

**An Investigation of Two Animal Models of
Anxiety: Central Administration of Corticotropin-
Releasing Factor on the Behaviour and
Neurochemistry of Rats, and the Effect of
Pharmacotherapy on Spontaneous Stereotypical
Behaviour and NMDA Receptor Function in Mice**



Thesis presented in partial fulfillment of the requirements for the
degree of Master of Science in Medical Sciences` at the University of
Stellenbosch

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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature:

Date:

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Summary

Corticotropin-releasing factor (CRF) study

Background: Hypothalamic pituitary adrenal (HPA)-axis dysfunction is a common symptom of patients with anxiety disorders like posttraumatic stress disorder (PTSD), panic disorder and obsessive-compulsive disorder (OCD). Depressive patients also have HPA-axis dysfunction similar to patients with anxiety disorders. PTSD patients usually show decreased basal plasma cortisol levels whereas OCD and depressive patients show an increase in plasma cortisol. A blunted adrenocorticotropin hormone (ACTH) response to CRF is seen in these patients and mostly an increased level of CRF in their cerebrospinal fluid (CSF). It has been proposed that the CSF level of CRF is a reflection of activity of both hypothalamic and extra-hypothalamic CRF systems. Since the amygdala has been shown to be involved in the endocrine and behavioural response to stress, and CRF is involved in the mediation of this response, we investigated whether chronic elevation of CRF in the amygdala is involved in development of the symptoms of psychiatric disorders. We chronically injected rats with CRF in this area to see what the effect is on their behaviour and HPA-axis response. It has been shown that the hippocampal serotonergic (5-HT) system is involved in both anxiety and depression and that the 5-HT system is regulated by CRF. We therefore measured hippocampal 5-HT_{1A} receptor density and affinity in CRF-injected and control rats.

Materials and methods: Male Sprague-Dawley and Wistar rats were stereotaxically implanted with unilateral and bilateral chronic cannulae in the basolateral amygdala (BLA). After recovery unilaterally implanted rats were injected with 10ng (n=7) or 100ng (n=6) of CRF or saline (n=6) daily for 5 days. Behaviour was tested on day 5 in the elevated plus-maze and open field. Rats with bilateral implants were injected with 100ng CRF (n=19) or saline (n=17) on either side for 5 days. On day 5, behaviour was tested on the elevated plus-maze and open field. Group 1 was tested at baseline levels (n=6 for saline and n=7 for CRF) and group 2 after 5 min. of restraint (n=11 for saline and n=12 for CRF). The stress response of all rats was tested 2 days later. The rats were divided into 3 groups: The first group was decapitated at baseline level (n=6 for each group), the second and third groups restrained for 10 min. and decapitated 15 min. (n=6 for saline and n=7 for CRF) and 60 min. (n=5 for saline and n=6 for CRF) after restraint stress. Blood was collected for plasma ACTH and corticosterone determinations. A group of naïve rats was also included in this experiment to control for the possible effect of the operation (n=6 for each time point). Hippocampi were dissected out and used for 5-HT_{1A} radioligand binding studies.

Results: Rats that were unilaterally injected with 100ng CRF, showed significant increase in the amount of entries into the open arms as well as the amount of time spent in the open arms of the elevated plus-maze compared to controls and

rats injected with 10ng CRF. There were no differences between the groups in other parameters of the elevated plus-maze or open field behaviour. There were no significant differences in behaviour of bilateral injected rats compared to controls, but an increase of grooming in the open field was observed in CRF-injected rats that were stressed before behavioural tests. The ACTH and corticosterone response of rats were normal as seen by a significant increase in both concentrations 15 min. after stress and a return to near basal values 60 min. post stress. There were no differences in plasma ACTH concentrations between the groups at any time point. The basal corticosterone level of CRF-injected rats was however significantly lower than controls, but no difference were found 15 or 60 min. post stress. There were no significant differences in hippocampal 5-HT_{1A} receptor densities or affinities of CRF-injected and control rats.

Conclusions: We did not observe increased anxiety levels or decreased activity in CRF injected rats. Instead, we observed increased activity in unilateral injected rats. We suspect that the lower dosage of CRF may have this effect on the behaviour of rats, since other authors have also found this result. The lack of differences in the 5-HT_{1A} receptor populations of CRF-injected rats and control rats also confirms this result, since there was no increase in anxiety levels of CRF-injected rats. The chronic elevation of CRF in the BLA caused decreased basal levels of corticosterone in the rats and we speculate that CRF caused adrenal insufficiency although the mechanism is unknown.

Stereotypical behaviour study

Background: OCD affects 1-2% of the adult human population and is amongst the most common psychiatric disorders. This disorder is characterized by obsessions and compulsions. These compulsions or repetitive behaviours are suggested to be the cause of a hyperactive cortical-striatal-thalamic-cortical (CSTC) circuit in the brain, because of imbalance of the direct and indirect pathways. CSTC pathways are modulated by both 5-HT and dopaminergic (DA) neurons and it has been suggested that a hyperglutamatergic state exists in the frontal cortex of OCD patients. Two types of animal models of stereotypical behaviour are used to investigate neurotransmitter abnormalities related to OCD. These are drug induced stereotypies and environmentally induced (spontaneous) stereotypies. It has been shown that in deermice (*Peromyscus maniculatis*), drug induced stereotypies are topographically different from spontaneous stereotypies, and our aims were to characterize a deermice model of spontaneous stereotypy for OCD in terms of face, predictive and construct validity. We injected adult deermice, showing spontaneous stereotypies, for a time period of 8 weeks with risperidone ($D_2/5\text{-HT}_2$ antagonist), citalopram (selective serotonin reuptake inhibitor) and inositol (a metabolic precursor to the phosphatidylinositol second messenger cycle). All these drugs have been shown to improve symptoms of OCD in humans, and we investigated whether the drugs can reduce stereotypies in deermice.

Materials and methods: 40 Adult deermice were raised and housed in standard laboratory cages and randomly divided into four groups (6 females and 4 males per group). After baseline recordings of behaviour (3 times per week for a total of 15 min.) the mice were injected daily for 8 weeks with risperidone, citalopram, inositol or saline. Video recordings were made for the 8 week trial and rated afterwards by raters that were blind to the medication status of the animals. A 5 sec. interval scoring system was used for ratings in which the absence or presence of a stereotypy (backward somersault) was noted. After 8 weeks of treatment, the mice were decapitated, brains were dissected and the frontal cortices were stored in liquid nitrogen until radioligand binding studies were performed on NMDA receptors.

Results: There were no significant differences in the amount of somersaults between saline injected and drug treated groups when the data was analysed using an ANOVA with repeated measures. There was a significant difference between the control group and drug treated groups at week 8 in male mice when the data was analysed using a Mann-Whitney test. The amount of somersaults shown by saline injected mice increased over the 8 week trial while it stayed more constant in all three drug treated groups. There were no significant differences between the control group and treatment groups in B_{max} and K_d values of NMDA receptors in the frontal cortex. There was a trend towards increased receptor densities in all treatment groups compared to the control group and a decrease in affinity in the risperidone group.

Conclusions: We found limited evidence for the involvement of both 5-HT and DA systems in the development of spontaneous stereotypical behaviour of deermice. Risperidone, citalopram and inositol were useful in suppressing the increase in somersaults observed in the control group towards week 8 of the trial. This increase was presumably due to stress from handling and injections. The fact that there was a trend towards increased receptor densities in all treatment groups and decreased affinity in the risperidone group also point to the involvement of 5-HT and DA in spontaneous stereotypies. The limitation of this study was small group numbers and excessive stress experienced by the animals.

Opsomming

Kortikotropien vrystellingsfaktor (CRF) studie

Agtergrond: Hipotalamus-hipofise-adrenale (HPA)-as abnormaliteite is 'n algemene simptome van pasiente wat gediagnoseer is met verskeie psigiatriese afwykings soos posttraumatiese stres steuring (PTSD), obsessiewe-kompulsiewe steuring (OCD) paniek steuring en depressie. PTSD pasiente toon gewoonlik 'n verlaging in basale kortisol vlakke, terwyl OCD en depressie pasiente 'n verhoging in die basale konsentrasie van kortisol in die bloed toon. 'n Verlaagde adrenokortikotropien hormoon (ACTH) respons na toediening van CRF word ook gewoonlik in al die bogenoemde afwykings waargeneem. Pasiente toon ook 'n verhoging in CRF konsentrasies in hul cerebrospinale vloeistof (CSF). Daar is voorheen al bewyse gevind dat die CRF konsentrasie in CSF 'n aanduiding van beide die aktiwiteit van die HPA-as en ander CRF sisteme verteenwoordig. Die amygdala is 'n belangrike deel van die brein wat betrokke is by die endokriene en gedragsrespons op stres. Omdat CRF aangedui is om 'n rol te speel in die stres respons, het ons ondersoek ingestel na die rol van kroniese verhoging van CRF in die amygdala in die ontwikkeling van simptome van psigiatriese steuringe. Rotte is kronies ingespuut in hierdie brein area en die effek daarvan is waargeneem deur na die gedrag en stres respons van die rotte te kyk. Dit is ook al aangedui dat die serotonergiese (5-HT) sisteem in die hippokampus betrokke is in angstigheids en depressie, en dat hierdie sisteem gereguleer word deur CRF.

Daarom het ons ook ondersoek ingestel of daar verskille is in die 5-HT_{1A} reseptor populasies van CRF en kontrole rotte.

Materiale en metodes: Manlike Sprague-Dawley en Wistar rotte is onderskeidelik met behulp van stereotaksis met chroniese kannules unilateraal en bilateraal geïmplanteer in die basolaterale nukleus van die amygdala (BLA). Nadat die rotte herstel het, is rotte met unilaterale inplanterings ingespuut met 10ng (n=7) of 100ng (n=6) CRF of 'n soutoplossing (n=6) daaglik vir 5 agtereenvolgende dae. Hierdie rotte se gedrag is waargeneem op die 5de dag met behulp van die "elevated plus-maze" en "open field". Rotte met bilaterale inplanterings is daaglik ingespuut met 100ng CRF (n=19) of soutoplossing (n=17) vir 5 dae. Die gedrag is op dieselfde manier getoets op die vyfde dag behalwe dat een groep vir 5 min gestres was voor die toets (n=11 vir kontrole en n=12 vir CRF) terwyl die ander groep rotte waargeneem is by basale vlakke (n=6 vir kontrole en n=7 vir CRF). Die stres respons van hierdie rotte is ook 2 dae later getoets. Die rotte is verdeel in 3 groepe waarvan die eerste groep gedekapiteer is by basale vlakke (n=6 vir kontrole en CRF). Die tweede en derde groepe is blootgestel aan 10 min. stres en gedekapiteer onderskeidelik 15 min. (n=6 vir kontrole en n=7 vir CRF) en 60 min. (n=5 vir kontrole en n=6 vir CRF) na die stres periode. Bloed is opgevang vir plasma ACTH en kortikosteroon bepaling. 'n Groep naiewe rotte is ook ingesluit in hierdie eksperiment om te kontroleer vir die effek wat die operasie kon hê (n=6 vir elke

tydstip). Die breine is gedissekteer en hippokampusse verwyder en gestoor in vloeibare stikstof vir radioligand bindingstudies op 5-HT_{1A} reseptore.

Resultate: Rotte wat unilaterale CRF inspuitings gekry het, het 'n beduidende verhoging getoon in die aantal tyd gespandeer en die aantal kere wat hulle die oop arms van die "elevated plus-maze" binnegegaan het in vergelyking met kontrole rotte. Daar was geen verskil tussen die groepe in enige van die ander parameters waargeneem in die "elevated plus-maze" of "open field" gedrag nie. Daar was ook geen beduidende verskil in enige gedrag van rotte wat bilateraal ingespuit is en kontrole rotte nie, behalwe dat daar 'n verhoging was in die totale aantal kere wat die rot versorgingsgedrag getoon het. Die ACTH en kortikosteroon respons van die rotte was normaal in vergelyking met kontrole rotte soos waargeneem deur die beduidende verhoging in konsentrasies 15 minute na stres en die verlaging daarvan na 60 minute. Daar was geen verskille tussen die groepe in die konsentrasies van ACTH nie, maar daar was wel 'n beduidend verlaagde basale konsentrasie van kortikosteroon in die CRF groep in vergelyking met kontrole rotte. Daar was ook geen verskil in die 5-HT_{1A} reseptor populasies van die CRF en kontrole rotte nie.

Gevolgtrekkings: Ons het geen verhoging in angstigheid of verlaging in aktiwiteitsvlakke waargeneem in die CRF rotte nie. Ons het intendeel verhoogde aktiwiteitsvlakke waargeneem by CRF rotte wat unilateraal ingespuit was. Ons vermoed dat die lae dosis van CRF hierdie effek teweeg gebring het in

die gedrag van die rotte aangesien vorige outeurs ook al hierdie resultaat waargeneem het. Die afwesigheid van verskille in die reseptor populasies van die CRF en kontrole rotte bevestig ons resultaat van die gedragstudie aangesien daar geen verhoging was in anstigtheid in CRF rotte nie. Die kroniese verhoging van CRF vlakke in die BLA het 'n verlaging in basale vlakke van kortikosteroon veroorsaak en, alhoewel die meganisme onbekend is, moontlik die adrenale funksie verlaag.

Stereotiepe gedrag studie

Agtergrond: 1-2% Van volwassene mense word gediagnoseer met OCD, wat beteken dat dit een van die mees algemene psigiatriese afwykings is. Hierdie afwyking word gekarakteriseer deur obsessies en kompulsies. Dit is bekend dat kompulsies of stereotiepe gedrag voorkom as gevolg van 'n ooraktiewe korteks-striatum-talamus-korteks (CSTC) sisteem in die brein, as gevolg van 'n wanbalans tussen die direkte en indirekte paaie. Die CSTC sisteem word gereguleer deur 5-HT en dopamien (DA) neurone en dit is al voorgestel dat 'n hiperglutamatergiese toestand in die frontale korteks van OCD pasiente bestaan. Twee tipes dier modelle word gebruik om stereotiepe gedrag te bestudeer in terme van neurotransmitter abnormaliteite. Die eerste is dwelm-geïnduseerde stereotiepe gedrag en die tweede omgewing-geïnduseerde of spontane stereotiepe gedrag. 'n Vorige studie op muise (*Peromyscus maniculatis*) het getoon dat dwelm-geïnduseerde en spontane stereotiepe gedrag verskillend is in

hierdie spesie. Die doel van ons studie was om 'n muis model vir OCD te karakteriseer. Ons het dus volwasse muise wat spontane stereotiepe gedrag toon vir 'n tydperk van 8 weke ingespuut met risperidoon, citalopram, inositol of 'n soutoplossing. Hierdie medikasie word reeds gebruik vir die behandeling van mense met OCD en die doel van ons studie was om te sien of dit ook spontane stereotiepe gedrag in muise kan verminder.

Materiale en metodes: 40 Volwasse muise is geteel en aangehou in standaard laboratorium hokke en lukraak verdeel in vier groepe met 6 wyfies en 4 mannetjies per groep. Video opnames is vir 9 weke gemaak vir 5 minute per keer met 'n totaal van 15 minute per week. Die eerste week is geneem as basislyn en die muise is daaglik ingespuut met die onderskeie medikasie vir die volgende 8 weke. Die onderskeie groepe is ingespuut met risperidoon, citalopram, inositol of 'n soutoplossing. 'n 5 Sekonde interval sisteem is gebruik om gedrag waar te neem, en die teenwoordigheid of afwesigheid van stereotiepe gedrag is genoteer. Na 8 weke van behandeling is die muise opgeoffer deur dekapitasie en die breine is gedissekteer en die frontale korteks gebruik vir radioligand studies op NMDA reseptore.

Resultate: Daar was geen beduidende verskille in stereotiepe gedrag tussen die kontrole groep en behandelde groepe tussen die basislyn en week 8 wanneer die data geanaliseer is met behulp van 'n ANOVA met herhaalde waarnemings nie. Daar was wel 'n beduidende verskil tussen die kontrole groep en die behandelde

groepe by mannetjies in week 8 wanneer die data met 'n Mann-Whitney toets geanaliseer word. Die aantal stereotiepe gedrag in die kontrole groep het vermeerder oor die 8 week tydperk terwyl dit meer konstant gebly het in behandelde groepe. Daar was geen beduidende verskille in die digtheid en affiniteit van NMDA reseptore van kontrole en behandelde groepe nie. Daar was wel 'n neiging tot 'n toename in reseptor digtheid by al die behandelde groepe in vergelyking met die kontrole groep en 'n afname in affiniteit in die risperidoon groep in vergelyking met die kontrole groep.

Gevolgtrekkings: Ons het beperkte bewyse gevind vir die betrokkenheid van beide 5-HT en DA neurotransmitter sisteme in die ontwikkeling van spontane stereotiepe gedrag in die muis. Risperidoon, citalopram en inositol het die toename in stereotiepe gedrag, soos waargeneem in die kontrole groep, onderdruk. Hierdie toename in stereotiepe gedrag was waarsynlik as gevolg van uitermatige stres wat die muis ervaar het as gevolg van hantering en inspuitings. Die feit dat daar 'n neiging tot toename in reseptor digtheid in al die behandelde groepe en afname in affiniteit was in die risperidoon groep dui ook op die betrokkenheid van 5-HT en DA in spontane stereotiepe gedrag. Die beperkings van hierdie studie was die klein groep nommers en uitermatige stres wat die muis ervaar het.

