tusher, V. G., Tibshirani, R. & Chu, G. (2001) Significance analysis of microarrays applied to the ionizing radiation response. Proc.Natl.Acad.Sci.U.S.A, 98, 5116-5121.

Twamley, E. W., Allard, C. B., Thorp, S. R., Norman, S. B., Hami, C. S., Hughes, B. K., Grimes, E. M. & Stein, M. B. (2009) Cognitive impairment and functioning in PTSD related to intimate partner violence. J Int.Neuropsychol.Soc., 15, 879-887.

Tyagi, N., Qipshidze, N., Sen, U., Rodriguez, W., Ovechkin, A. & Tyagi, S. C. (2011) Cystathionine beta synthase gene dose dependent vascular remodeling in murine model of hyperhomocysteinemia. Int.J Physiol Pathophysiol.Pharmacol., 3, 210-222.

Uchida, S., Nishida, A., Hara, K., Kamemoto, T., Suetsugi, M., Fujimoto, M., Watanuki, T., Wakabayashi, Y., Otsuki, K., McEwen, B. S. & Watanabe, Y. (2008) Characterization of the vulnerability to repeated stress in Fischer 344 rats: possible involvement of microRNA-mediated down-regulation of the glucocorticoid receptor. Eur.J.Neurosci., 27, 2250-2261.

Uchida, S., Hara, K., Kobayashi, A., Funato, H., Hobara, T., Otsuki, K., Yamagata, H., McEwen, B. S. & Watanabe, Y. (2010) Early life stress enhances behavioural vulnerability to stress through the activation of REST4-mediated gene transcription in the medial prefrontal cortex of rodents. J Neurosci., 30, 15007-15018.

Uchida, S., Hara, K., Kobayashi, A., Funato, H., Hobara, T., Otsuki, K., Yamagata, H., McEwen, B. S. & Watanabe, Y. (2010) Early life stress enhances behavioural vulnerability to stress through the activation of REST4-mediated gene transcription in the medial prefrontal cortex of rodents. J Neurosci., 30, 15007-15018.

Uchida, Y. (1994) Growth-inhibitory factor, metallothionein-like protein, and neurodegenerative diseases. Biol.Signals, 3, 211-215.

Uddin, M., Aiello, A. E., Wildman, D. E., Koenen, K. C., Pawelec, G., de Los, S. R., Goldmann, E. & Galea, S. (2010) Epigenetic and immune function profiles associated with posttraumatic stress disorder. Proc.Natl.Acad.Sci.U.S.A, 107, 9470-9475.

Valente, N. L., Vallada, H., Cordeiro, Q., Miguita, K., Bressan, R. A., Andreoli, S. B., Mari, J. J. & Mello, M. F. (2011) Candidate-gene approach in posttraumatic stress disorder after urban violence: association analysis of the genes encoding serotonin transporter, dopamine transporter, and BDNF. J Mol.Neurosci., 44, 59-67.

Vamvakopoulos, N. C. & Chrousos, G. P. (1993) Evidence of direct estrogenic regulation of human corticotropin-releasing hormone gene expression. Potential implications for the sexual dimophism of the stress response and immune/inflammatory reaction. J Clin.Invest, 92, 1896-1902.

van der Kolk, B. A., Dreyfuss, D., Michaels, M., Shera, D., Berkowitz, R., Fisler, R. & Saxe, G. (1994) Fluoxetine in posttraumatic stress disorder. J Clin.Psychiatry, 55, 517-522.

van der Kolk, B. A., Spinazzola, J., Blaustein, M. E., Hopper, J. W., Hopper, E. K., Korn, D. L. & Simpson, W. B. (2007) A randomized clinical trial of eye movement desensitization and reprocessing (EMDR), fluoxetine, and pill placebo in the treatment of posttraumatic stress disorder: treatment effects and long-term maintenance. J Clin.Psychiatry, 68, 37-46.

van Gaalen, M. M., Stenzel-Poore, M. P., Holsboer, F. & Steckler, T. (2002) Effects of transgenic overproduction of CRH on anxiety-like behaviour. Eur.J Neurosci., 15, 2007-2015.

van Heerden, J. H., Conesa, A., Stein, D. J., Montaner, D., Russell, V. & Illing, N. (2009) Parallel changes in gene expression in peripheral blood mononuclear cells and the brain after maternal separation in the mouse. BMC Res Notes, 2, 195.

van Lookeren, C. M., Thibodeaux, H., van, B. N., Cairns, B., Gerlai, R., Palmer, J. T., Williams, S. P. & Lowe, D. G. (1999) Evidence for a protective role of metallothionein-1 in focal cerebral ischemia. Proc.Natl.Acad.Sci.U.S.A, 96, 12870-12875.

van Praag, H. M. (1996) Faulty cortisol/serotonin interplay. Psychopathological and biological characterisation of a new, hypothetical depression subtype (SeCA depression). Psychiatry Res., 65, 143-157.

van, P. H. (1996) Serotonin-related, anxiety/aggression-driven, stressor-precipitated depression. A psycho-biological hypothesis. Eur.Psychiatry, 11, 57-67.