List of Abbreviations

α	alpha
γ	gamma
$^{\circ}\text{C}$	degrees Celsius
%	percent
μg	microgram
μl	microliter
μm	micrometer
μM	micromolar
[^3H]-8-OH-DPAT	2-(N,N-di[2,3(n)- ^3H]propylamino)-8-hydroxy-1,2,3,4-tetrahydronaphthalene
5-HT	serotonin/serotonergic
ACTH	adrenocorticotropin hormone
ALD	acral lick dermatitis
ANOVA	analysis of variance
B_{max}	receptor density
BLA	basolateral amygdala
BSA	bovine serum albumin
CaCl_2	calcium chloride
CeA	central amygdala
cm	centimeter
CPM	counts per minute
CRF	corticotropin-releasing factor

CSF	cerebrospinal fluid
CSTC	cortical-striatal-thalamic-cortical
CuSO ₄	copper sulphate
DA	dopamine/dopaminergic
EDTA	ethylenediaminetetraacetic acid
<i>et al.</i>	and co-authors
Fig.	figure
fMRI	functional magnetic resonance imaging
g	gram
GABA	gamma-amino-butyric acid
GR	glucocorticoid receptor
h	hour
HPA	hypothalamic-pituitary-adrenal
ICV	intracerebroventricular
i.p.	intra-peritoneal
irCRF	immuno-reactive CRF
K	potassium
K _d	receptor affinity
kg	kilogram
m	meter
mg	milligram
min	minutes
ml	millilitre

mm	millimeter
mMol	millimolar
MR	mineralocorticoid receptor
mRNA	messenger ribonucleic acid
n	sample number
NA ₂ CO ₃	sodium bicarbonate
NaOH	sodium hydroxide
nMol	nanomolar
NMDA	N-methyl-D-aspartate
OCD	obsessive-compulsive disorder
p	probability
PD	panic disorder
PET	positron emission tomography
PI	phosphatidylinositol
pmol	picomole
PTSD	posttraumatic stress disorder
Rpm	revolutions per minute
sec.	seconds
SEM	standard error of the mean
SPECT	single photon emission computed tomography
SSRI	selective serotonin reuptake inhibitors
Tris	tris(hydroxymethyl) aminomethane
vs.	versus

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Chapter 1:

Literature Review

1.1 Corticotropin-Releasing Factor Study

1.1.1 Stress response

When an animal is exposed to stressors (hostile environment), a series of coordinated responses are initiated, namely alterations in behaviour, autonomic function and the secretion of multiple stress hormones (Van de Kar & Blair, 1999). Collectively, these events are called the stress response. The stressor can either be psychological, for example exposing animals to a novel environment, or physical, such as giving an animal an electrical foot shock (Van de Kar & Blair, 1999). An important part of the neuroendocrine pathways in the stress response is the activation of the hypothalamic-pituitary-adrenal (HPA) axis. Corticotropin-releasing factor (CRF) is a 41 amino acid peptide characterised by Vale *et al.* in 1981 and was the first of the hypothalamic-releasing factors to be named. CRF is synthesized by neurons of the paraventricular nucleus of the hypothalamus and, during stress, released into the portal circulation to reach the anterior pituitary, where it stimulates the release of ACTH (Cole & Sawchenko, 2002). Adrenocorticotropin hormone (ACTH) in turn stimulates glucocorticoid release from the adrenal cortex, mainly cortisol in humans and corticosterone in rats. This is necessary for maintaining homeostasis in the body (Habib *et al.*, 2001).

Glucocorticoids regulate the HPA-axis and terminate the stress response by means of negative feedback inhibition at the level of the hypothalamus and pituitary (De Kloet 1995); see fig.1.1. In addition, extrahypothalamic structures also participate in the regulation of HPA-axis activity eg. the limbic system. There are two types of glucocorticoid receptors involved in this regulation. The type 1 mineralocorticoid receptor (MR) has a high affinity for glucocorticoids and mediates the effects of corticosterone on the maintenance of basal HPA activity. These receptors appear to set the threshold of the central stress response system. The type 2 glucocorticoid receptor (GR) is found in high densities in the limbic system and is occupied during times of higher circulating corticosterone levels such as during stress when the glucocorticoid concentration may increase to about 100-fold. Glucocorticoid binding to these receptors suppresses subsequent HPA activation. The affinity of corticosterone is about 10 times higher for the MR receptor than the GR receptor (McEwen *et al.*, 1986; De Kloet *et al.*, 1998).

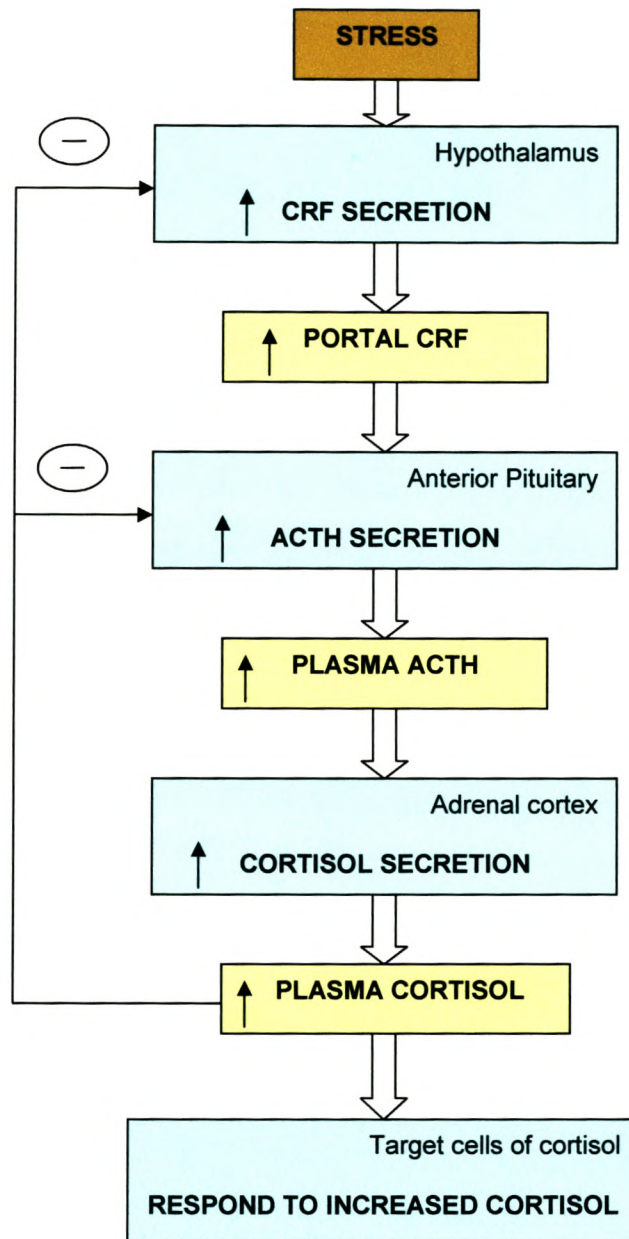


Fig.1.1. The HPA-axis and negative feedback inhibition (Adapted from: Vander, Sherman & Luciano, 1994).

1.1.2 CRF and behavioural responses to stress

Activation of the HPA-axis is not the only function of CRF in the brain. Adaptive behavioural changes are an important component of the stress response, and this may involve extrahypothalamic CRF systems (Koob *et al.*, 1993). Immunochemical studies have shown the presence of CRF cell bodies and fibres in the cortex, limbic system, substantia nigra and locus coeruleus. There is also evidence that CRF has a neurotransmitter role in the brain (Dunn & Berridge, 1990). Smith *et al.* (1986) found irCRF release from minced tissue of the amygdala, striatum and midbrain after potassium stimulation in the presence of calcium, while Suda *et al.* (1985) found potassium-induced release of irCRF from rat hypothalami in vitro. The actions of CRF are mediated through two G-protein coupled receptors, CRF₁ and CRF₂. The two receptor subtypes have a unique distribution in the rat brain and CRF₁ receptors are abundant in the pituitary, cerebellum, brain stem, amygdala and cortex, while the CRF₂ receptors are found in the lateral septum, ventromedial hypothalamus, choroid plexus and olfactory bulb (Chalmers *et al.*, 1995). It has been demonstrated that, during stress, CRF enhanced behavioural suppression and increased freezing, facilitated conditioned fear, and decreased exploration and feeding (Koob *et al.*, 1993). Selective blocking of CRF₁ receptors with antagonists promoted anxiolytic responses in the elevated plus-maze, light-dark box, mouse defense test battery and the fear-potentiated startle test (Griebel *et al.*, 1998; Lundkvist *et al.*, 1996; Okuyama *et al.*, 1999). Finally, CRF₁ knockout mice showed increased anxiolytic

activity in the elevated plus-maze and a tendency to enter the illuminated region of the light-dark box (Smith *et al.*, 1998). Findings such as these suggest that CRF has anxiety provoking behavioural effects. Considering the important role for CRF in mediating the physiological and behavioural response to stress, it can be argued that disturbances in the control of CRF synthesis and release may compromise homeostasis and stress adaptation of an organism, and can result in pathophysiology, including major depression and anxiety (Groenink *et al.*, 2003).

The behavioural effect of intracerebroventricular (ICV) administration of CRF has been thoroughly investigated in rats. CRF administered in non-stressed rats produces a dose-dependent behavioural activation or increase in locomotion, rearing and grooming when animals are tested in a familiar environment. Animals that are exposed to a novel or stressful environment after CRF administration, show behaviour reminiscent of that observed during stress (Koob & Heinrichs, 1999).

Rats chronically infused with CRF at a dose of 4.9µg/day for 7 days ICV spent significantly less time in the open arms of the elevated plus-maze and made less entries into the open arms (Buwalda *et al.*, 1997), while 3µg/day CRF for 5 days decreased the amount of entries into the closed arms (Pietersen, 2001) and 1µg/day for 5 days decreased time spent in the open arms (Song *et al.*, 1995). In contrast to these observations, Song *et al.* (1995) also found that chronic infusions of 0.1, 0.5 and 1µg CRF dose dependently increase activity and rearing

in the open field and Linthorst *et al.* (1997) also found increased activity after CRF infusion of 1 µg/µl/hour for 7 days.

Acute ICV administration of 1 µg CRF in rats produced behavioural activation (Matsuzaki *et al.*, 1989). Britton *et al.* (1982) found decreased rearing and food approach behaviour in rats acutely injected with 150 pmol CRF. CRF produced a dose-dependent increase in locomotor activity while it increased freezing and decreased locomotion in a novel open field (Koob *et al.*, 1993). Sutton *et al.* (1982) found similar results as 0.15 and 1.5 nmol CRF increased locomotion, rearing and grooming in a familiar cage while 0.15 nmol CRF decreased locomotion and rearing and increased freezing in a novel open field.

These data show that some researchers found CRF to have anxiogenic properties and decreases locomotor activity, while others reported opposite effects. The exact role of CRF in behaviour therefore remains controversial and more animal studies are needed.

1.1.3 The Amygdala

An important part of the limbic system in the brain is the amygdala. This brain area serves as the major interface between the sensory experiences, such as seeing an aggressor and perceiving indications of its harmful intentions and the biochemical and behavioural systems that respond to this information (Le Doux,

1990). The amygdala determines whether there should be a stress response or not, and activates the neuroanatomical and neurochemical circuitry of fear (Davis, 1992). Projections from the central amygdala to the bed nucleus of the stria terminalis and hypothalamus initiate the HPA-axis response, and projections to the nucleus accumbens (part of the basal ganglia) influences motor responses (Crossman & Neary, 1995). The amygdala, which is important in the control of emotional and autonomic responses to stress, receives its information through lateral and basolateral nuclei (Davis, 1992), Fig.1.2.

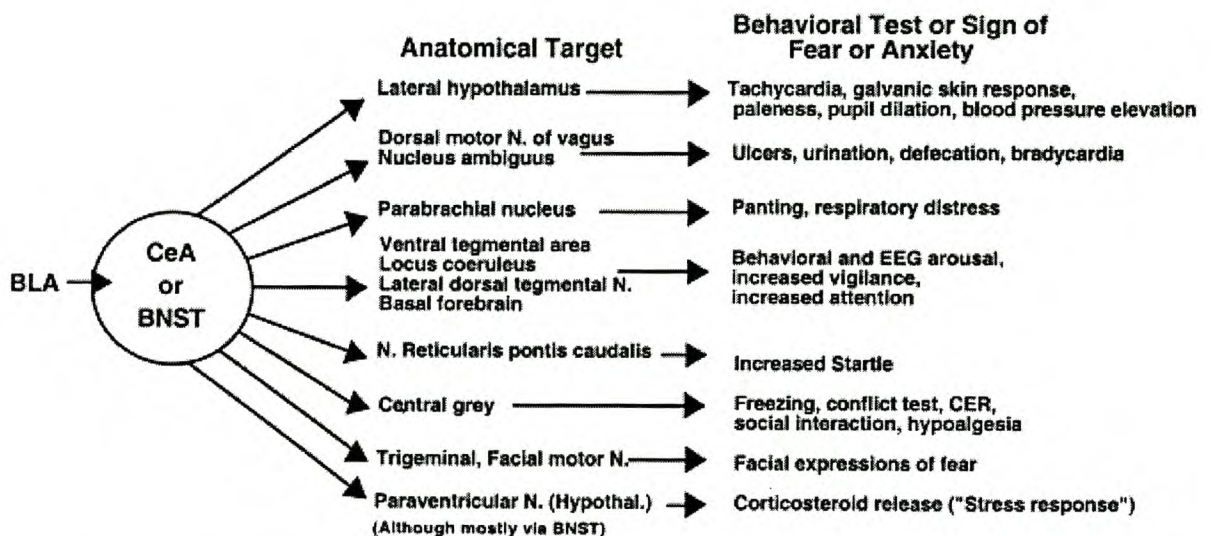


Fig. 1.2. The bed nucleus of the stria terminalis and central nucleus of the amygdala receive projections from the basolateral amygdala and project, in turn, to downstream target areas that mediate behavioural, autonomic and electrophysiological consequences of fear and anxiety. (From: Walker *et al.*, 2003).

It is evident from several studies that the amygdala facilitates the HPA response: In rats, electrical stimulation of the central amygdala (CeA) causes a rise in plasma ACTH and corticosterone levels (Feldman & Weidenfeld, 1998), and lesions of the amygdala attenuates HPA responses to stress (Feldman & Weidenfeld, 1998; Prewitt & Herman, 1994). Chronic implantations of corticosterone in the CeA caused corticosterone levels to be significantly elevated 45 and 90 min post stress in comparison to controls (Shepard *et al.*, 2003).

1.1.3.1 CRF and corticosterone in the amygdala

CRF release from brain regions such as the amygdala may be important for the stress response. The amygdala contains high levels of CRF receptors (De Souza *et al.*, 1985), cell bodies (Cummings *et al.*, 1983) and nerve terminals (Uryu *et al.*, 1992). A study by Chalmers *et al.* (1995) showed that the greatest density of CRF1 mRNA in the amygdala, is situated in the basolateral nucleus (BLA). In the BLA, a disruption of inhibitory neurotransmission, by injection of a γ -amino-butyric-acid (GABA) antagonist bicuculline methiodide in rats, induces behavioural and physiological responses similar to that seen in patients with anxiety disorders (Sanders & Shekar, 1995). Acute bilateral injections of CRF or urocortin in the BLA also reduced social interaction time of rats (Sajdyk *et al.*, 1999).

The role of CRF in the central nucleus of the amygdala has also been investigated and bilateral injection of α -helical CRF (a CRF antagonist) in the CeA reduced the freezing time of rats after re-exposure to a shock box (Swiergel *et al.*, 1993), while a reduction in exploration of the open arms of the elevated plus-maze following ethanol withdrawal, was antagonized by bilateral infusion of the antagonist (Rassnick *et al.*, 1993). Rats injected bilaterally with CRF at the dorsal margin of the amygdaloid complex exhibited decreased exploration and rearing in the open field (Liang & Lee, 1988). Hauger *et al.* (1993) showed that chronic intracisternal CRF administration also decreased CRF receptor density in the amygdala.

Neurons in the amygdala express both type 1 and type 2 corticosteroid receptors, with the highest density in the CeA (Morimoto *et al.*, 1996). Chronically elevated corticosterone increases CRF mRNA in the central amygdala (Makino *et al.*, 1994; Shepard *et al.*, 2000), and this upregulation may contribute to anxiety-like behaviours observed with elevated corticosterone (Schulkin *et al.*, 1998). Shepard *et al.* (2000) investigated the effect of bilateral corticosterone implants in the CeA of rats and found that after 7 days, the rats exhibited a decrease in exploration of the open arms of the elevated plus-maze indicating an anxiogenic effect of corticosterone. Similarly, Greenwood-Van Meerveld *et al.* (2001) found increased measures of anxiety in a different rat strain after chronic corticosterone implants. These studies indicate that the

interaction between CRF and/or corticosterone and the amygdala may contribute to the establishment of behavioural abnormalities.

1.1.4 Psychiatric disorders

Psychiatric disorders are common internationally, and an estimated 22.1% of Americans above the age of 18 are diagnosed with a mental disorder in a given year (Regier *et al.*, 1993). When this figure is applied to the 1998 United States census residential population estimate, it translates to 44.3 million people (Narrow, 1998). Additionally, 4 of the 10 leading causes of disability in the United States are mental disorders (Murray & Lopez, 1996). The anxiety disorders accounts for one third of the total costs of all the psychiatric disorders (Dupont *et al.*, 1996). Psychiatric disorders are probably a result of a complex interaction of genetic, behavioural and developmental factors. In order to develop better treatments, animal studies and clinical trials are used to pinpoint specific brain areas and neurotransmitters involved in anxiety and fear, the two behaviours that underlie anxiety and depressive disorders.

1.1.5 Psychiatric disorders and HPA-dysfunction

Conditions directly associated with impairment in the regulation of stress systems are most likely to be anxiety and depressive disorders (Sullivan & Gratton, 2002). Patients with anxiety disorders such as posttraumatic stress disorder (PTSD), panic disorder and obsessive-compulsive disorder (OCD), as well as patients diagnosed with major depression show symptoms of a dysregulated HPA-axis.

Several studies support the hypothesis of hypersecretion of CRF in anxiety disorders and depression (Nemeroff *et al.*, 1984). Various clinical studies have also been done to determine baseline plasma and urinary cortisol levels, as well as ACTH and cortisol levels after a challenge, in patients and compared it to normal persons.