van, R. E., Sutherland, L. B., Liu, N., Williams, A. H., McAnally, J., Gerard, R. D., Richardson, J. A. & Olson, E. N. (2006) A signature pattern of stress-responsive microRNAs that can evoke cardiac hypertrophy and heart failure. Proc.Natl.Acad.Sci.U.S.A, 103, 18255-18260.

van, Z. M., Geuze, E., Willemen, H. L., Vermetten, E., Maas, M., Heijnen, C. J. & Kavelaars, A. (2011) Pre-existing high glucocorticoid receptor number predicting development of posttraumatic stress symptoms after military deployment. Am J Psychiatry, 168, 89-96.

van, Z. M., Geuze, E., Willemen, H. L., Vermetten, E., Maas, M., Amarouchi, K., Kavelaars, A. & Heijnen, C. J. (2012) Glucocorticoid receptor pathway components predict posttraumatic stress disorder symptom development: a prospective study. Biol.Psychiatry, 71, 309-316.

van, Z. M., Kavelaars, A., Geuze, E., Olff, M. & Heijnen, C. J. (2013) Predicting PTSD: pre-existing vulnerabilities in glucocorticoid-signaling and implications for preventive interventions. Brain Behav.Immun., 30, 12-21.

Varty, G. B., Cohen-Williams, M. E., Morgan, C. A., Pylak, U., Duffy, R. A., Lachowicz, J. E., Carey, G. J. & Coffin, V. L. (2002) The gerbil elevated plus-maze II: anxiolytic-like effects of selective neurokinin NK1 receptor antagonists. Neuropsychopharmacology, 27, 371-379.

Vecil, G. G., Larsen, P. H., Corley, S. M., Herx, L. M., Besson, A., Goodyer, C. G. & Yong, V. W. (2000) Interleukin-1 is a key regulator of matrix metalloproteinase-9 expression in human neurons in culture and following mouse brain trauma in vivo. J Neurosci.Res, 61, 212-224.

Venolia, L., Gartler, S. M., Wassman, E. R., Yen, P., Mohandas, T. & Shapiro, L. J. (1982) Transformation with DNA from 5-azacytidine-reactivated X chromosomes. Proc.Natl.Acad.Sci.U.S.A, 79, 2352-2354.

Venter, J. C., Adams, M. D., Myers, E. W., Li, P. W., Mural, R. J., Sutton, G. G., Smith, H. O., Yandell, M., Evans, C. A., Holt, R. A., Gocayne, J. D., Amanatides, et al. (2001) The sequence of the human genome. Science, 291, 1304-1351.

Verkhratsky, A. (2010) Physiology of neuronal-glial networking. Neurochem.Int., 57, 332-343.

Vermetten, E. & Bremner, J. D. (2002) Circuits and systems in stress. II. Applications to neurobiology and treatment in posttraumatic stress disorder. Depress.Anxiety., 16, 14-38.

Vgontzas, A. N., Zoumakis, M., Bixler, E. O., Lin, H. M., Prolo, P., Vela-Bueno, A., Kales, A. & Chrousos, G. P. (2003) Impaired nighttime sleep in healthy old versus young adults is associated with elevated plasma interleukin-6 and cortisol levels: physiologic and therapeutic implications. J Clin.Endocrinol.Metab, 88, 2087-2095.

Vgontzas, A. N., Zoumakis, E., Bixler, E. O., Lin, H. M., Follett, H., Kales, A. & Chrousos, G. P. (2004) Adverse effects of modest sleep restriction on sleepiness, performance, and inflammatory cytokines. J Clin.Endocrinol.Metab, 89, 2119-2126.

Vgontzas, A. N., Pejovic, S., Zoumakis, E., Lin, H. M., Bixler, E. O., Basta, M., Fang, J., Sarrigiannidis, A. & Chrousos, G. P. (2007) Daytime napping after a night of sleep loss decreases sleepiness, improves performance, and causes beneficial changes in cortisol and interleukin-6 secretion. Am J Physiol Endocrinol.Metab, 292, E253-E261.

Vidal-Gonzalez, I., Vidal-Gonzalez, B., Rauch, S. L. & Quirk, G. J. (2006) Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear. Learn.Mem., 13, 728-733.

Viveros, M. P., Marco, E. M. & File, S. E. (2005) Endocannabinoid system and stress and anxiety responses. Pharmacol.Biochem.Behav., 81, 331-342.

Vo, N., Klein, M. E., Varlamova, O., Keller, D. M., Yamamoto, T., Goodman, R. H. & Impey, S. (2005) A cAMP-response element binding protein-induced microRNA regulates neuronal morphogenesis. Proc.Natl.Acad.Sci.U.S.A, 102, 16426-16431.

Vogel, J. R., Beer, B. & Clody, D. E. (1971) A simple and reliable conflict procedure for testing anti-anxiety agents. Psychopharmacologia., 21, 1-7.

Vogel, R. A., Frye, G. D., Wilson, J. H., Kuhn, C. M., Mailman, R. B., Mueller, R. A. & Breese, G. R. (1980) Attenuation of the effect of punishment by thyrotropin-releasing hormone: comparisons with chlordiazepoxide. J Pharmacol.Exp.Ther., 212, 153-161.

Voisey, J., Swagell, C. D., Hughes, I. P., Morris, C. P., van, D. A., Noble, E. P., Kann, B., Heslop, K. A., Young, R. M. & Lawford, B. R. (2009) The DRD2 gene 957C>T polymorphism is associated with posttraumatic stress disorder in war veterans. Depress.Anxiety, 26, 28-33.

Vreugdenhil, E., Verissimo, C. S., Mariman, R., Kamphorst, J. T., Barbosa, J. S., Zweers, T., Champagne, D. L., Schouten, T., Meijer, O. C., de Kloet, E. R. & Fitzsimons, C. P. (2009) MicroRNA 18 and 124a down-regulate the glucocorticoid receptor: implications for glucocorticoid responsiveness in the brain. Endocrinology, 150, 2220-2228.

Vurbic, D., Gold, B. & Bouton, M. E. (2011) Effects of D-cycloserine on the extinction of appetitive operant learning. Behav.Neurosci., 125, 551-559.

Vyas, A., Mitra, R., Shankaranarayana Rao, B. S. & Chattarji, S. (2002) Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. J Neurosci., 22, 6810-6818.

Wahlestedt, C. & Reis, D. J. (1993) Neuropeptide Y-related peptides and their receptors--are the receptors potential therapeutic drug targets? Annu.Rev.Pharmacol.Toxicol., 33, 309-352.

Walker, D. L., Ressler, K. J., Lu, K. T. & Davis, M. (2002) Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of D-cycloserine as assessed with fear-potentiated startle in rats. J Neurosci., 22, 2343-2351.

Walker, D. L., Toufexis, D. J. & Davis, M. (2003) Role of the bed nucleus of the stria terminalis versus the amygdala in fear, stress, and anxiety. Eur.J Pharmacol., 463, 199-216.

Walsh, R. N. & Cummins, R. A. (1976) The Open-Field Test: a critical review. Psychol.Bull., 83, 482-504.

Wang, G., Anrather, J., Huang, J., Speth, R. C., Pickel, V. M. & Iadecola, C. (2004) NADPH oxidase contributes to angiotensin II signaling in the nucleus tractus solitarius. J Neurosci., 24, 5516-5524.

Wang, G., Milner, T. A., Speth, R. C., Gore, A. C., Wu, D., Iadecola, C. & Pierce, J. P. (2008) Sex differences in angiotensin signaling in bulbospinal neurons in the rat rostral ventrolateral medulla. Am J Physiol Regul.Integr.Comp Physiol, 295, R1149-R1157.

Wang, H., Zuo, D., He, B., Qiao, F., Zhao, M. & Wu, Y. (2012) Conditioned fear stress combined with single-prolonged stress: a new PTSD mouse model. Neurosci.Res, 73, 142-152.

Wang, L., Huang, J. & Jiang, M. (2011) CREB5 computational regulation network construction and analysis between frontal cortex of HIV encephalitis (HIVE) and HIVE-control patients. Cell Biochem.Biophys., 60, 199-207.

Wang, X., Louden, C., Yue, T. L., Ellison, J. A., Barone, F. C., Solleveld, H. A. & Feuerstein, G. Z. (1998) Delayed expression of osteopontin after focal stroke in the rat. J Neurosci., 18, 2075-2083.

Wassel, C. L., Barrett-Connor, E. & Laughlin, G. A. (2010) Association of circulating C-reactive protein and interleukin-6 with longevity into the 80s and 90s: The Rancho Bernardo Study. J Clin.Endocrinol.Metab, 95, 4748-4755.

Watson, I. P., Muller, H. K., Jones, I. H. & Bradley, A. J. (1993) Cell-mediated immunity in combat veterans with post-traumatic stress disorder. Med.J Aust., 159, 513-516.

Watts, B. V., Schnurr, P. P., Mayo, L., Young-Xu, Y., Weeks, W. B. & Friedman, M. J. (2013) Meta-analysis of the efficacy of treatments for posttraumatic stress disorder. J Clin.Psychiatry, 74, e541-e550.

Waubant, E., Goodkin, D. E., Gee, L., Bacchetti, P., Sloan, R., Stewart, T., Andersson, P. B., Stabler, G. & Miller, K. (1999) Serum MMP-9 and TIMP-1 levels are related to MRI activity in relapsing multiple sclerosis. Neurology, 53, 1397-1401.

Weaver, I. C., Szyf, M. & Meaney, M. J. (2002) From maternal care to gene expression: DNA methylation and the maternal programming of stress responses. Endocr.Res, 28, 699.

Weaver, I. C., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R., Dymov, S., Szyf, M. & Meaney, M. J. (2004) Epigenetic programming by maternal behavior. Nat.Neurosci., 7, 847-854.

Weaver, I. C., Champagne, F. A., Brown, S. E., Dymov, S., Sharma, S., Meaney, M. J. & Szyf, M. (2005) Reversal of maternal programming of stress responses in adult offspring through methyl supplementation: altering epigenetic marking later in life. J Neurosci., 25, 11045-11054.