1.1.5.1 Anxiety disorders

Each of the anxiety disorders has a component comprising anxiety symptoms:

1. In PTSD, after a traumatic event, patients re-experience the past event
2. In OCD, patients have obsessive thoughts that increase anxiety and compulsive behaviours help to decrease anxiety
3. Panic disorder patients develop spontaneous periods of anxiety called panic attacks (Stein & Hugo, 2002).

1.1.5.2 OCD

Elevated baseline levels of plasma cortisol (Monteleone *et al.*, 1995) and urinary free cortisol (Gehris *et al.*, 1990) were measured in OCD patients. Altemus *et al.* (1992) measured elevated cerebrospinal fluid (CSF) concentrations of CRF in adult OCD patients compared to controls, while Fossey *et al.* (1996) measured increased CRF only in male patients. A blunted ACTH response was observed after CRF administration, suggesting a chronic increase in CRF release resulting from a hyperactive HPA-axis (Servant, 1997).

1.1.5.3 Panic Disorder

Most studies found elevated or normal basal cortisol plasma levels in patients with Panic Disorder (Charney & Bremner, 1999). Similarly, Marshall *et al.* (2002) measured significantly elevated baseline cortisol levels in panic patients vs. controls, but CSF levels of CRF were not elevated (Jolkkonen *et al.*, 1993; Fossey *et al.*, 1996). A blunted ACTH response was observed after CRF administration, similar to that observed in patients with OCD (Servant, 1997).

1.1.5.4 PTSD

Studies on PTSD patients showed decreased urinary cortisol levels compared to controls (Mason *et al.*, 1986; Yehuda *et al.* 1990, 1995) and, although results are conflicting, a majority of studies observed decreased baseline plasma cortisol levels in PTSD patients (Boscarino, 1996; Stein *et al.*, 1997; Goenjian *et al.*, 1996, Marshall *et al.* 2002). CSF concentrations of CRF are normally higher in PTSD patients than in healthy persons and could be an indication of a hyperactive hypothalamus (Bremner *et al.*, 1997). A blunted ACTH response to CRF is also observed in PTSD patients (Smith *et al.*, 1989; DeBellis *et al.*, 1994).

1.1.5.5 Depression

Depressive patients show increased basal cortisol as well as increased levels of CRF in their CSF (Plotsky *et al.*, 1998; Wong *et al.*, 2000). These patients, like PTSD and panic disorder and OCD patients also show a blunted ACTH response to CRF administration (Holsboer *et al.*, 1986).

1.1.5.6 Extrahypothalamic CRF systems in psychiatric disorders

The above-mentioned studies provide some evidence for increased CRF secretion in the brain. Neuropeptides appear to be secreted directly into CSF from brain tissue, therefore CRF found in CSF is unlikely to be derived from systemic circulation. Studies with non-human primates suggest that this elevation in CRF concentrations may reflect function of extrahypothalamic CRF systems (Post *et al.*, 1982; Kalin, 1990)

1.1.6 Serotonin and psychiatric disorders

Dysfunction in serotonergic (5-HT) neurotransmission has been associated with depression, anxiety, panic disorder and obsessive-compulsive disorder (Graeff *et al.*, 1997; Levy & Van de Kar, 1992). 5-HT_{1A} Receptors are densely distributed in limbic brain areas such as the hippocampus, amygdala and entorhinal cortex (Pazos & Palacios, 1985) and the serotonergic input into these brain areas is

mainly from the raphe nucleus (Vizi & Kiss, 1998). A reduced number of 5-HT₁ receptors have been found in post-mortem brains of depressed suicide patients (Cheetam *et al.*, 1990).

Antidepressant drugs, or serotonin reuptake inhibitors, are effective in improving both depression and anxiety, specifically in patients with OCD, panic disorder and generalized anxiety disorder (Nutt, 1991). Effective antidepressant treatment has been found to enhance 5-HT_{1A} function in the hippocampus (Blier *et al.*, 1987; De Montigny & Blier, 1992), while acute tryptophan depletion produces a return in depressive symptoms in 80% of treated patients (Delgado *et al.*, 1990). A possible mechanism may be an increase in synaptic 5-HT levels to maximize binding to depleted 5-HT_{1A} receptors.

Activation of postsynaptic 5-HT_{1A} receptors inhibits various other neurons for example GABA-ergic interneurons (Barnes & Sharp, 1999) and agonists for this receptor subtype modulates anxiety in humans. In clinical studies, 5-HT_{1A} agonists have been efficient in clinical studies to reduce depression and anxiety in depressive patients and chronic treatment does not desensitize this receptor in the hippocampus (Blier & De Montigny 1987). One hypothesis for the efficacy of 5-HT_{1A} agonists in both depression and anxiety, is that 5-HT transmission would be decreased in patients with depression since the exogenous agonist would act in synergy with the low levels of endogenous 5-HT, while the agonist would

compete with high levels of endogenous 5-HT and decrease 5-HT transmission in patients with anxiety (De Vry, 1995).

The hippocampus is activated by stress, since 30 min restraint stress in rats induced *c-fos* mRNA expression, which is a marker of neuronal activity (Morgan & Curran, 1991). Microdialysis studies on rats provide evidence that handling, tail pinch and exposure to the elevated plus-maze increases 5-HT release in the hippocampus (Bickerdike *et al.*, 1993; Kalen *et al.*, 1989). Acute stress either increases or decreases hippocampal and/or cortical 5-HT_{1A} receptors (Raghupathi & Mcgonigle, 1997), and postsynaptic 5-HT_{1A} hippocampal receptors have been postulated to play a role in adaptation to stress (Meijer & De Kloet, 1998). 2 Weeks of chronic, unpredictable stress also reduced 5-HT_{1A} receptor binding in rat hippocampi (Lopez *et al.* 1998). In a study on ultrasonic vocalizations in rat pups during separation anxiety, Olivier *et al.* (1994) found that both 5-HT_{1A} agonists and SSRI's decrease the calling of the pups. Studies using 5-HT_{1A} receptor knockout mice as a model of anxiety and depression, found that the knockout mice are less active than controls in stressful situations, implicating that these mice are more anxious (Olivier *et al.*, 2001). It is evident from these animal studies that anxiety is regulated at least in part by the hippocampal 5-HT_{1A} receptor.

1.2 Stereotypical Behaviour Study

1.2.1 Stereotypical behaviour

Stereotypies are described as movements that are combined into rhythmic or complex sequences of obscure purpose and have attained a level of functional autonomy (Woodworth, 1918). Another definition depicts them as sequences of motor behaviour that are repetitive, topographically invariant and purposeless (Ridley, 1994). Stereotypical behaviour is a characteristic feature of a range of psychiatric disorders, for example obsessive-compulsive disorder, Tourette's disorder and trichotillomania. Stereotypical behaviour is not limited to human psychiatric disorders, but results from brain damage, environmental stimuli or as a result of drug treatment (Mason, 1991). Although many studies on stereotypical behaviour have been done, the complex neurobiology of this phenomenon is still not clear.

1.2.1.1 Environmental vs. drug induced stereotypies

'Cage' stereotypies, which are commonly seen in caged laboratory animals such as rats, mice and monkeys, are elicited by the frustration of specific motivated behaviours for example escaping the cage (Rushen *et al.*, 1993), in combination with stress (Wurbel & Stauffacher, 1997), and / or a reduction in environmental complexity (Toates, 2000). Some examples of 'cage' stereotypies are pacing, backward somersaulting and jumping on all fours in monkeys (Berksen, 1967),

bar gnawing and jumping in mice (Wurbel *et al.*, 1996), backward somersaults and jumping in bank voles (Schoenecker & Heller, 2003). These behaviours can generally be abolished by environmental enrichment or removing the animal from the cage. It has been proposed that stereotypies in laboratory animals serve a coping function to reduce stress and, if this was the case, prevention of stereotypy would increase stress levels. Kennes & De Rycke (1988) found that bank voles with stereotypy had longer lasting and more pronounced levels of plasma corticosterone than individuals without stereotypy, while Wurbel and Stauffacher (1996) measured significant elevations in serum corticosterone 24h after mice were prevented from wire-gnawing. These results therefore confirm the stress-relieving function of stereotypic behaviour.

An animal model of OCD, acral lick dermatitis (ALD) in dogs, is an example of environmentally induced stereotypical behaviour. The development of the stereotypies in dogs is believed to be secondary to boredom, loneliness and confinement (Veith, 1986). It is interesting that ALD is more likely to develop in certain large breeds of dogs, such as Labradors, German sheperds, Great Danes and Saint Bernards as well as in individual families of dogs. This phenomenon suggests that some dogs are genetically predisposed to develop ALD in a similar environment (Rapoport *et al.*, 1992).

Psychomotor stimulant drugs such as amphetamine and apomorphine can also induce stereotypies. These substances increase dopamine release from nerve

terminals in the ventral (nucleus accumbens) and dorsal striatum (caudate putamen). The stereotypy usually becomes more intense with increasing doses of the drug (Lyon & Robbins 1975). In monkeys, amphetamine induces repetitive movements such as self-grooming, staring at the hands, snatching the air (Ellison *et al.*, 1980) and excessive grooming when injected locally in the dorsal striatum (Robbins *et al.*, 1990).

The dopaminergic system is not the only neurotransmitter system implicated in stereotypical behaviour. Manipulation of postsynaptic 5-HT receptors by 5-HT agonists also leads to stereotypical behaviour including forepaw-treading, 'wet-dog' shakes and head weaving (Gerson & Baldasserini, 1980; Curzon, 1990). Morphine, and other opioids given to rats and mice, induces stereotyped locomotion and oral stereotypies (Robbins & Sahakian, 1981). Direct injections of opiate agonists in the substantia nigra produce intense stereotypies in rats presumably because of disinhibition of nigrostriatal dopaminergic projections (Wood & Richard, 1982). Similarly, central injection of a large number of active neuropeptides induces excessive grooming in rats (Isaacson & Gispen, 1990).

1.2.1.2 Animal models of psychiatric disorders

Three types of validity characterize animal models for human disorders. Face validity of a model is where symptoms of the model are phenotypically similar to that of the human disorder, which can be measured by behavioural changes.

Good predictive validity of an animal model is where the animal responds to drugs used in treatment of the human disorder and symptoms are decreased. Construct validity refers to the underlying mechanism of the symptoms, and whether the cause of the abnormal behaviour is similar in both the animal model and human disorder (Overall, 2000).

1.2.1.3 Anatomy of the basal ganglia

Stereotypies are associated with basal ganglia dysfunction in the brain. The anatomy of the brain and basal ganglia that is involved in motor control is shown in fig. 1.3.

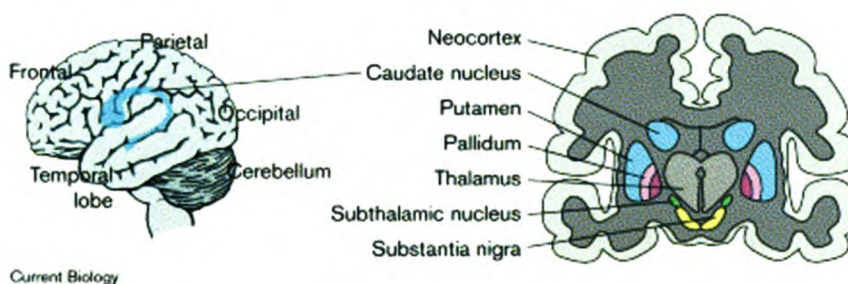


Fig 1.3. The anatomy of the basal ganglia. (From: Graybiel, 2000)

Selection and sequencing of normal behaviour and movement is mainly regulated by the interaction of two antagonistic cortico-striatal circuit loops in the dorsal basal ganglia. The direct pathway activates behaviour while the indirect pathway is inhibitory and allows for proper transitions between behaviours (Fig. 1.4). Behaviour is elicited by the activation of different subtypes of dopamine receptors and negative feedback within the circuits are done by dynorphin

releasing neurons in the direct pathway and enkephalin releasing neurons in the indirect pathway (Alexander & Crutcher, 1990; Hauber, 1998).

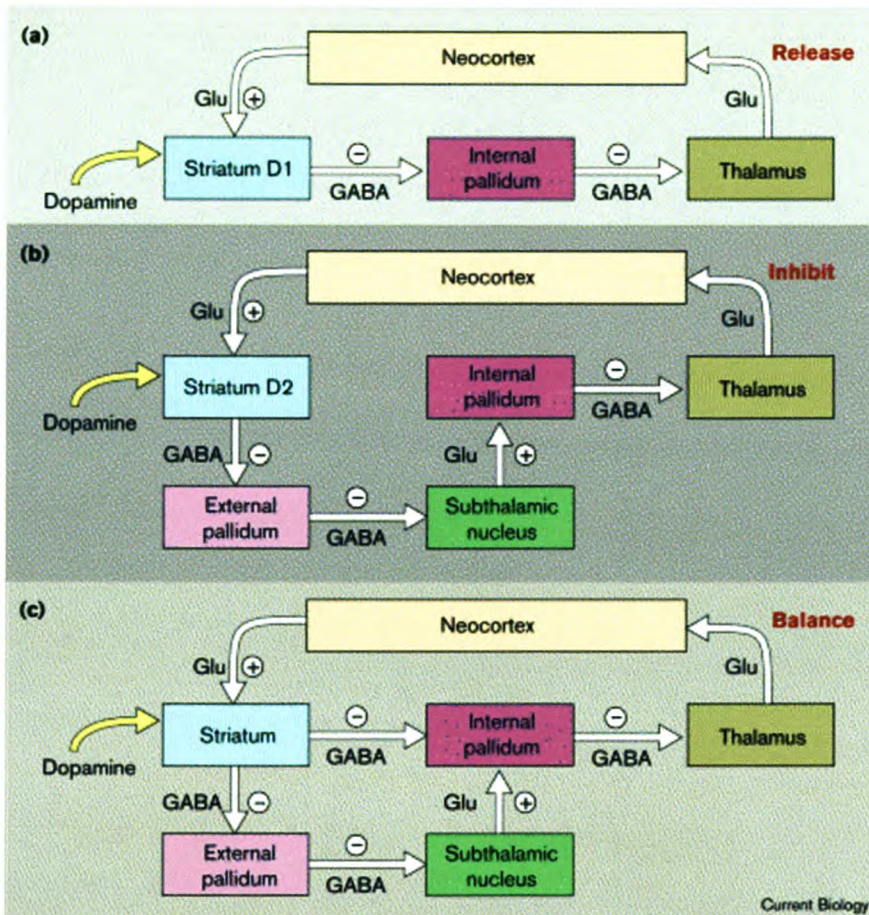


Fig. 1.4. (a) The direct pathway consists of two successive GABAergic connections from the striatum to the internal pallidum and from the internal pallidum to the thalamus. Glutamate input from the neocortex to the striatum disinhibits thalamic neurons, and dopamine modulates the system at the level of the striatum. (b) In the indirect pathway there is an extra step after the external pallidum, so that the subthalamic nucleus excites the internal pallidum. (c) Balance is achieved when these antagonist systems are combined under normal circumstances. (From: Graybiel, 2000).

1.3 Aims

The aim of both these studies was to investigate possible models for psychiatric disorders. The CRF study focused on a rat model for anxiety disorders (OCD, PTSD and Panic Disorder) and depression. In these disorders, HPA-axis abnormalities are common and an increase in CRF is observed in CSF of patients. We investigated the role of elevated CRF in the BLA of rats in the development of anxious behaviour and HPA-axis abnormalities. The stereotypical behaviour study focused on OCD. Compulsions observed in patients with OCD are compared with spontaneous stereotypical behaviour of deermice. The aim was to characterize a mouse model of OCD in terms of predictive, face and construct validity.

Chapter 2:

Methods

2.1 Behavioural experiments

2.1.1 The Elevated Plus-maze

The Elevated Plus-maze has been validated by various authors (Pellow *et al.*, 1985; Rodgers & Dalvi, 1997) and is used widely in rat behavioural studies as a measure of anxiety and locomotor activity. Fig.2.1. shows a photograph of the Elevated Plus Maze, which is constructed of black Perspex. The maze is elevated half a meter from the ground, and consists of 2 opposed closed arms (50x10x40cm) and 2 opposed open arms (50x10cm). The arms are situated at 90° angles from each other with an open square in the middle (10x10cm). The maze is designed to test the rat's level of anxiety based on the following principle: When placed in the maze, the animal experiences two conflicting behavioural states. The maze provides a novel or unfamiliar environment that the rat would like to explore, but the rat simultaneously experiences a fear of the open spaces and height. If the rat is more anxious, it will tend to avoid the open arms of the maze and exploratory behaviour will be reduced. At the same time the maze can be used to test locomotor activity levels by recording the amount of entries into the arms. Rats that are more anxious will show decreased activity (Pellow *et al.*, 1985; Rodgers & Dalvi, 1997).

In our study, rats were placed on the maze for 5 min. to evaluate their level of anxiety and locomotion. The room was dark and only dim light used on the elevated plus-maze during testing. After an acclimation period of to 1 hour to the

behavioural test room, rats were placed in the middle of the maze, facing an open arm, at the beginning of the 5 min time period, and an entry into an arm was recorded when one leg crossed the line at the entrance of the arm, and out again when all 4 legs crossed the line to the middle of the maze. This criterion was chosen since we found that there is no significant difference in total time when using one or two arms as entry into an arm. The maze was cleaned with a 70% ethanol solution and dried between recordings, in order to avoid the possibility of chemical cues affecting the behaviour of the rats.

The following parameters were used for scoring: Amount of time spent in the open and closed arms; the amount of entries into the open and closed arms; rearing (standing on its hind paws), grooming (washing of the coat) and freezing (absolute stillness of the rat and whiskers for 2 seconds or longer) as anxiety-related behaviours (Escorihuela *et al.*, 1999). Three independent individuals performed the scoring and rated the video recordings at the same time, each noting a different set of behaviours.