Weaver, I. C., Meaney, M. J. & Szyf, M. (2006) Maternal care effects on the hippocampal transcriptome and anxiety-mediated behaviors in the offspring that are reversible in adulthood. Proc.Natl.Acad.Sci.U.S.A, 103, 3480-3485.

Wei, H., Wang, C., Zhang, C., Li, P., Wang, F. & Zhang, Z. (2010) Comparative profiling of microRNA expression between neural stem cells and motor neurons in embryonic spinal cord in rat. Int.J Dev.Neurosci., 28, 545-551.

Weiss, E. L., Longhurst, J. G. & Mazure, C. M. (1999) Childhood sexual abuse as a risk factor for depression in women: psychosocial and neurobiological correlates. Am J Psychiatry, 156, 816-828.

Weizman, R., Laor, N., Schujovitsky, A., Wolmer, L., bramovitz-Schnaider, P., Freudstein-Dan, A. & Rehavi, M. (1996) Platelet imipramine binding in patients with posttraumatic stress disorder before and after phenelzine treatment. Psychiatry Res, 63, 143-150.

West, A. K., Hidalgo, J., Eddins, D., Levin, E. D. & Aschner, M. (2008) Metallothionein in the central nervous system: Roles in protection, regeneration and cognition. Neurotoxicology, 29, 489-503.

Whalen, P. J., Shin, L. M., McInerney, S. C., Fischer, H., Wright, C. I. & Rauch, S. L. (2001) A functional MRI study of human amygdala responses to facial expressions of fear versus anger. Emotion., 1, 70-83.

Whishaw, I. Q. (1998) Place learning in hippocampal rats and the path integration hypothesis. Neurosci.Biobehav.Rev., 22, 209-220.

White, P. & Faustman, W. (1989) Coexisting physical conditions among inpatients with post-traumatic stress disorder. Mil.Med., 154, 66-71.

Wilhelm, S., Buhlmann, U., Tolin, D. F., Meunier, S. A., Pearlson, G. D., Reese, H. E., Cannistraro, P., Jenike, M. A. & Rauch, S. L. (2008) Augmentation of behavior therapy with D-cycloserine for obsessive-compulsive disorder. Am J Psychiatry, 165, 335-341.

Wingenfeld, K., Driessen, M., Schlosser, N., Terfehr, K., Carvalho, F. S. & Wolf, O. T. (2013) Cortisol effects on autobiographic memory retrieval in PTSD: an analysis of word valence and time until retrieval. Stress., 16, 581-586.

Wittmann, G., Fuzesi, T., Liposits, Z., Lechan, R. M. & Fekete, C. (2009) Distribution and axonal projections of neurons coexpressing thyrotropin-releasing hormone and urocortin 3 in the rat brain. J Comp Neurol., 517, 825-840.

Wojdacz, T. K. & Dobrovic, A. (2007) Methylation-sensitive high resolution melting (MS-HRM): a new approach for sensitive and high-throughput assessment of methylation. Nucleic Acids Res, 35, e41.

Wolf, S. F., Jolly, D. J., Lunnen, K. D., Friedmann, T. & Migeon, B. R. (1984) Methylation of the hypoxanthine phosphoribosyltransferase locus on the human X chromosome: implications for X-chromosome inactivation. Proc.Natl.Acad.Sci.U.S.A, 81, 2806-2810.

Wonderlich, S. A., Brewerton, T. D., Jocic, Z., Dansky, B. S. & Abbott, D. W. (1997) Relationship of childhood sexual abuse and eating disorders. J Am Acad.Child Adolesc.Psychiatry, 36, 1107-1115.

Wong, C. M. (2002) Post-traumatic stress disorder: advances in psychoneuroimmunology. Psychiatr.Clin.North Am, 25, 369-83, vii.

Wong, M. L., Kling, M. A., Munson, P. J., Listwak, S., Licinio, J., Prolo, P., Karp, B., McCutcheon, I. E., Geracioti, T. D., Jr., DeBellis, M. D., Rice, K. C., Goldstein, D. S., Veldhuis, J. D., Chrousos, G. P., Oldfield, E. H., McCann, S. M. & Gold, P. W. (2000) Pronounced and sustained central hypernoradrenergic function in major depression with melancholic features: relation to hypercortisolism and corticotropin-releasing hormone. Proc.Natl.Acad.Sci.U.S.A, 97, 325-330.

Wood, G. E., Norris, E. H., Waters, E., Stoldt, J. T. & McEwen, B. S. (2008) Chronic immobilization stress alters aspects of emotionality and associative learning in the rat. Behav.Neurosci., 122, 282-292.

Wood, J. D., Peesker, S. J., Gorecki, D. K. & Tsui, D. (1978) Effect of L-cycloserine on brain GABA metabolism. Can.J Physiol Pharmacol., 56, 62-68.

Woodward, S. H., Kaloupek, D. G., Grande, L. J., Stegman, W. K., Kutter, C. J., Leskin, L., Prestel, R., Schaer, M., Reiss, A. L. & Eliez, S. (2009) Hippocampal volume and declarative memory function in combat-related PTSD. J Int.Neuropsychol.Soc., 15, 830-839.