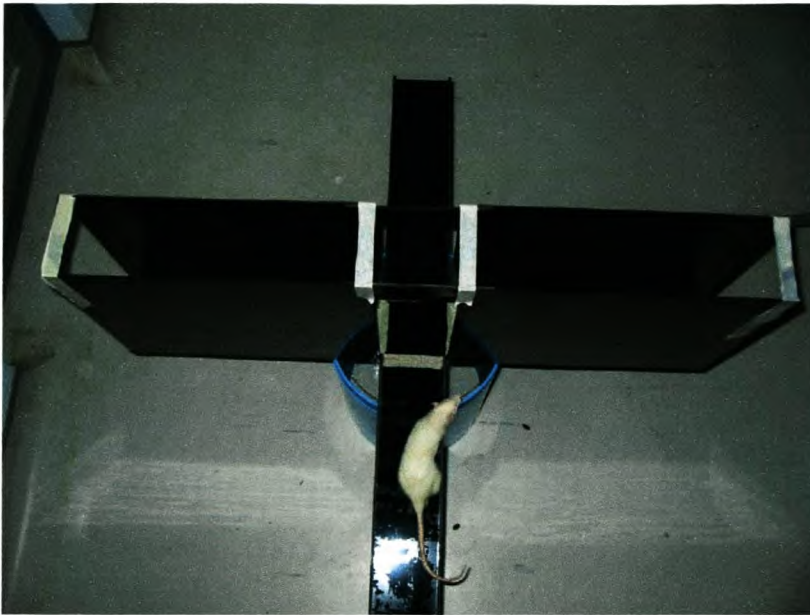


Fig.2.1. The Elevated Plus-maze viewed from above.

2.1.2 The Open Field test

The Open Field test is used in behavioural studies as an indication of locomotor activity as well as anxiety of rats (Prut & Belzung, 2003; Royce, 1977). Fig.2.2 shows a photograph of the Open Field arena, which is constructed from white perspex sides and gray floor with dimensions of 1x1x0.5m. The floor is divided into 25 squares as well as an outer and inner zone demarcated by a line 15cm from the sides. A fluorescent lamp is positioned above the middle of the box, about 20cm from the ground and illuminates the inner zone while the outer zone is dimmer. The level of anxiety of a rat is tested while moving in the Open Field, since the more anxious the rat is, the more it tends to avoid the brightly lit inner zone, and stays closer to the darker outer zone. The locomotor activity is

simultaneously tested as the rat moves across the floor by counting the amount of squares crossed.

The rats were always placed in the same corner of the open field, and video recordings were made for 5 min. Parameters that were used to test the behaviour of the rats included the following: number of blocks crossed; time spent in the outer zone vs. time spent in the inner zone, number of crossings between the outer and inner zone; rearing and grooming. The light in the room was dimmed prior to testing in the Open Field so that the effect of the lamp in the center of the box was more pronounced. The Open Field was cleaned with a 70% ethanol solution and dried after each rat was tested.

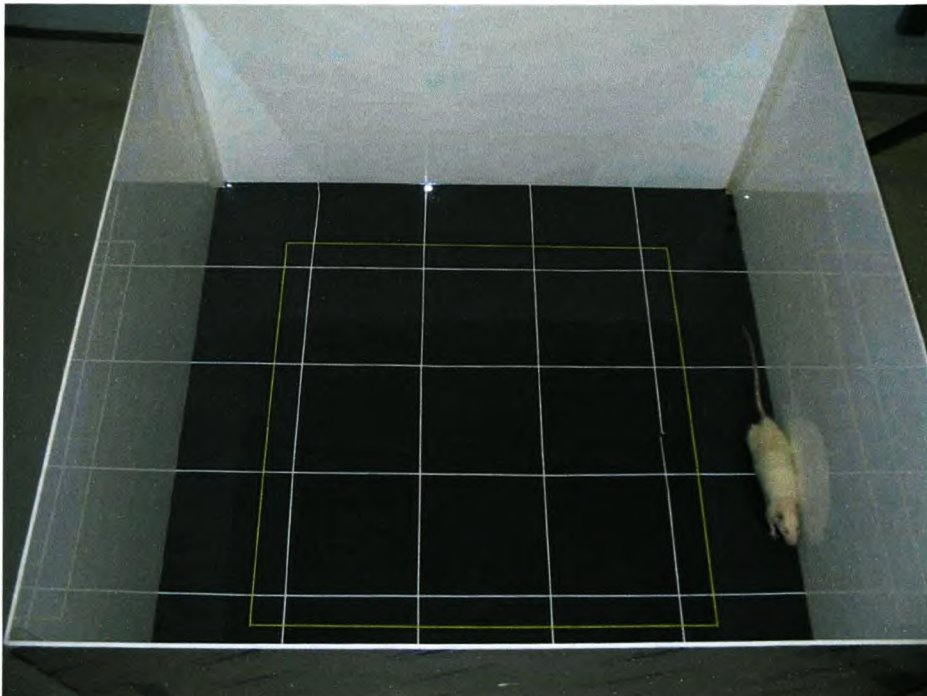


Fig.2.2. The Open Field arena viewed from above.

2.1.3 Time of behavioural tests

In order to assess the best time to perform the behavioural experiments, 12 naïve male Wistar rats were acclimated to the test room for 1 hour and placed on the elevated plus maze and open field (5 min each). Video recordings were made and rated afterwards. The first group (n=4) was recorded between 10:00 and 12:00 (morning), the second group (n=4) between 14:00 and 16:00 (afternoon) and the third group (n=4) between 20:00 and 21:00 (evening). Locomotor activity was noted as the amount of entries into the open and closed arms of the elevated plus maze, the amount of blocks crossed and the number of crossings between the outer and inner zone of the open field, as well as rearing. Statistical analyses were done using Kruskal-Wallis non-parametric test with Dunn's multiple comparison post-hoc test. There was no significant difference in rearing, the amount of entries in the elevated plus maze or number of crossings between the outer and inner zone of the open field. There was, however, a significant increase in the number of blocks crossed in rats tested in the morning as opposed to the other two groups ($p < 0.05$), Fig.2.3. Following this result, we decided to record all behaviour in the morning when activity levels were highest.

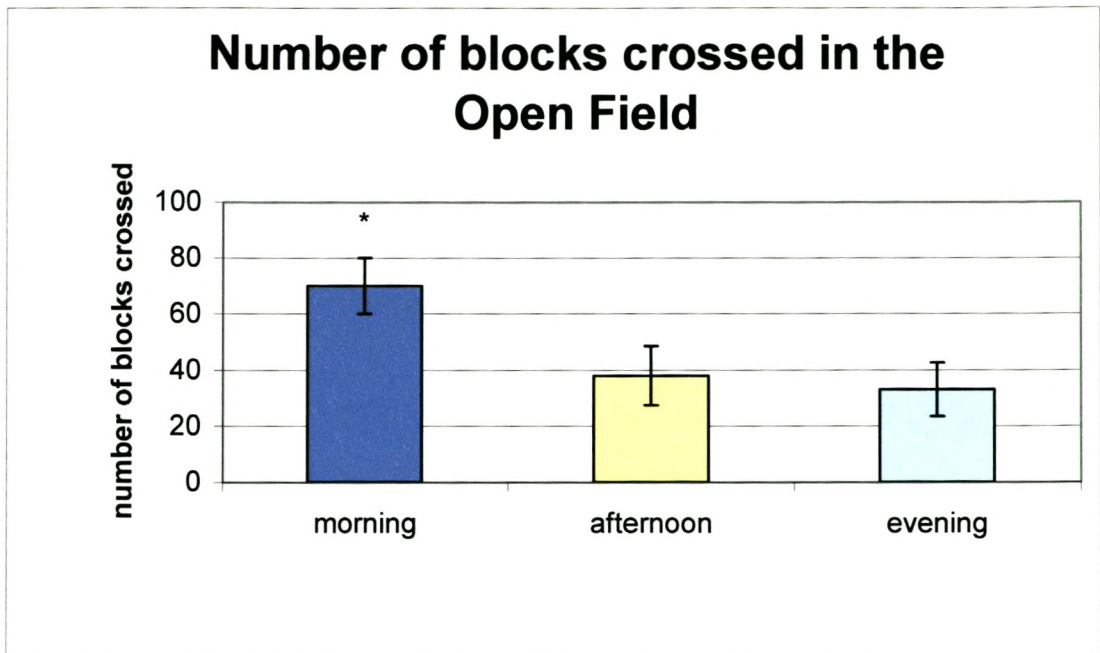


Fig. 2.3. Significant differences in the number of blocks crossed in the open field were found between rats tested in the morning and rats tested in the afternoon or evening (* = $p < 0.05$). Values are shown as means \pm SEM.

2.2 Stereotaxis

Stereotaxis was performed using a David Kopf stereotaxis apparatus. Rats were left in the theatre for at least one hour before experiments. Prior to surgical manipulation, animals were anaesthetised with equithesin (1.5ml/300g bodyweight). When the rat was fully sedated, the hair on the head was shaved and the area cleaned with a 70% ethanol solution. The rat was placed in the stereotaxis apparatus, and carefully positioned by inserting the ear bars and centering the head. The incisors were put in the incisor bar. A heating pad, attached to a temperature controller, maintaining the heat at a temperature of 37°C, was placed under the rat. An incision was made with a scalpel, and subcutaneous tissue scraped away with a spatula until the skull was exposed. The surface was cleaned with saline solution, and dried with Kleenex tissue. Using a dissecting microscope (Zeiss), the coordinates of bregma and lambda as well as the midline were determined. The coordinates for the basolateral amygdala were the following: Anterior-Posterior -3.3 from Bregma, Medial-Lateral -4.6 and Dorsal-Ventral -8.7, according to the atlas of Paxinos & Watson (1986). An electrical drill was used to drill holes in the skull, and the excess bone was removed using forceps. Four stainless steel screws (1.6mm) were inserted into the skull to serve as anchors in order to fix the dental cement to the skull. The Dura Mater was punctured with a needle, and the guide cannulae were lowered into the brain. Surgicell was inserted around the cannulae into the holes that were drilled, and dental cement was used to fix the cannulae in this position.

After the cement hardened, dummy cannulae were inserted into the guide cannulae (purchased from Plastic Products Company, Virginia) and rats were left in their home cages to recover for 3-4 days before the start of any experimental procedures.

2.3 Histology

Histology was performed on rat brains to verify placement of the cannulae. Histology was only performed on a number of rats since other brains were used for dissection of the hippocampus. After rats were anaesthetized, 1µl of Indian black ink was injected, the rats were sacrificed by decapitation and the brain removed and stored in 10% formalin solution. After fixation, the brains were processed and imbedded in paraffin wax. The brains were sectioned (20µm) and stained with Heamatoxylin and Eosin. Fig. 2.4 is a photograph of a slide showing unilateral implantation and Fig. 2.5 a bilateral implantation showing only the right side of the brain.

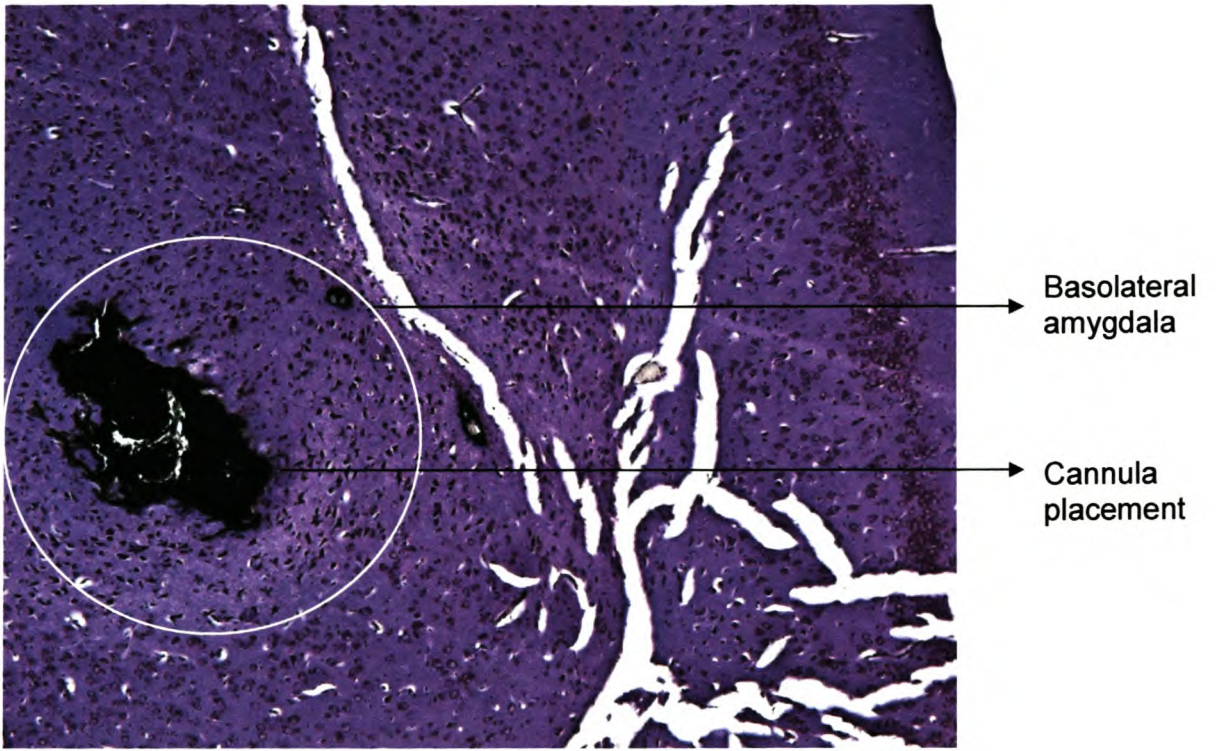


Fig. 2.4. Unilateral implantation in the basolateral amygdala.

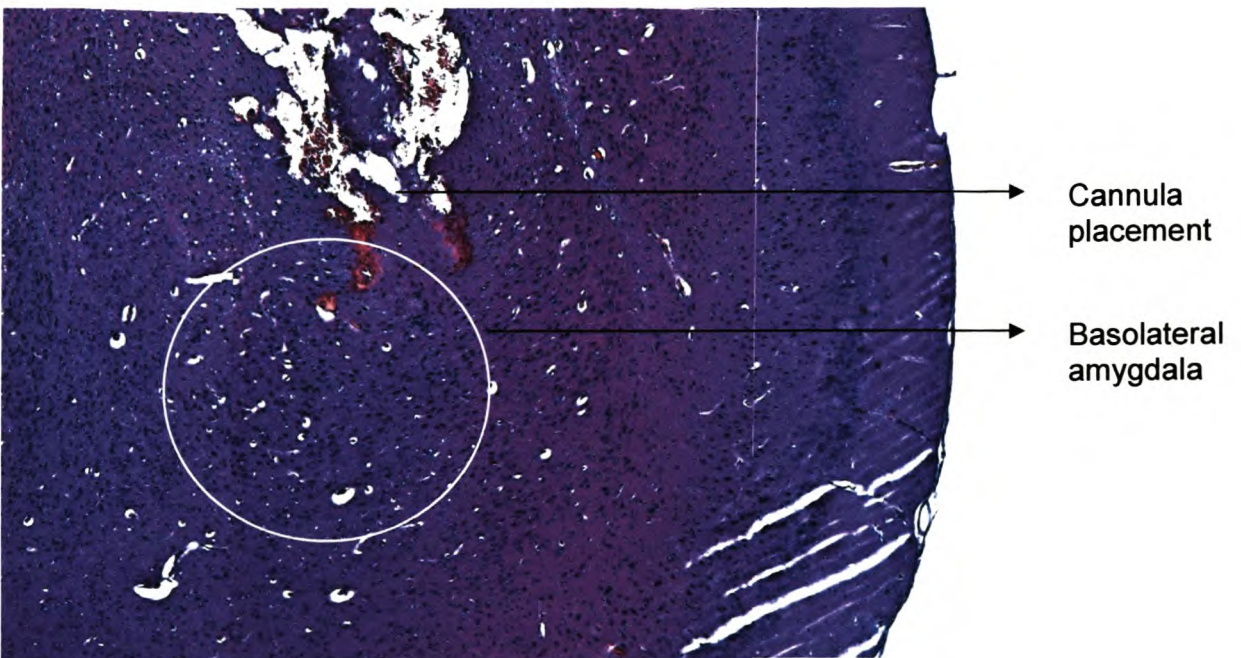


Fig. 2.5. Bilateral implantation in the basolateral amygdala.

2.4 Radioligand binding

2.4.1 CRF study: serotonin (5HT)_{1A} receptors in the rat hippocampus

The hippocampi were removed on an ice cold glass slab, snap frozen in liquid nitrogen and stored at -80°C until radioligand binding assays were performed using the method of Jackson & Etgen (2001) and Keck & Lakoski (2000).

2.4.1.1 Membrane preparation

Tissue samples were weighed, thawed and suspended in 20ml cold buffer (50mM Tris, 4mM CaCl₂, pH 7,7). The sample was homogenised for 5 sec using a Polytron. The suspension was then centrifuged in a Beckman Ultracentrifuge (4°C) for 10 min at 15000rpm. The supernatant was discarded and the pellet resuspended in 20 ml buffer, homogenized with a glass teflon homogeniser and centrifuged. This process was repeated twice and before the last centrifugation, incubated at 37°C in a shaking waterbath. The pellet was resuspended in 20 volumes buffer and homogenized using the polytron.

2.4.1.2 Binding assay

2-(N,N-di[2,3(n)-³H]propylamino)-8-hydroxy-1,2,3,4-tetrahydronaphthalene ([³H]-8-OH-DPAT) binding assays were performed at room temperature. The total incubation volume for each tube was 500µl. Each tube contained 100µl membrane suspension (1mg/ml protein), 300µl buffer and 100µl [³H]-8-OH-DPAT (197 Ci/mMol, Amersham Biosciences). A concentration range of 0.25 to 20nM with 8 different concentrations was used for [³H]-8-OH-DPAT. Non-specific binding was measured by adding 100µl 5-HT (20µM) instead of 100µl buffer. The radioligand was added in increasing concentrations and the homogenate was vortexed before incubation for 15min at 37°C. Assays were terminated by rapid filtration through Whatman GF/C glass fibre filters, which were saturated with buffer before filtration. Tubes and filters were washed with cold buffer, and filters were transferred into counting vials (Packard) and covered with 10ml Filter Count scintillation fluid (Packard). After at least 3 hours, radioactivity on the filters was determined using a Beckman scintillation counter. Receptor density (B_{max}) and affinity (K_d) were calculated using GraphPad Prism version 2.01.

2.4.2 Stereotypical behaviour study: NMDA receptors in the mouse frontal cortex

Frontal cortices of the brains were removed on an ice cold glass slab, snap frozen in liquid nitrogen and stored at -80°C until radioligand binding assays were performed on NMDA receptors using a modified method of Foster and Wong

(1987), in collaboration with the Department of Pharmacology, North West University, South Africa.

2.4.2.1 Membrane preparation

Tissue samples were thawed and suspended in 5ml cold buffer (100ml 5mM HEPES and 100ml 4,5mM Tris made up to 1l with distilled water, pH 7.8) and homogenised for 6 sec using a Brinkman Polytron. The suspension was centrifuged at 18000 rpm for 20min using a Beckman Ultracentrifuge (4°C). The pellet was resuspended in buffer and the process was repeated twice. The final pellet was suspended in 5ml buffer and homogenised for 6 sec.

2.4.2.2 Binding assay

[³H]MK801 binding assays were performed at room temperature. The total incubation volume for each tube was 500µl. Each tube contained 300µl membrane suspension, 50µl glutamate (10µM, Sigma), 50µl glycine (30µM, Sigma), 50µl buffer and 50µl [³H]MK801 (28.9 Ci/mMol, Amersham Biosciences). A concentration range of 0.1 to 10nM with 8 different concentrations was used for [³H]MK801. Non-specific binding was measured by adding 50µl MK801 (5µM, Sigma) instead of buffer. The radioligand was added in increasing concentrations and the homogenate was vortexed before incubation for 90min at room temperature. Assays were terminated by rapid filtration through Whatman

GF/C glass fibre filters, which were saturated with buffer before filtration. Tubes and filters were washed with cold buffer, and filters were transferred into counting vials (Packard) and covered with 4ml Filter Count scintillation fluid (Packard). After at least 3 hours, radioactivity on the filters was determined using a Packard Tri-Carb 4660 counter. Receptor density (B_{max}) and affinity (K_d) were calculated using the Combiccept program (Packard).

2.4.3 Protein determination

Protein determinations were done using the method described by Lowry *et al.* (1951). For the standard curve, 5 concentrations of BSA (1mg/ml) were used (Table 2.1.).

Table 2.1. Contents of tubes used for drawing a standard curve in protein determination.

	Distilled water	Buffer	BSA
Standard 1	940µl	50µl	10µl
Standard 2	930µl	50µl	20µl
Standard 3	920µl	50µl	30µl
Standard 4	910µl	50µl	40µl
Standard 5	900µl	50µl	50µl

All protein determinations were done in duplicate, and an average value was used for assay analyses. Sample tubes contained 950 μ l distilled water and 50 μ l tissue homogenate, obtained from membrane preparation for the binding assay. 1ml of Tartrate reagent (Table 2.2) was added to each tube, and incubated for 10 min at room temperature. 3ml Folin reagent (3ml folin +27ml distilled water) was added to each tube, which was then vortexed and incubated for 10 min at 50°C. A blank tube containing 950 μ l distilled water and 50 μ l buffer, was used to zero the spectrophotometer (Spectronic 20 Genesys), and absorbance of standards and sample tubes were read at 650nm at room temperature. The standard curve was used to determine protein contents of tissue samples.

Table 2.2: Contents of Tartrate reagent used for protein determination.

NaOH	0.5g in 25ml distilled water
Na ₂ CO ₃	2.5g
K Tartrate	2.5ml of 10% solution
CuSO ₄	250 μ l of 5% solution

2.5 Adrenocorticotropin Hormone (ACTH) determinations

Blood samples of rats were collected in EDTA tubes after decapitation and centrifuged at 4°C at 5000rpm. Plasma was collected and stored in liquid nitrogen until the assay was done. An ACTH radioisotopic assay was purchased from Nichols Institute Diagnostics. The kit included 7 standards for establishing a standard curve, as well as two controls. Each standard and control was assayed in duplicate, and the average CPM was determined using a Packard gamma counter. The plasma samples of the rats were also assayed in duplicate.

2.6 Corticosterone determinations

Blood samples of rats were collected in EDTA tubes after decapitation and centrifuged at 4°C at 5000rpm. Plasma was collected and stored in liquid nitrogen until the assay was done. Corticosterone determinations were done using an ADVIA centaur system, in collaboration with the Department of Chemical Pathology, University of Stellenbosch.

Chapter 3:

Corticotropin-Releasing Factor Study

3.1 Introduction

Psychiatric disorders are common internationally and almost 22% of adult Americans are diagnosed with a mental disorder in a given year (Regier *et al.*, 1993). This figure translates to 44.3 million people when applied to the 1998 United States census residential population estimate (Narrow, 1998). Anxiety disorders are amongst the most prevalent of the psychiatric disorders (Kessler *et al.*, 1994), and they account for one third of the costs of all the psychiatric disorders (Dupont *et al.*, 1996).

Patients diagnosed with certain mood and anxiety disorders show symptoms of a dysregulated hypothalamic-pituitary-adrenal (HPA) axis. Patients diagnosed with major depression show increased plasma cortisol and corticotropin-releasing factor (CRF) in their cerebrospinal fluid (CSF) (Plotsky *et al.*, 1998; Wong *et al.*, 2000) as well as a blunted adrenocorticotropin hormone (ACTH) response to CRF administration (Holsboer *et al.*, 1986). Elevated plasma or urinary cortisol levels have been observed in patients with obsessive-compulsive disorder (OCD) (Monteleone *et al.*, 1995; Gehris *et al.*, 1990) as well as in patients with panic disorder (PD) (Charney & Bremner, 1999; Marshall *et al.*, 2002). However, in patients with posttraumatic stress disorder (PTSD), decreased levels of urinary (Mason *et al.*, 1986; Yehuda *et al.*, 1990, 1995) and plasma cortisol levels have been observed (Boscarino, 1996; Stein *et al.*, 1997; Goenjian *et al.*, 1997; Marshall *et al.*, 2002). A blunted ACTH response to CRF administration is observed in OCD, PD and PTSD patients (Servant, 1997; Smith *et al.*, 1989; DeBellis *et al.*, 1994;

Bremner *et al.*, 2003). Elevated concentrations of CRF have been found in CSF of both OCD and PTSD patients (Altemus *et al.*, 1992; Fossey *et al.*, 1996; Bremner *et al.*, 1997) but not in PD patients (Fossey *et al.*, 1996; Jolkkonen *et al.*, 1993).

These studies provide evidence for increased CRF secretion in the brains of some patients with psychiatric disorders and, since neuropeptides appear to be secreted directly into CSF from brain tissue, CRF found in CSF is unlikely to be derived from systemic circulation (Post *et al.*, 1982). It is therefore suggested that these elevated concentrations of CRF reflect extrahypothalamic CRF systems (Kalin, 1990).

CRF administered intracerebroventricularly (ICV) in rats produce a dose-dependant behavioural activation when rats are tested in a familiar environment. When CRF injected rats are exposed to a novel environment, they show behaviour reminiscent of that observed during stress (Koob & Heinrichs, 1999).

The amygdala is part of the limbic system in the brain and is important in the regulation of stress responses through its excitatory influence on the hypothalamus as indicated by a rise in plasma ACTH and corticosterone levels after electrical stimulation of the central nucleus (Feldman & Weidenfeld, 1998). The amygdala also influences motor responses via connections to the nucleus accumbens (Crossman & Neary, 1995). The amygdala contains high levels of CRF receptors (De Souza *et al.*, 1985), cell

bodies (Cummings *et al.*, 1983) and nerve terminals (Uryu *et al.*, 1992). Chalmers *et al.* (1995) showed that the greatest density of CRF₁ mRNA in the amygdala is found in the basolateral nucleus (BLA). Acute bilateral CRF or urocortin (a peptide similar to CRF) injections in the BLA reduced social interaction time in rats (Sajdyk *et al.*, 1999) and chronic administration of urocortin also increased anxiety (Sajdyk *et al.*, 2003). Disruption of inhibitory neurotransmission in the BLA, by injection of a GABA antagonist, induced behavioural and physiological responses in rats similar to that seen in patients with anxiety disorders (Sanders & Shekar, 1995).

The aim of this study was therefore to determine the effect of chronic injections of CRF in the BLA on the behaviour and HPA-axis response of rats. In addition to this, serotonin (5-HT)_{1A} receptor activity in the hippocampus was determined as 5-HT_{1A} agonists have been efficient in clinical studies to reduce both anxiety and depression and it has been shown to enhance these receptors in the hippocampus (Blier & De Montigny, 1987). Kagamiishi *et al.* (2003) also showed that CRF injected ICV increased levels of serotonin in the hippocampus and this elevation was attenuated by injection of a 5-HT_{1A} agonist prior to CRF injection. The present study therefore hypothesized that chronic elevation of CRF levels in the BLA would lead to behavioural, HPA-axis and serotonin abnormalities normally associated with anxiety disorders or depression.

3.2 Materials and Methods

3.2.1 Animals

Male Sprague-Dawley and Wistar rats were bred and housed in the animal facility of the University of Stellenbosch Medical School. The rats were kept under standard laboratory conditions with free access to food and water.

3.2.2 Unilateral injections

Stereotaxis and behaviour

Male Sprague-Dawley rats weighing 250-300g were used for unilateral stereotaxical implantations in the basolateral amygdala. Rats were acclimated to the theatre 1 hour before the procedure. Rats were implanted in the right basolateral amygdala at coordinates: Anterior-Posterior -3.3 from Bregma, Medial-Lateral -4.6 and Dorsal-Ventral -8.7 according to the atlas of Paxinos & Watson (1986). Histology was done to verify the accuracy of the implantations. Rats were singly housed after the operation for handling purposes, and left to recover for 3-4 days before the injection period. Rats were randomly divided into 3 groups and received saline (n=6), 10ng CRF (n=7) or 100ng CRF (n=6) injections daily for 5 days, between 8:00 and 11:00 in the morning. After injection, a period of 2 min was allowed for infusion of the fluid before the needle was removed from the cannula. On day 5, video recordings were made of the behaviour of the rats in the elevated plus-maze and open field.

3.2.3 Bilateral injections

Stereotaxis and behaviour

Male Wistar rats weighing 300-350g were stereotaxically implanted with chronic bilateral cannulae in the basolateral amygdala. The coordinates used were: Anterior-Posterior -3.3 from Bregma, Medial-Lateral ± 4.6 and Dorsal-Ventral -8.7 from the surface of the skull according to the atlas of Paxinos & Watson (1986). Histology was done to verify the accuracy of the implantations. The rats were singly housed and allowed to recover for 4 days before injections. Rats were randomly divided into 2 groups, receiving either $1\mu\text{l}$ saline injections ($n=17$) or $100\text{ng}/\mu\text{l}$ CRF injections ($n=19$) daily for 5 days between 8h00 and 11h00. This dosage of CRF was chosen as it yielded significant results in the unilaterally injected rats. On day 5, 1 hour after the last injection, the behaviour of the rats was recorded for 5 min on the elevated plus-maze and open field. The behaviour of the first group of animals was tested at baseline levels, $n=6$ for saline injected rats and $n=7$ for CRF injected rats. A second group of animals were tested after restraint stress for 5 min (fear potentiated behaviour; Korte & De Boer, 2003), $n=11$ for saline injected rats and $n=12$ for CRF injected rats. Three evaluators rated video recordings afterwards, each noting a different set of parameters.

3.2.4 Stress response

On day 8, between 8:00 and 12:00 in the morning, rats were subjected to 10 min restraint stress, and decapitated either 15 min post stress (n=6 for control; n=7 for CRF) or 60 min post stress (n=5 for control; n=6 for CRF). A third group was decapitated at baseline levels (not subjected to restraint stress; n=6 for each group). Trunk blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes for corticosterone and ACTH assays using a glass funnel. Blood was kept on ice and centrifuged at 4°C at 5000 rpm and 1ml plasma was collected and stored in liquid nitrogen until the assays were done. A group of naïve rats was also decapitated at baseline (n=6), 15 min post stress (n=6) and 60 min post stress (n=6), to compare their levels of ACTH and corticosterone to that of the injected rats in order to see if the operational procedures had any effect on HPA-activity. ACTH determinations were done using a radioisotopic assay purchased from Nichols Institute Diagnostics, while corticosterone levels were determined using an ADVIA centaur system.

3.2.5 Receptor binding

After decapitation, brains of bilateral injected rats were removed, dissected on ice and hippocampi were stored in liquid nitrogen until radioligand binding assays were performed on 5-HT_{1A} receptors. Hippocampi were pooled (2 animals per group) to increase the sensitivity of the assays (n=6 for control and CRF).

3.2.6 Statistical analyses

Statistical analyses for behaviour were done using Mann-Whitney U tests or Kruskal-Wallis tests. Data for the stress response was analysed using Kruskal-Wallis tests. Receptor binding data was analysed using Student's t-test.

3.3 Results

3.3.1 Unilateral injections

Unilateral injections of CRF induced a significant increase in the number of entries into the open arms of the elevated plus-maze in the CRF 100ng group ($p < 0.05$), Fig. 3.1. There was also a significant increase in the time spent in the open arms of the elevated plus-maze in the CRF 100ng group ($p < 0.05$), Fig. 3.2. There were no significant differences in the amounts of rearing, freezing or grooming between any of the groups ($p > 0.05$), Fig. 3.3.

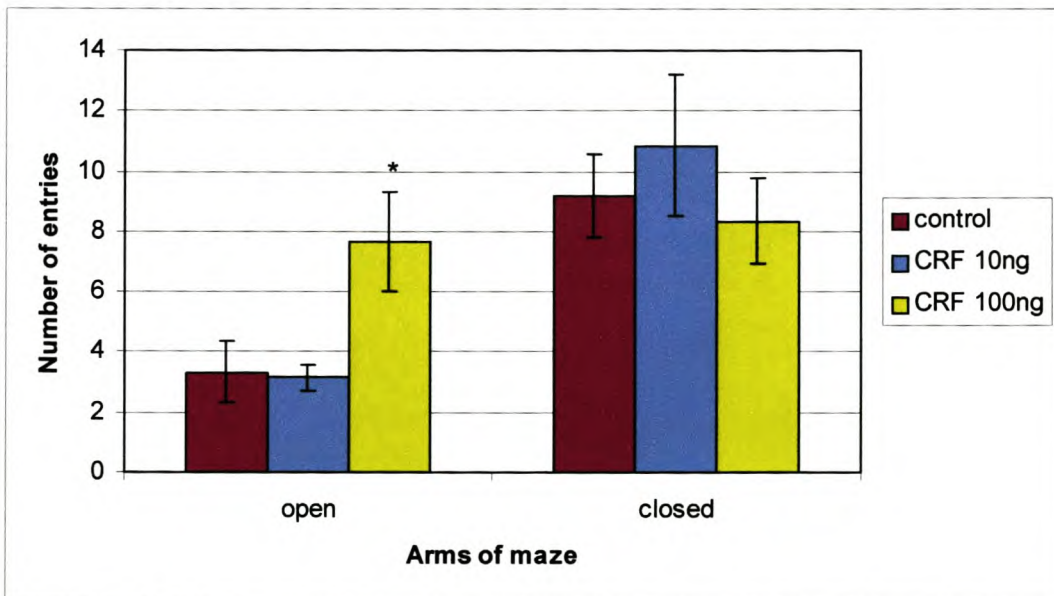


Fig. 3.1. There was a significant increase in the number of entries into the open arms of the elevated plus-maze in the CRF 100ng group (* = $p < 0.05$). There were no significant differences in the number of entries into the closed arms of the elevated plus-maze between any of the groups. Values are shown as means \pm SEM.

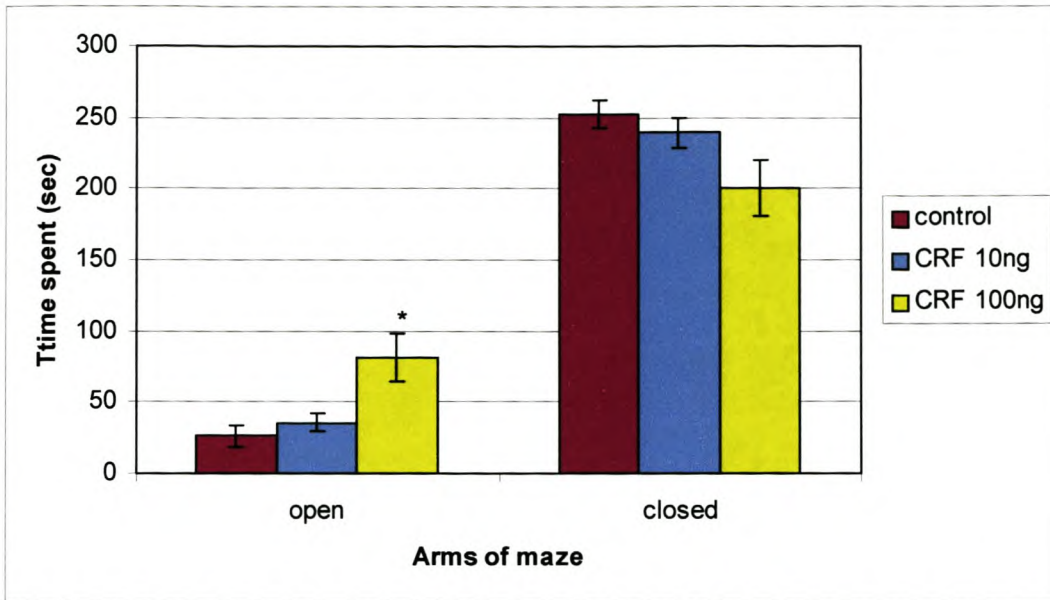


Fig. 3.2. There was a significant increase in the amount of time spent in the open arms of the elevated plus-maze (* = $p < 0.05$) in the CRF 100ng group. There was no significant differences in the amount of time spent in the closed arms of the elevated plus-maze. Values are shown as means \pm SEM.