Worthylake, R. A. & Burridge, K. (2001) Leukocyte transendothelial migration: orchestrating the underlying molecular machinery. Curr.Opin.Cell Biol., 13, 569-577.

Wright, C. I., Martis, B., McMullin, K., Shin, L. M. & Rauch, S. L. (2003) Amygdala and insular responses to emotionally valenced human faces in small animal specific phobia. Biol.Psychiatry, 54, 1067-1076.

Wright, J. W. & Harding, J. W. (2004) The brain angiotensin system and extracellular matrix molecules in neural plasticity, learning, and memory. Prog.Neurobiol., 72, 263-293.

Wright, J. W., Brown, T. E. & Harding, J. W. (2007) Inhibition of hippocampal matrix metalloproteinase-3 and -9 disrupts spatial memory. Neural Plast., 2007, 73813.

Xiao, C., Srinivasan, L., Calado, D. P., Patterson, H. C., Zhang, B., Wang, J., Henderson, J. M., Kutok, J. L. & Rajewsky, K. (2008) Lymphoproliferative disease and autoimmunity in mice with increased miR-17-92 expression in lymphocytes. Nat.Immunol., 9, 405-414.

Xie, P., Kranzler, H. R., Poling, J., Stein, M. B., Anton, R. F., Brady, K., Weiss, R. D., Farrer, L. & Gelernter, J. (2009) Interactive effect of stressful life events and the serotonin transporter 5-HTTLPR genotype on posttraumatic stress disorder diagnosis in 2 independent populations. Arch.Gen.Psychiatry, 66, 1201-1209.

Xie, P., Kranzler, H. R., Poling, J., Stein, M. B., Anton, R. F., Farrer, L. A. & Gelernter, J. (2010) Interaction of FKBP5 with childhood adversity on risk for post-traumatic stress disorder. Neuropsychopharmacology, 35, 1684-1692.

Xie, P., Kranzler, H. R., Yang, C., Zhao, H., Farrer, L. A. & Gelernter, J. (2013) Genome-wide Association Study Identifies New Susceptibility Loci for Posttraumatic Stress Disorder. Biol.Psychiatry.

Xie, S., Wang, Z., Okano, M., Nogami, M., Li, Y., He, W. W., Okumura, K. & Li, E. (1999) Cloning, expression and chromosome locations of the human DNMT3 gene family. Gene, 236, 87-95.

Xie, W., Barr, C. L., Kim, A., Yue, F., Lee, A. Y., Eubanks, J., Dempster, E. L. & Ren, B. (2012) Base-resolution analyses of sequence and parent-of-origin dependent DNA methylation in the mouse genome. Cell, 148, 816-831.

Xu, H., He, J., Richardson, J. S. & Li, X. M. (2004) The response of synaptophysin and microtubule-associated protein 1 to restraint stress in rat hippocampus and its modulation by venlafaxine. J Neurochem., 91, 1380-1388.

Xu, J. J., Chan, M. J. & Yang, Y. C. (2011) Fluoxetine as a treatment for post-traumatic stress disorder. Neurosciences.(Riyadh.), 16, 257-262.

Yagle, M. K. & Palmiter, R. D. (1985) Coordinate regulation of mouse metallothionein I and II genes by heavy metals and glucocorticoids. Mol.Cell Biol., 5, 291-294.

Yamada, T., McGeer, P. L. & McGeer, E. G. (1992) Lewy bodies in Parkinson's disease are recognized by antibodies to complement proteins. Acta Neuropathol., 84, 100-104.

Yamamoto, S., Morinobu, S., Fuchikami, M., Kurata, A., Kozuru, T. & Yamawaki, S. (2008) Effects of single prolonged stress and D-cycloserine on contextual fear extinction and hippocampal NMDA receptor expression in a rat model of PTSD. Neuropsychopharmacology, 33, 2108-2116.

Yamamoto, S., Morinobu, S., Takei, S., Fuchikami, M., Matsuki, A., Yamawaki, S. & Liberzon, I. (2009) Single prolonged stress: toward an animal model of posttraumatic stress disorder. Depress.Anxiety, 26, 1110-1117.

Yamamoto, S., Morinobu, S., Iwamoto, Y., Ueda, Y., Takei, S., Fujita, Y. & Yamawaki, S. (2010) Alterations in the hippocampal glycinergic system in an animal model of posttraumatic stress disorder. J Psychiatr.Res, 44, 1069-1074.

Yang, C. H., Huang, C. C. & Hsu, K. S. (2011) Generalization of fear inhibition by disrupting hippocampal protein synthesis-dependent reconsolidation process. Neuropsychopharmacology, 36, 1992-2008.

Yang, X., Ewald, E. R., Huo, Y., Tamashiro, K. L., Salvatori, R., Sawa, A., Wand, G. S. & Lee, R. S. (2012) Glucocorticoid-induced loss of DNA methylation in non-neuronal cells and potential involvement of DNMT1 in epigenetic regulation of Fkbp5. Biochem.Biophys.Res Commun., 420, 570-575.