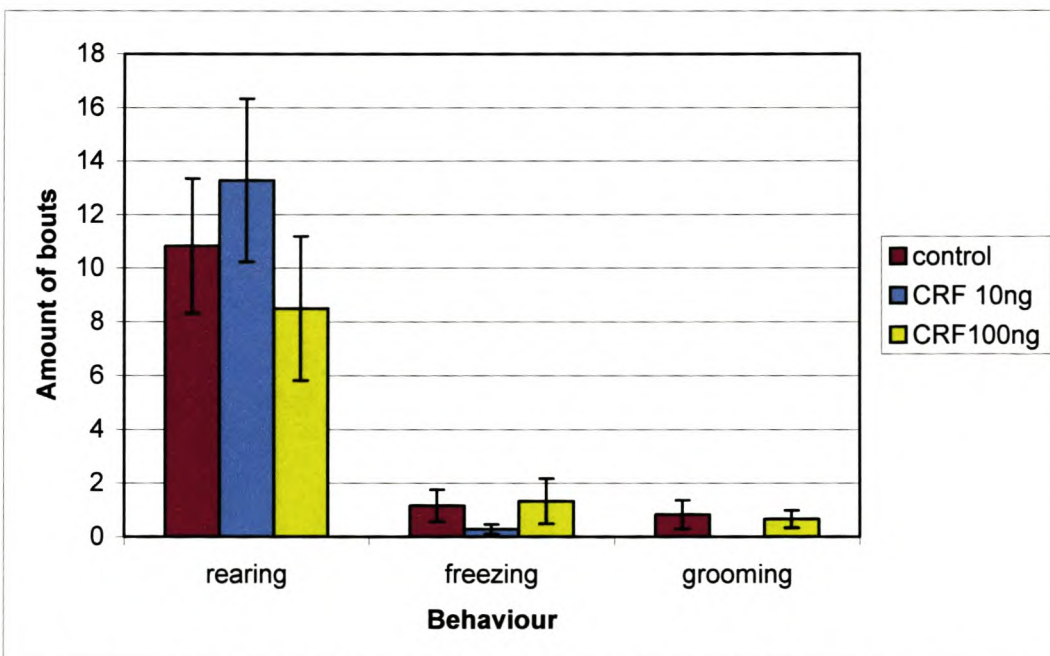


Fig. 3.3. There were no significant differences in rearing, freezing and grooming behaviour in the elevated plus-maze. Values are shown as means \pm SEM.

There were no significant differences in time spent in the inner or outer zone of the open field between any of the groups ($p > 0.05$), Fig. 3.4. There were also no significant differences in the number of blocks crossed in the open field or the amount of crossings between the outer and inner zones of the open field between any of the groups ($p > 0.05$), Fig. 3.5. There was no significant difference in the amount of rearing and freezing behaviour in the open field ($p > 0.05$), Fig. 3.6.

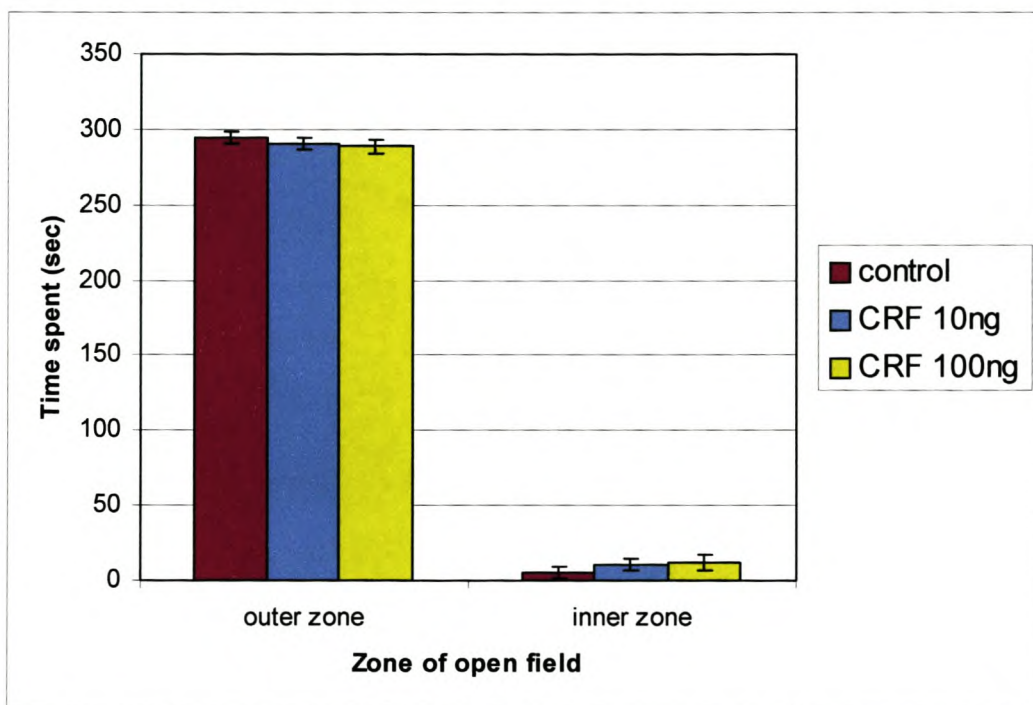


Fig. 3.4. No significant differences were found between groups in time spent in the outer or inner zone of the open field. Values are shown as means \pm SEM.

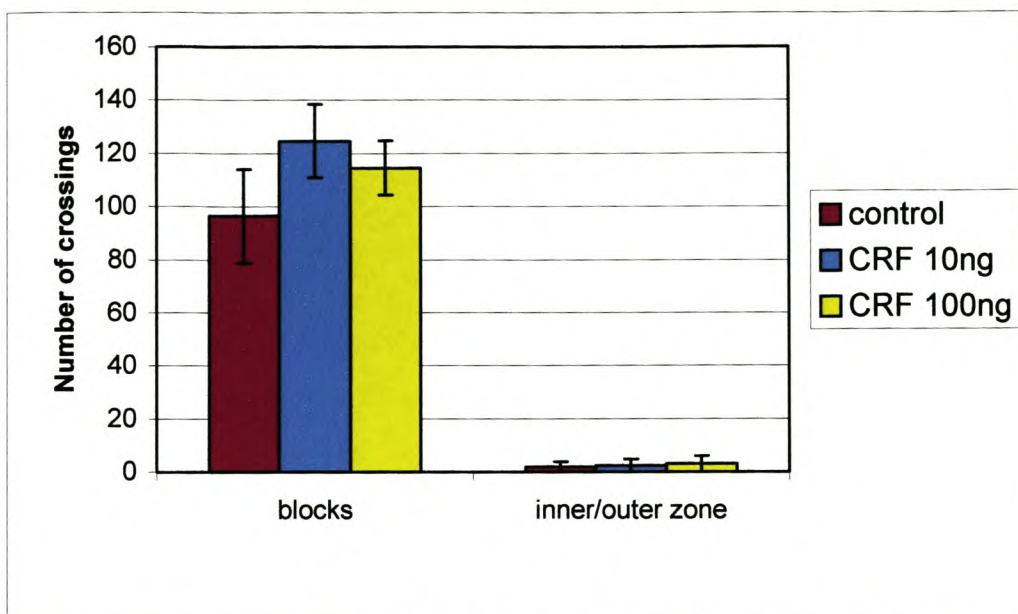


Fig. 3.5. There were no significant differences in the amount of blocks crossed or the number of crossings between zones in the open field. Values are shown as means \pm SEM.

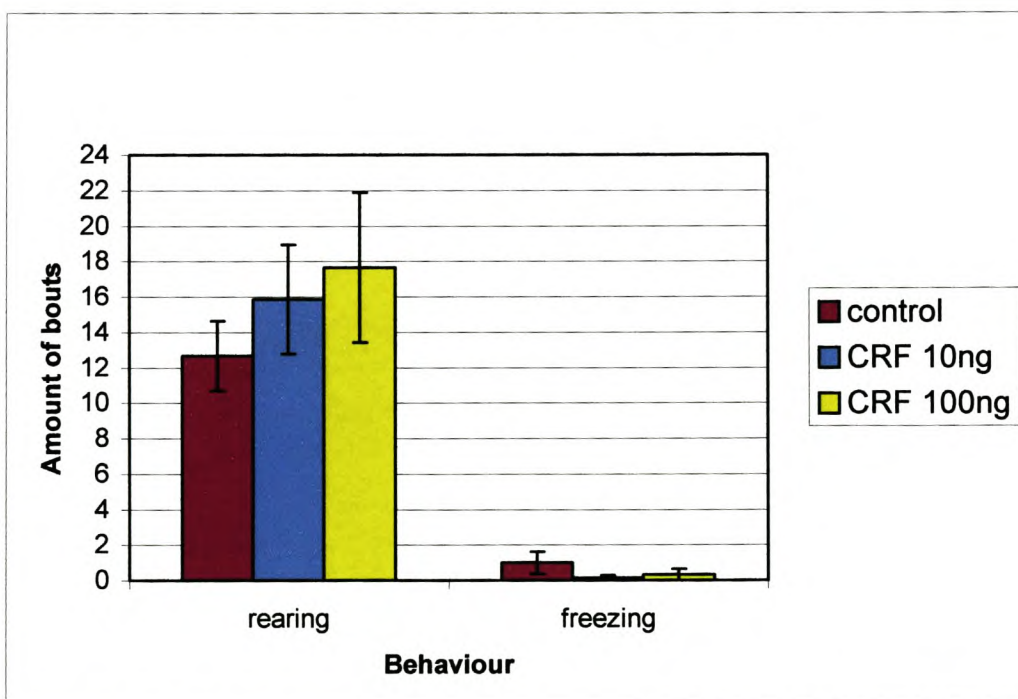


Fig. 3.6. There were no significant differences in the amount of rearing or freezing behaviour measured in the open field. Values are shown as means \pm SEM.

3.3.2 Bilateral injections

There were no significant differences in elevated plus-maze (Fig. 3.7-3.9) and open field behaviour (Fig. 3.10-3.12) of rats that were not stressed before measuring their behaviour ($p > 0.05$).

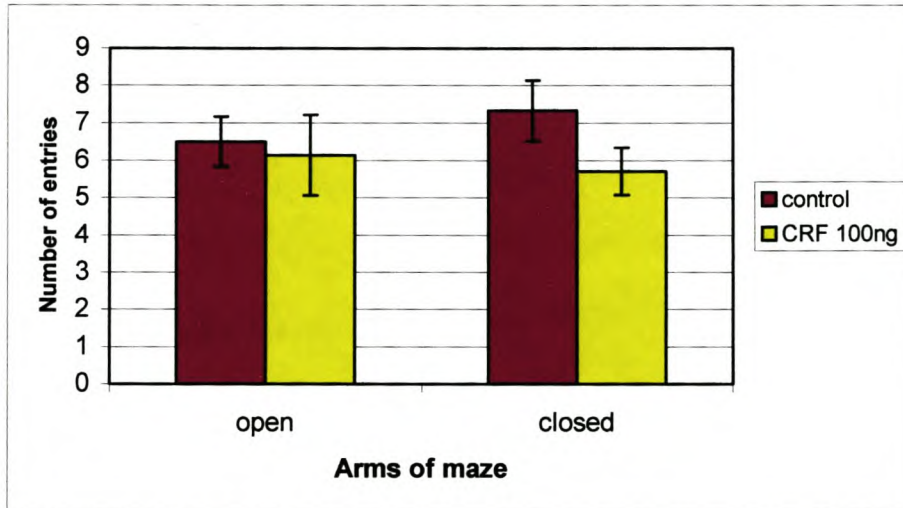


Fig. 3.7. No significant differences were found between groups for number of entries into the open or closed arms of the elevated plus-maze. Values are shown as means \pm SEM.

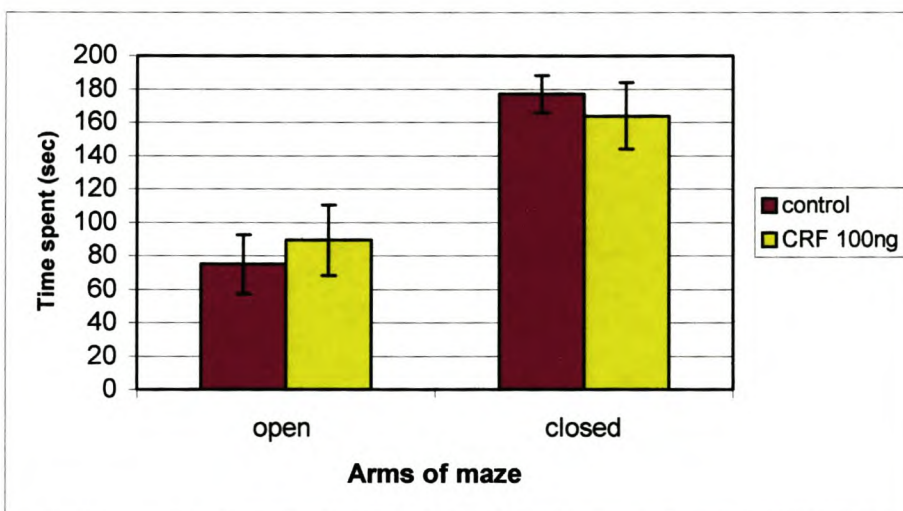


Fig. 3.8. No significant differences were found between groups for the amount of time spent in the open and closed arms of the elevated plus-maze. Values are shown as means \pm SEM.

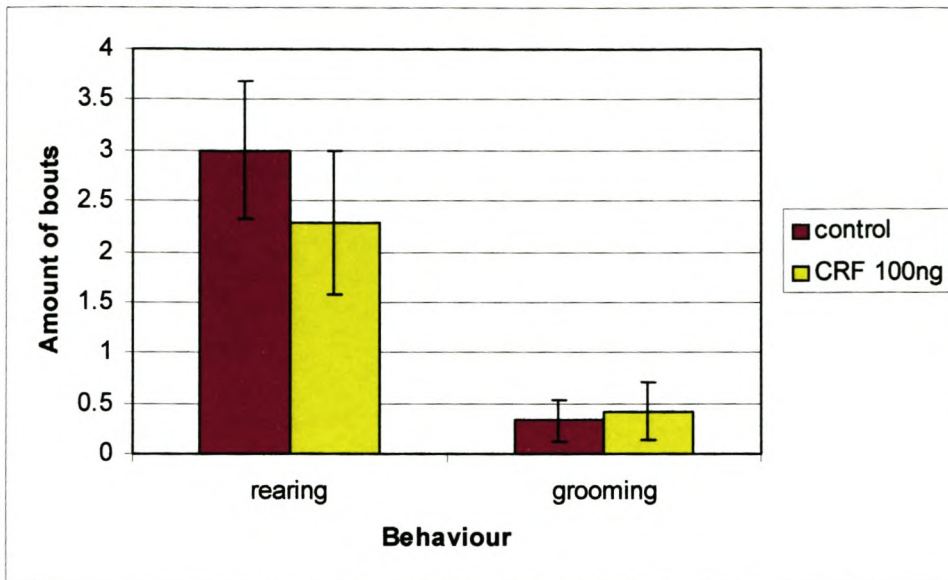


Fig. 3.9. No significant differences were found between groups in rearing or grooming behaviour in the elevated plus-maze. Values are shown as means \pm SEM.

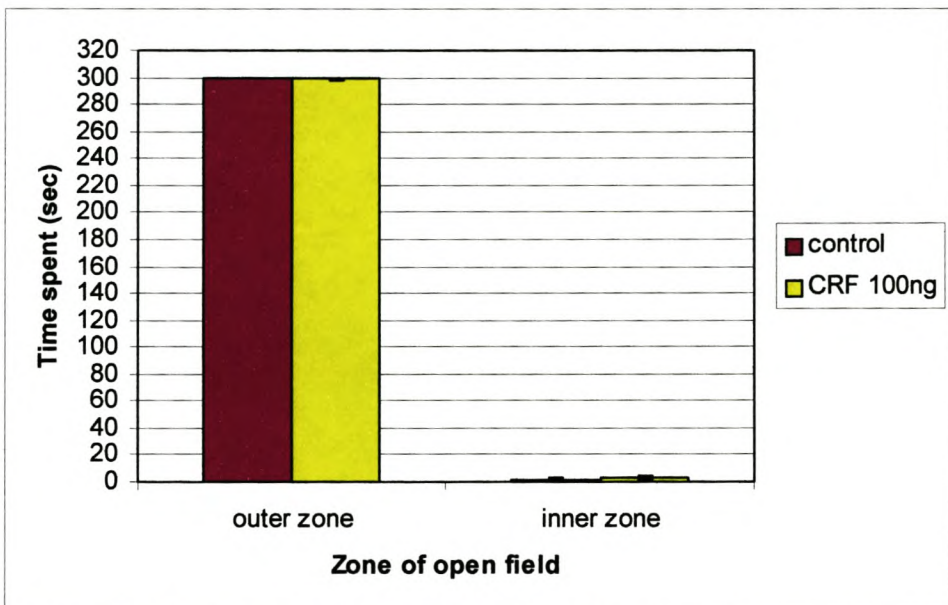


Fig. 3.10. No significant differences were found between groups in time spent in the outer and inner zones of the open field. Values are shown as means \pm SEM.