Yang, Y. L. & Lu, K. T. (2005) Facilitation of conditioned fear extinction by d-cycloserine is mediated by mitogen-activated protein kinase and phosphatidylinositol 3-kinase cascades and requires de novo protein synthesis in basolateral nucleus of amygdala. Neuroscience, 134, 247-260.

Yang, Z. Q. (2005) Agonists and antagonists for group III metabotropic glutamate receptors 6, 7 and 8. Curr.Top.Med.Chem. , 5, 913-918.

Yehuda, R., Southwick, S. M., Nussbaum, G., Wahby, V., Giller, E. L., Jr. & Mason, J. W. (1990) Low urinary cortisol excretion in patients with posttraumatic stress disorder. J Nerv.Ment.Dis., 178, 366-369.

Yehuda, R., Southwick, S., Giller, E. L., Ma, X. & Mason, J. W. (1992) Urinary catecholamine excretion and severity of PTSD symptoms in Vietnam combat veterans. J Nerv.Ment.Dis., 180, 321-325.

Yehuda, R., Teicher, M. H., Levengood, R. A., Trestman, R. L. & Siever, L. J. (1994) Circadian regulation of basal cortisol levels in posttraumatic stress disorder. Ann.N.Y.Acad.Sci., 746, 378-380.

Yehuda, R., Boisoneau, D., Lowy, M. T. & Giller, E. L., Jr. (1995) Dose-response changes in plasma cortisol and lymphocyte glucocorticoid receptors following dexamethasone administration in combat veterans with and without posttraumatic stress disorder. Arch.Gen.Psychiatry, 52, 583-593.

Yehuda, R., Teicher, M. H., Trestman, R. L., Levengood, R. A. & Siever, L. J. (1996) Cortisol regulation in posttraumatic stress disorder and major depression: a chronobiological analysis. Biol.Psychiatry, 40, 79-88.

Yehuda, R., Halligan, S. L. & Bierer, L. M. (2001) Relationship of parental trauma exposure and PTSD to PTSD, depressive and anxiety disorders in offspring. J.Psychiatr.Res., 35, 261-270.

Yehuda, R., Halligan, S. L., Grossman, R., Golier, J. A. & Wong, C. (2002) The cortisol and glucocorticoid receptor response to low dose dexamethasone administration in aging combat veterans and holocaust survivors with and without posttraumatic stress disorder. Biol.Psychiatry, 52, 393-403.

Yehuda, R., Halligan, S. L., Golier, J. A., Grossman, R. & Bierer, L. M. (2004) Effects of trauma exposure on the cortisol response to dexamethasone administration in PTSD and major depressive disorder. Psychoneuroendocrinology, 29, 389-404.

Yehuda, R. (2005) Neuroendocrine aspects of PTSD. Handb.Exp.Pharmacol., 371-403.

Yehuda, R. (2006) Advances in understanding neuroendocrine alterations in PTSD and their therapeutic implications. Ann.N.Y.Acad.Sci., 1071, 137-166.

Yehuda, R. & LeDoux, J. (2007) Response variation following trauma: a translational neuroscience approach to understanding PTSD. Neuron, 56, 19-32.

Yehuda, R. & Bierer, L. M. (2009) The relevance of epigenetics to PTSD: implications for the DSM-V. J Trauma Stress., 22, 427-434.

Yehuda, R., Cai, G., Golier, J. A., Sarapas, C., Galea, S., Ising, M., Rein, T., Schmeidler, J., Muller-Myhsok, B., Holsboer, F. & Buxbaum, J. D. (2009) Gene expression patterns associated with posttraumatic stress disorder following exposure to the World Trade Center attacks. Biol.Psychiatry, 66, 708-711.

Yehuda, R., Flory, J. D., Pratchett, L. C., Buxbaum, J., Ising, M. & Holsboer, F. (2010) Putative biological mechanisms for the association between early life adversity and the subsequent development of PTSD. Psychopharmacology (Berl), 212, 405-417.

Yeomans, J. S. & Frankland, P. W. (1995) The acoustic startle reflex: neurons and connections. Brain Res Brain Res Rev., 21, 301-314.

Yew, W. W., Wong, C. F., Wong, P. C., Lee, J. & Chau, C. H. (1993) Adverse neurological reactions in patients with multidrug-resistant pulmonary tuberculosis after coadministration of cycloserine and ofloxacin. Clin.Infect.Dis., 17, 288-289.

Yirmiya, R. & Goshen, I. (2011) Immune modulation of learning, memory, neural plasticity and neurogenesis. Brain Behav.Immun., 25, 181-213.

Yoder, J. A., Walsh, C. P. & Bestor, T. H. (1997) Cytosine methylation and the ecology of intragenomic parasites. Trends Genet., 13, 335-340.

Yong, V. W., Power, C., Forsyth, P. & Edwards, D. R. (2001) Metalloproteinases in biology and pathology of the nervous system. Nat.Rev.Neurosci., 2, 502-511.