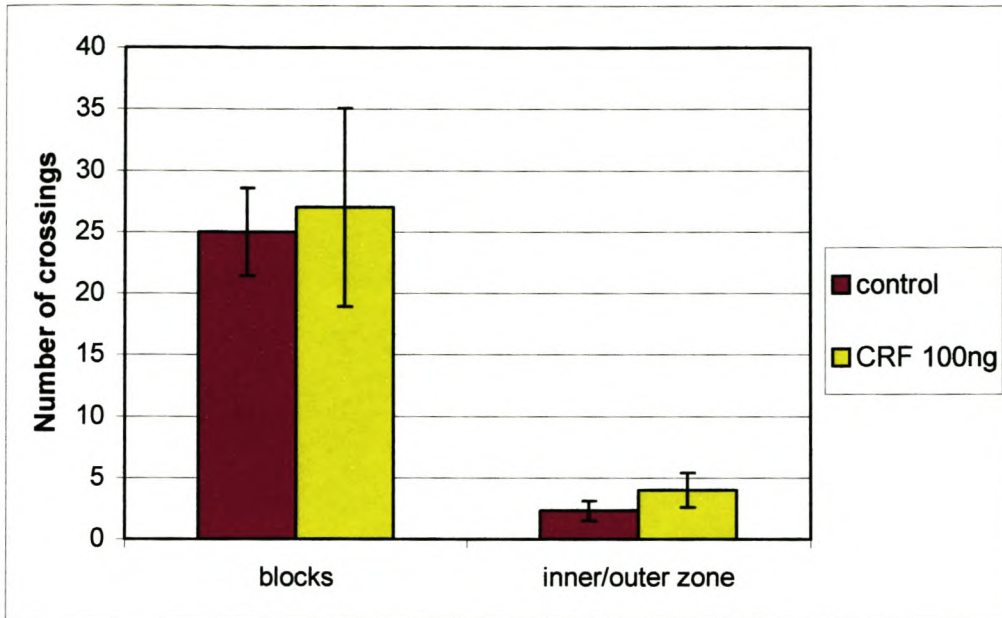


Fig. 3.11. No significant differences were found between groups for the amount of blocks crossed and crossings between the inner and outer zones in the open field. Values are shown as means \pm SEM.

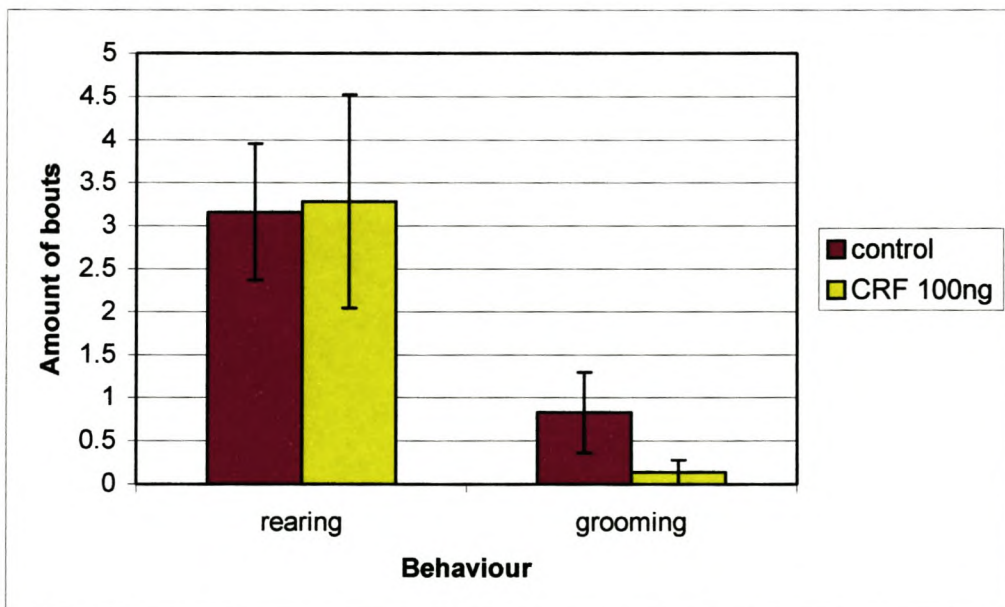


Fig. 3.12. No significant differences were found between groups for rearing and grooming behaviour in the open field. Values are shown as means \pm SEM.

There were no significant differences between groups in elevated plus-maze behaviour of fear-potentiated rats ($p > 0.05$), Fig. 3.13 - 3.15. There was no significant difference in time spent in outer or inner zones of the open field (Fig. 3.16), number of blocks crossed or crossings between the outer and inner zone of the open field (Fig. 3.17), and rearing behaviour (Fig. 3.18). There was, however, a significant increase in the amount of times the CRF injected rats displayed grooming behaviour ($p < 0.05$), Fig. 3.18.

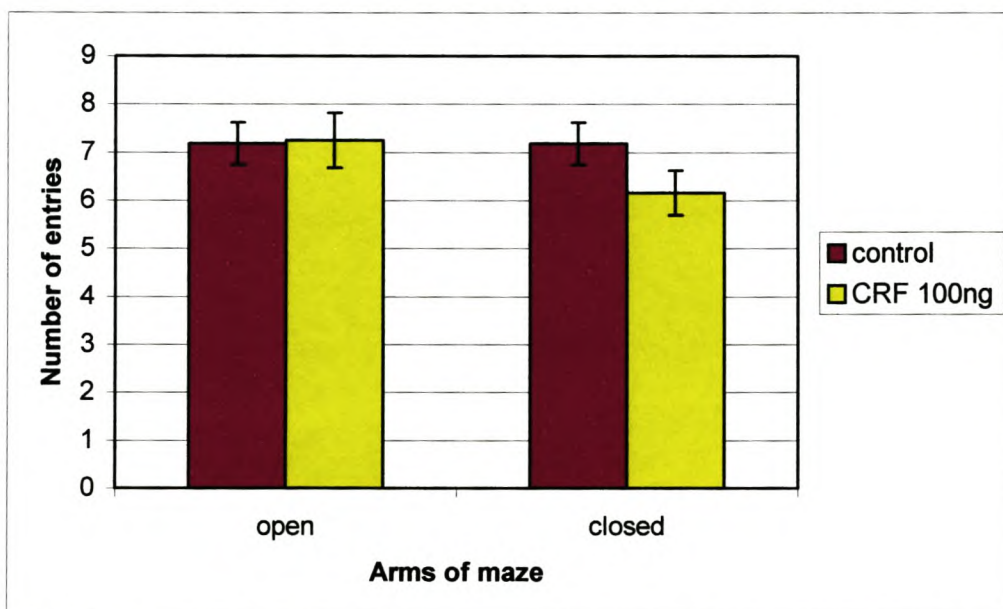


Fig. 3.13. No significant differences were found between groups for the number of entries into the open and closed arms of the elevated plus-maze. Values are shown as means \pm SEM.

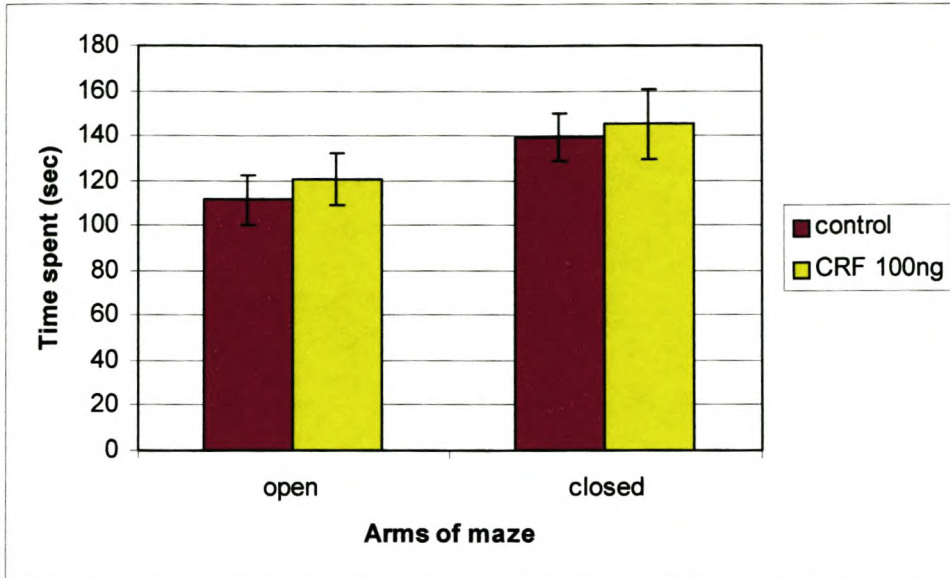


Fig. 3.14. No significant differences were found between groups for time spent in the open and closed arms of the elevated plus-maze. Values are shown as means \pm SEM.

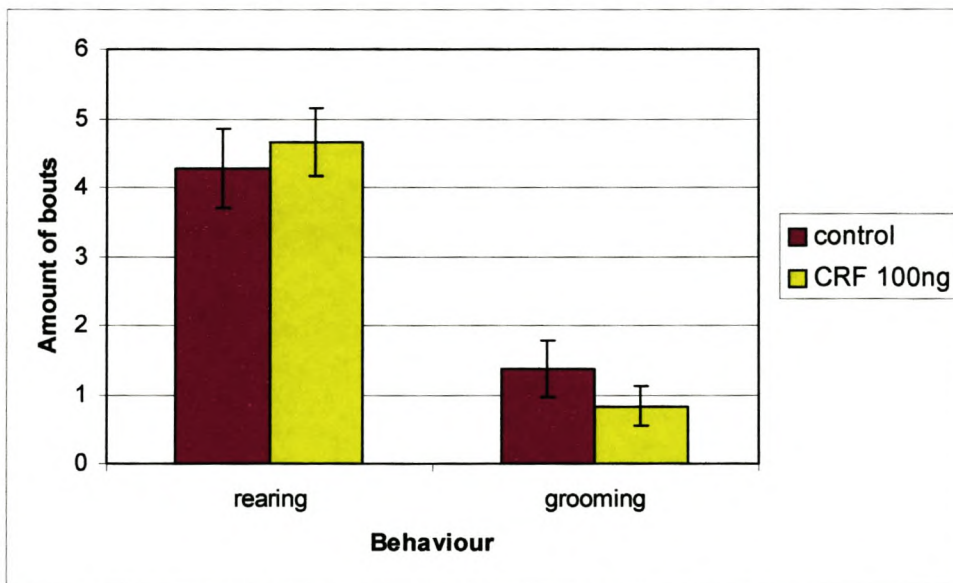


Fig. 3.15. No significant differences were found between groups for rearing and grooming behaviour in the elevated plus-maze. values are shown as means \pm SEM.

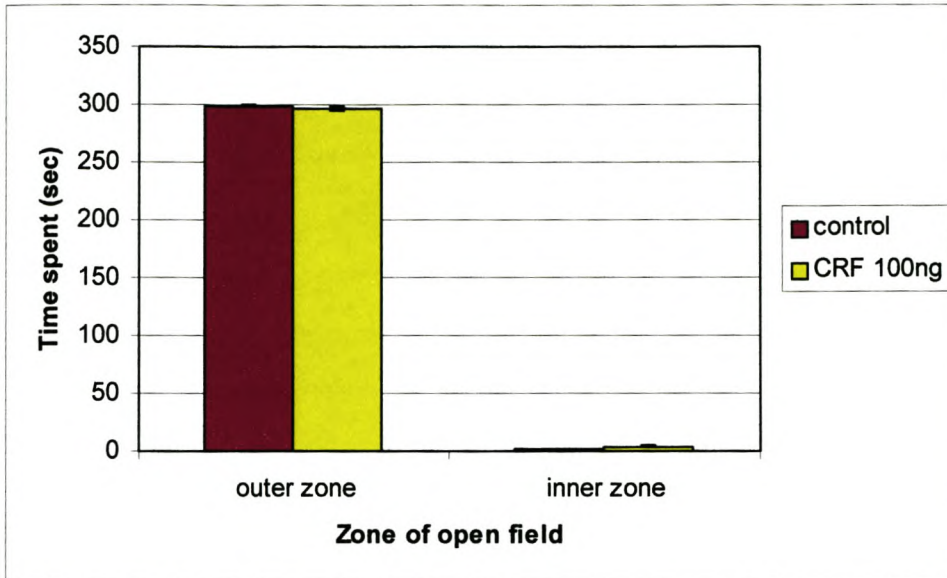


Fig. 3.16. No significant differences were found between groups for time spent in the outer and inner zones of the open field. Values are shown as means \pm SEM.

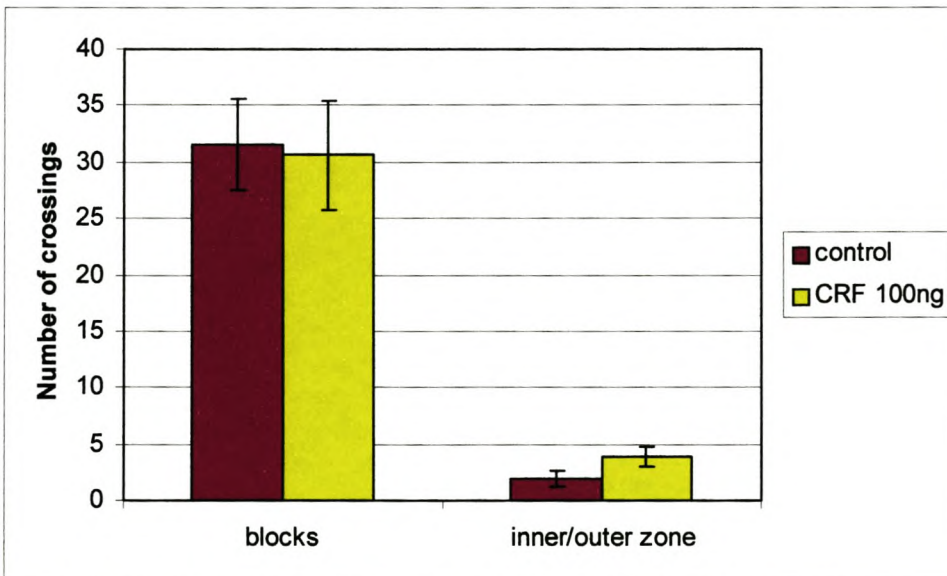


Fig. 3.17. No significant differences were found between groups for the number of crossings and crossings between the outer and inner zones of the open field. Values are shown as means \pm SEM.

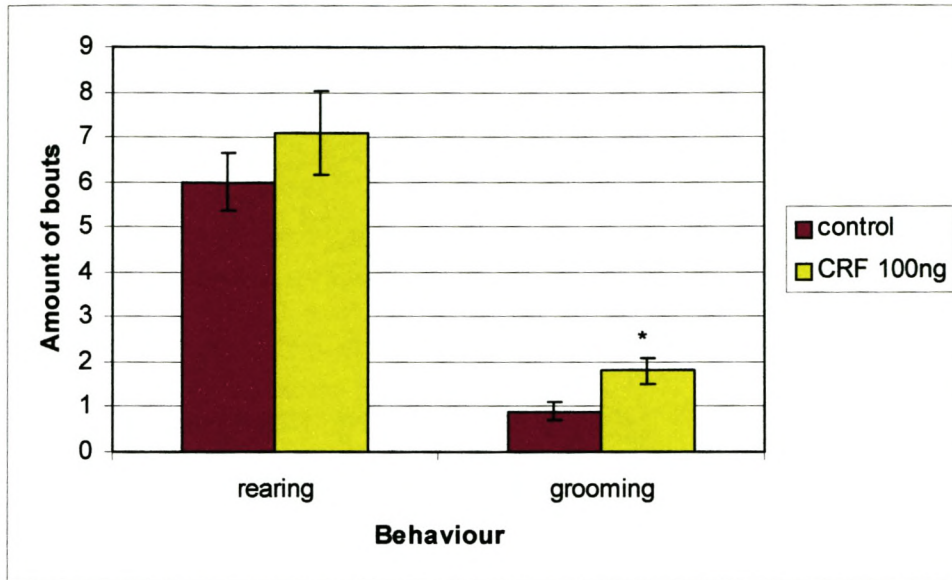


Fig. 3.18. A significant increase in the amount of grooming bouts in the open field was found in CRF injected rats compared to control rats (* = $p < 0.05$). No significant difference were found between groups for rearing behaviour. Values are shown as means \pm SEM.

3.3.3 Stress response

The ACTH and corticosterone response to stress of the CRF group was normal compared to naïve and control rats. As evident from the graphs, both ACTH and corticosterone values increased to peak values 15 min after stress and returned to basal levels 60 min after stress. There were no significant differences between the groups in ACTH values at any of the time points ($p>0.05$) Fig. 3.19. The basal corticosterone level of the CRF injected rats was significantly lower compared to the control group ($p<0.05$), but there was no significant differences 15 and 60 min post stress (Fig. 3.20).

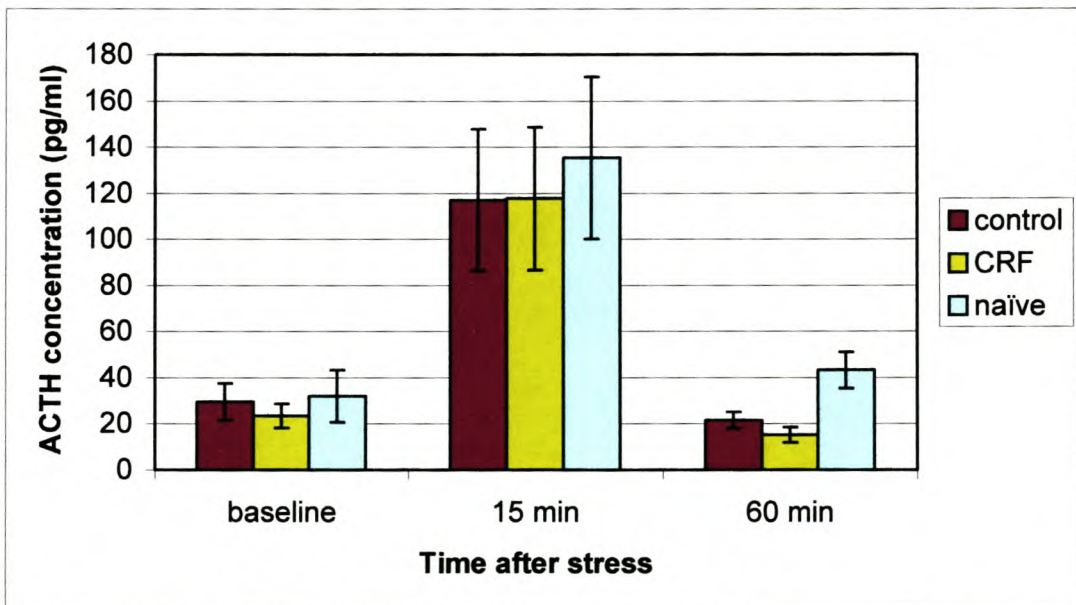


Fig. 3.19. Plasma ACTH concentrations at baseline, 15 min and 60 min after restraint stress. Values are shown as means \pm SEM. There were no significant differences between the groups.