Yong, V. W. (2005) Metalloproteinases: mediators of pathology and regeneration in the CNS. Nat.Rev.Neurosci., 6, 931-944.

Yoshitomi, H., Sakaguchi, N., Kobayashi, K., Brown, G. D., Tagami, T., Sakihama, T., Hirota, K., Tanaka, S., Nomura, T., Miki, I., Gordon, S., Akira, S., Nakamura, T. & Sakaguchi, S. (2005) A role for fungal {beta}-glucans and their receptor Dectin-1 in the induction of autoimmune arthritis in genetically susceptible mice. J Exp.Med., 201, 949-960.

Youn, J., Hwang, S. H., Ryoo, Z. Y., Lynes, M. A., Paik, D. J., Chung, H. S. & Kim, H. Y. (2002) Metallothionein suppresses collagen-induced arthritis via induction of TGF-beta and down-regulation of proinflammatory mediators. Clin.Exp.Immunol., 129, 232-239.

Young, L. D., Suomi, S. S., Harlow, H. F. & McKinney, W. T., Jr. (1973) Early stress and later response to separation in rhesus monkeys. Am J Psychiatry, 130, 400-405.

Young, R. M., Lawford, B. R., Noble, E. P., Kann, B., Wilkie, A., Ritchie, T., Arnold, L. & Shadforth, S. (2002) Harmful drinking in military veterans with post-traumatic stress disorder: association with the D2 dopamine receptor A1 allele. Alcohol Alcohol, 37, 451-456.

Yu, H., Wang, Y., Pattwell, S., Jing, D., Liu, T., Zhang, Y., Bath, K. G., Lee, F. S. & Chen, Z. Y. (2009) Variant BDNF Val66Met polymorphism affects extinction of conditioned aversive memory. J Neurosci., 29, 4056-4064.

Yu, J., Xiao, F., Zhang, Q., Liu, B., Guo, Y., Lv, Z., Xia, T., Chen, S., Li, K., Du, Y. & Guo, F. (2013) Prolactin Receptor (PRLR) regulates hepatic insulin sensitivity in mice via Signal Transducer and Activator of Transcription (STAT)5. Diabetes.

Yumru, M., Savas, H. A., Kalenderoglu, A., Bulut, M., Celik, H. & Erel, O. (2009) Oxidative imbalance in bipolar disorder subtypes: a comparative study. Prog.Neuropsychopharmacol.Biol.Psychiatry, 33, 1070-1074.

Zachrisson, O., Mathe, A. A., Stenfors, C. & Lindefors, N. (1995) Limbic effects of repeated electroconvulsive stimulation on neuropeptide Y and somatostatin mRNA expression in the rat brain. Brain Res Mol.Brain Res, 31, 71-85.

Zambenedetti, P., Giordano, R. & Zatta, P. (1998) Metallothioneins are highly expressed in astrocytes and microcapillaries in Alzheimer's disease. J Chem.Neuroanat., 15, 21-26.

Zarate, R. & Agras, W. S. (1994) Psychosocial treatment of phobia and panic disorders. Psychiatry, 57, 133-141.

Zarate, C. A. & Manji, H. K. (2008). The promising role of the glutamate system in mood disorders. In: Heresco-Levy U, Javitt DC (Eds.), Glutamate in Neuropsychiatric Disorders (pp. 213–251). Kerala, India : Research Signpost.

Zatzick, D. F., Marmar, C. R., Weiss, D. S., Browner, W. S., Metzler, T. J., Golding, J. M., Stewart, A., Schlenger, W. E. & Wells, K. B. (1997) Posttraumatic stress disorder and functioning and quality of life outcomes in a nationally representative sample of male Vietnam veterans. Am J Psychiatry, 154, 1690-1695.

Zhang, C. (2008) MicroRNomics: a newly emerging approach for disease biology. Physiol Genomics, 33, 139-147.

Zhang, H., Ozbay, F., Lappalainen, J., Kranzler, H. R., van Dyck, C. H., Charney, D. S., Price, L. H., Southwick, S., Yang, B. Z., Rasmussen, A. & Gelernter, J. (2006) Brain derived neurotrophic factor (BDNF) gene variants and Alzheimer's disease, affective disorders, posttraumatic stress disorder, schizophrenia, and substance dependence. Am J Med.Genet.B Neuropsychiatr.Genet., 141B, 387-393.

Zhang, L., Zhou, R., Li, X., Ursano, R. J. & Li, H. (2006) Stress-induced change of mitochondria membrane potential regulated by genomic and non-genomic GR signaling: a possible mechanism for hippocampus atrophy in PTSD. Med.Hypotheses, 66, 1205-1208.

Zhang, L., Ding, L., Cheung, T. H., Dong, M. Q., Chen, J., Sewell, A. K., Liu, X., Yates, J. R., III & Han, M. (2007) Systematic identification of C. elegans miRISC proteins, miRNAs, and mRNA targets by their interactions with GW182 proteins AIN-1 and AIN-2. Mol.Cell, 28, 598-613.

Zhang, L., Li, H., Su, T. P., Barker, J. L., Maric, D., Fullerton, C. S., Webster, M. J., Hough, C. J., Li, X. X. & Ursano, R. (2008) p11 is up-regulated in the forebrain of stressed rats by glucocorticoid acting via two specific glucocorticoid response elements in the p11 promoter. Neuroscience, 153, 1126-1134.

Zhang, L., Benedek, D. M., Fullerton, C. S., Forsten, R. D., Naifeh, J. A., Li, X. X., Hu, X. Z., Li, H., Jia, M., Xing, G. Q., Benevides, K. N. & Ursano, R. J. (2013) PTSD risk is associated with BDNF Val66Met and BDNF overexpression. Mol.Psychiatry.

Zhang, L. F., Shi, L., Liu, H., Meng, F. T., Liu, Y. J., Wu, H. M., Du, X. & Zhou, J. N. (2011) Increased hippocampal tau phosphorylation and axonal mitochondrial transport in a mouse model of chronic stress. Int.J Neuropsychopharmacol., 1-12.

Zhang, Y., Ng, H. H., Erdjument-Bromage, H., Tempst, P., Bird, A. & Reinberg, D. (1999) Analysis of the NuRD subunits reveals a histone deacetylase core complex and a connection with DNA methylation. Genes Dev., 13, 1924-1935.

Zhang, Y., Sheng, H., Qi, J., Ma, B., Sun, J., Li, S. & Ni, X. (2012) Glucocorticoid acts on a putative G protein-coupled receptor to rapidly regulate the activity of NMDA receptors in hippocampal neurons. Am J Physiol Endocrinol.Metab, 302, E747-E758.

Zhao, M. G., Toyoda, H., Lee, Y. S., Wu, L. J., Ko, S. W., Zhang, X. H., Jia, Y., Shum, F., Xu, H., Li, B. M., Kaang, B. K. & Zhuo, M. (2005) Roles of NMDA NR2B subtype receptor in prefrontal long-term potentiation and contextual fear memory. Neuron, 47, 859-872.

Zhao, Q., Li, X., Fang, X., Zhao, G., Zhao, J., Lin, X. & Stanton, B. (2010) Difference in psychosocial well-being between paternal and maternal AIDS orphans in rural China. J Assoc.Nurses AIDS Care, 21, 335-344.

Zhou, R., Yuan, P., Wang, Y., Hunsberger, J. G., Elkahloun, A., Wei, Y., mschroder-Williams, P., Du, J., Chen, G. & Manji, H. K. (2009) Evidence for selective microRNAs and their effectors as common long-term targets for the actions of mood stabilizers. Neuropsychopharmacology, 34, 1395-1405.

Zhu, S. W., Yee, B. K., Nyffeler, M., Winblad, B., Feldon, J. & Mohammed, A. H. (2006) Influence of differential housing on emotional behaviour and neurotrophin levels in mice. Behav.Brain Res, 169, 10-20.

Zieker, J., Zieker, D., Jatzko, A., Dietzsch, J., Nieselt, K., Schmitt, A., Bertsch, T., Fassbender, K., Spanagel, R., Northoff, H. & Gebicke-Haerter, P. J. (2007) Differential gene expression in peripheral blood of patients suffering from post-traumatic stress disorder. Mol.Psychiatry, 12, 116-118.

Zimmerman, M. C. (2011) Angiotensin II and angiotensin-1-7 redox signaling in the central nervous system. Curr.Opin.Pharmacol., 11, 138-143.

Zlotnick, C., Johnson, J., Kohn, R., Vicente, B., Rioseco, P. & Saldivia, S. (2006) Epidemiology of trauma, post-traumatic stress disorder (PTSD) and co-morbid disorders in Chile. Psychol.Med., 36, 1523-1533.

Zobel, A. W., Nickel, T., Sonntag, A., Uhr, M., Holsboer, F. & Ising, M. (2001) Cortisol response in the combined dexamethasone/CRH test as predictor of relapse in patients with remitted depression. a prospective study. J Psychiatr.Res, 35, 83-94.

Zohar, R., Suzuki, N., Suzuki, K., Arora, P., Glogauer, M., McCulloch, C. A. & Sodek, J. (2000) Intracellular osteopontin is an integral component of the CD44-ERM complex involved in cell migration. J Cell Physiol, 184, 118-130.

Zoladz, P. R., Conrad, C. D., Fleshner, M. & Diamond, D. M. (2008) Acute episodes of predator exposure in conjunction with chronic social instability as an animal model of post-traumatic stress disorder. Stress., 11, 259-281.

Zoladz, P. R., Fleshner, M. & Diamond, D. M. (2012) Psychosocial animal model of PTSD produces a long-lasting traumatic memory, an increase in general anxiety and PTSD-like glucocorticoid abnormalities. Psychoneuroendocrinology, 37, 1531-1545.

Zovkic, I. B. & Sweatt, J. D. (2013) Epigenetic mechanisms in learned fear: implications for PTSD. Neuropsychopharmacology, 38, 77-93.

Zucker, R. S. & Regehr, W. G. (2002) Short-term synaptic plasticity. Annu.Rev.Physiol, 64, 355-405.