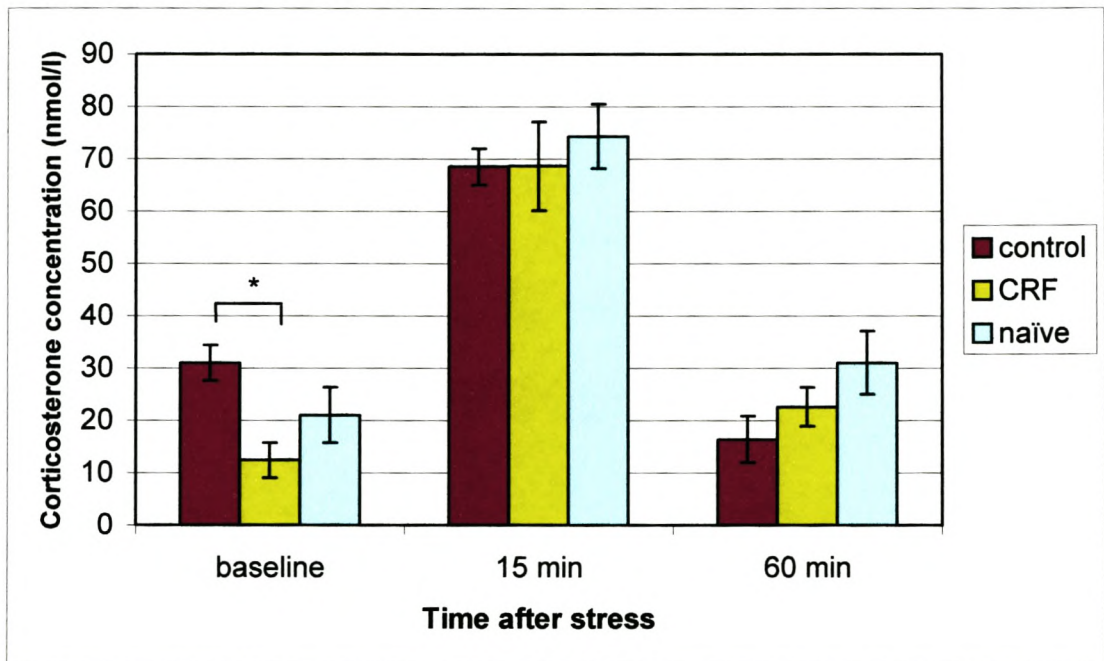


Fig. 3.20. There was a significant decrease in basal corticosterone levels of CRF injected rats compared to controls (* = $p < 0.05$). There were no significant differences between the groups 15 or 60 min post stress. Values are shown as means \pm SEM.

3.3.4 Receptor binding

There were no significant differences in the B_{max} (Fig. 3.21) or K_d (Fig. 3.22) values of 5-HT_{1A} receptor populations in the hippocampus of CRF-injected and control rats ($p > 0.05$).

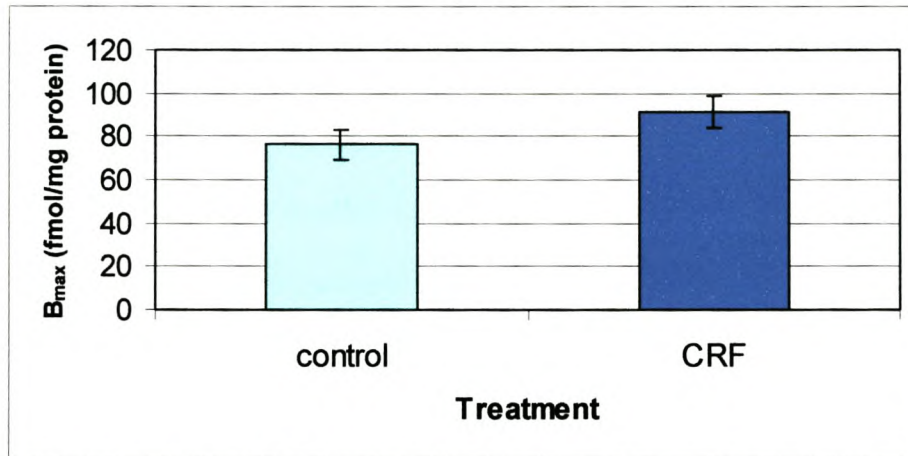


Fig. 3.21. B_{max} values of 5-HT_{1A} receptors in the hippocampus of control and CRF injected rats. Values are shown as means \pm SEM. No significant differences were found.

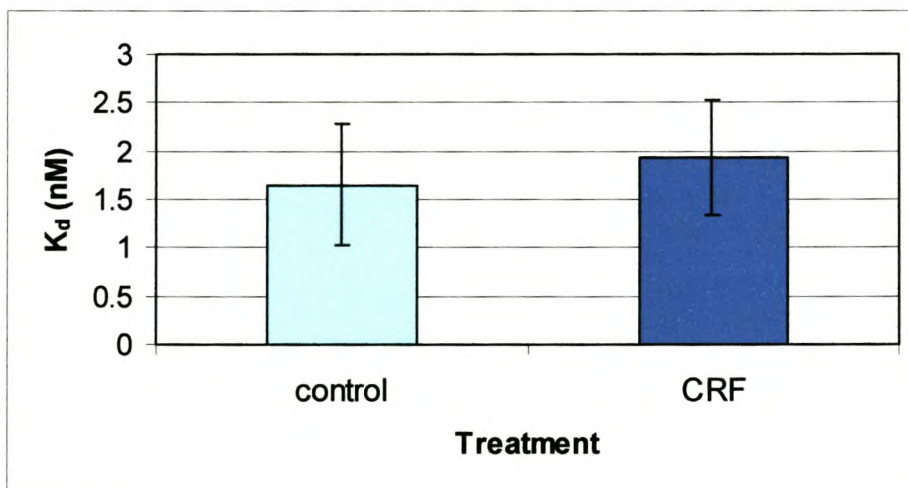


Fig. 3.22. K_d values of 5-HT_{1A} receptors in the hippocampus of control and CRF-injected rats. Values are shown as means \pm SEM. No significant differences were found.

3.4 Discussion

The elevated plus-maze is commonly used in rat behavioural studies to measure anxiety as well as locomotor activity (Rodgers & Dalvi, 1997; Pellow *et al.*, 1985). The more important parameters for anxiety is time spent in the open and closed arms of the maze. In addition, rearing, freezing and grooming are also used to differentiate between levels of anxiety (Escorihuela *et al.*, 1999). Locomotor activity is assessed by counting the amount of entries made into the open and closed arms of the maze. The open field test is used mainly for measuring locomotor activity, but may also indicate anxious behaviour (Prut & Belzung, 2002). Number of blocks crossed in a 5 min period is used as a measure of activity, while rearing and grooming as well as time spent in the inner vs. outer zone can be indicators of anxiety.

3.4.1 Unilateral injections

When we injected rats unilaterally with 100ng of CRF for 5 days, we found that they were less anxious than controls as indicated by an increase in the amount of time spent in the open arms of the elevated plus maze. An increase in the amount of entries also indicates a higher activity level than controls. Song *et al.* (1995) also found chronic ICV injections of 0.1, 0.5 and 1 μg to dose dependently increase activity and rearing in the open field. Similarly, Linthorst *et al.* (1997) reported increased activity after chronic infusion of 1 μg /day for 7 days. It is evident from these studies that dosages

up to 1 µg/day induced decreased levels of anxiety while it increased locomotor activity. Interestingly, similar studies with higher dosages of CRF injected ICV resulted in decreased locomotor activity and/or increased anxiety in the elevated plus-maze (Buwalda *et al.*, 1997; Song *et al.*, 1995). Acute ICV injections of CRF showed that 0.3 and 3 µg of CRF dose dependently increased grooming and locomotion in a novel environment and the effect of CRF was seen in both novel and familiar environments (Sherman & Kalin, 1987). Our initial results of increased exploration and locomotion in unilateral injected rats are therefore in agreement and confirm the findings obtained with low CRF concentrations. These results suggested that mild elevations of CRF in the brain led to a state of arousal and relaxation and that the BLA contributes to this emotional state.

3.4.2 Bilateral injections

Contrary to what we expected, bilateral injection of 100ng CRF did not enhance the effects obtained with unilateral injections. Most of the findings obtained with unilateral injections were lost, with only a significant increase in grooming recorded with the open field test. An increase in number of grooming bouts, as observed in CRF injected rats that were stressed before behavioural testing, is usually interpreted as decreased anxiety levels. In contrast to our findings, Sajdyk *et al.* (2003) found increased social anxiety in rats in the social interaction test after chronic bilateral administration of urocortin to the BLA. Reduced social interaction time was also observed after acute CRF or urocortin injections in the BLA (Sajdyk *et al.*, 1999). These

results could indicate that the social interaction test is more sensitive than our behavioural tests. Liang & Lee (1988) observed decreased exploration and rearing in the open field after acute bilateral CRF injection at the dorsal margin of the amygdala. Shepard *et al.* (2000) implanted corticosterone bilaterally in the central nucleus of the amygdala (CeA) and also found reduced open arm exploration in the elevated plus-maze. Similarly, increased anxiety in the elevated plus maze was measured in Fisher-344 rats after chronic corticosterone implants (Geenwood-Van Meerveld 2001). These results indicate that corticosterone is also important in the amygdala in modulating animal behaviour.

After we observed no increase in anxiety in the elevated plus-maze in our first group of rats, we employed the fear-potentiated strategy in the second group of rats by restraining the animals for 5 min prior to behavioural testing. An unconditioned stressor prior to behavioural testing enhances the anxiety state of rats in the elevated plus-maze (Korte & De Boer, 2003). The restraint stress method has been shown to reduce exploration in the elevated plus-maze (Heinrichs *et al.*, 1994). Restraint stress one day before exposure to the elevated plus-maze reduced the percentage of time spent in the open arms and as well as the number of entries into the open arms (Martijena *et al.*, 1997). When we used 5 min restraint as stressor on the second group of rats, the elevated plus behaviour again showed no significant differences to controls.

3.4.3 Stress response

We observed a normal ACTH and corticosterone response in CRF injected rats compared to control and naïve rats. We expected to see a blunted ACTH/corticosterone response to stress in the CRF injected rats because of the stimulatory effect of the amygdala on the HPA-axis causing a downregulation of CRF receptors in the pituitary through negative feedback inhibition. This effect has previously been observed in CRF-injected rats after 10 min of restraint stress. The level of ACTH was lower 15 min after stress in rats chronically injected ICV (Pietersen, 2001). Our observations suggest that injection of CRF in the BLA did not significantly increase its stimulatory effect on the HPA-axis to the extent that pituitary receptors are downregulated. Interestingly it has been shown that corticosterone regulates CRF in the amygdala (Honkaniemi *et al.*, 1992), and chronic implants of corticosterone in the CeA in rats produces a prolongation of corticosterone release 45 and 90 min post stress (Shepard *et al.*, 2003). In this study, the stress response was tested after 7 days, suggesting that a blunted response may therefore only be observed after a longer period of elevated CRF in the amygdala. It is possible that the duration of the CRF injections in our study was too short to change receptor populations at the level of the pituitary.

We found that CRF-injected rats had a significantly lower basal corticosterone value compared to saline-injected rats. This is an interesting observation since patients with PTSD may also have lower basal cortisol levels compared to normal individuals (Boscarino, 1996; Stein *et al.*, 1997; Goenjian *et al.*,

1997; Marshall *et al.*, 2002). Increased, persistent negative feedback inhibition is most likely the cause of decreased release of cortisol from the adrenal gland. This takes place after activation of the HPA-axis and the subsequent release of cortisol acts on receptors in the hippocampus, amygdala, pituitary and hypothalamus (De Kloet & Reul, 1987). Mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) are found in equivalent numbers in the hippocampus (Dioro *et al.*, 1993) and the hippocampus has an inhibitory effect on the HPA-axis (Jacobson & Sapolsky, 1991; Dallman; 1993). While MR are involved in the regulation of basal HPA-activity, GR are occupied during stress and high levels of circulating corticosterone in rats (McEwen *et al.*, 1986; De Kloet *et al.*, 1998). The frontal cortex also exerts an inhibitory effect on the HPA-axis, but rats with corticosterone implants in this region showed only a blunted ACTH and corticosterone effect after stress, but no effect on basal HPA-axis activity (Dioro *et al.*, 1993). As expected, the frontal cortex has a 4 to 5 fold higher concentration of GR compared to MR (Meany & Aitken 1985) and is more likely to be involved in negative feedback inhibition during times of stress.

Patients with PTSD generally have an increased lymphocyte GR number, and it has been hypothesised that PTSD is associated with increased sensitivity of the HPA-axis to negative feedback inhibition (Yehuda *et al.*, 1991; 1993). Hypersecretion of CRF activates the HPA-axis and increased sensitivity of GR receptors in PTSD patients causes the HPA-axis to become progressively more sensitive to cortisol (Yehuda, 1998). Besides increased negative feedback sensitivity of the HPA-axis, other mechanisms have been proposed

for decreased basal cortisol in PTSD. The first mechanism could be reduced biosynthesis of cortisol because of adrenal insufficiency. Decrease in responsiveness of the adrenal glands could explain hypocortisolemia with no change in cortisol response to CRF in PTSD patients (Bremner *et al.*, 2003). Secondly, structural changes like atrophy or decreased volume of the adrenal glands could cause a reduction in cortisol levels (Heim *et al.*, 2000). In contrast to this, increased adrenal volumes have been found in patients with major depression that is associated with hypercortisolemia (Nemeroff *et al.*, 1993; Rubin *et al.*, 1995). Both these mechanisms are more likely to explain our observation of decreased basal corticosterone in CRF-injected rats than downregulation of pituitary receptors, since this would also cause a reduction in ACTH levels.

3.4.4 Receptor binding

Both CRF and 5-HT are mediators of the behavioural response to stress, and have been reported to be related to the symptoms of depression or anxiety-related disorders (Owens & Nemeroff, 1991; Owens & Nemeroff, 1994; Stout *et al.*, 2002). Activation of the serotonergic system is considered to have an anxiogenic effect while reduction of serotonergic function is considered to have an anxiolytic effect (Marsden, 1990). In both animal (Katoaka *et al.*, 1991; Rittenhouse *et al.*, 1992) and clinical (Feighner & Boyer, 1989; Pecknold *et al.*, 1989) studies, 5-HT_{1A} receptor agonists had an anxiolytic effect, showing an inhibitory action of these receptors on the 5-HT system. Kagamiishi *et al.* (2003) showed that CRF injected ICV increased levels of

serotonin in the hippocampus. This elevation was attenuated by systemic injection of a 5-HT_{1A} agonist prior to CRF injection. This finding shows that there is an interaction between CRF and the serotonergic system and that an increase in hippocampal 5-HT may lead to anxiety.

Further evidence for the involvement of 5-HT_{1A} receptors in anxiety is provided by 5-HT_{1A} knockout mice showing increased anxiety compared to normal mice (Olivier et al., 2001). We did not, however, find any differences in hippocampal 5-HT_{1A} receptors of CRF-injected and saline-injected rats. Similarly, Montgomery *et al.* (2001) also found no differences in 5-HT_{1A} binding in the hippocampus in clinical subjects chronically exposed to high levels of cortisol compared to normal controls. Since we did not find an increase in anxious behaviour in CRF-injected rats either, we can assume that the chronically injected CRF had no significant effect on 5-HT levels in the hippocampus and therefore we did not observe any changes in the 5-HT_{1A} receptor population.

3.4.5 Conclusion

Our findings of decreased anxiety and increased locomotor activity in unilateral CRF-injected rats are probably dependent on the dosage of CRF injected, since we did not observe these effects when animals were bilaterally injected. The same results have also been found by other authors, when they injected low dosages of CRF (chronically or acutely) in rats, while most other

studies using higher dosages reported anxious behaviour and decreased locomotor activity.

Chronic CRF injections in rats caused changes in basal HPA-axis activity. Since we did not observe a decrease in ACTH levels, we can argue that the decreased basal corticosterone level is independent of downregulation of receptors in the HPA-axis, and that increased CRF somehow caused adrenal insufficiency in the rats. This finding is comparable with clinical studies on PTSD patients showing decreased basal cortisol levels, therefore it is possible that chronically elevated levels of CRF in the BLA is involved in the pathophysiology of PTSD.

From previous literature it seems as if both the BLA and CeA are important in anxiety. Our results do not directly support the involvement of the BLA in anxious behaviour, but chronic injections of CRF in this region causes a decreased basal corticosterone level in rats. Further research is therefore necessary to unravel the exact nature of this finding and its relevance to psychiatric disorders.

Chapter 4:

Stereotypical Behaviour Study

4.1 Introduction

Recent studies have shown that obsessive-compulsive disorder (OCD) affects 1-2% of the adult human population, and it is therefore amongst the most common psychiatric disorders (Micallef & Blin, 2001). OCD is characterised by intrusive thoughts (obsessions) and repetitive behaviours (compulsions) associated with marked distress and impairment (Saxena *et al.*, 1998). Compulsions can be described as unreasonable or excessive attempts to avoid anxiety caused by obsessions (Wright & Hewlett, 1994).

Clinical neuroimaging studies on OCD patients have provided strong evidence for the involvement of frontal-subcortical structures in the neurobiology of OCD. In particular, cortical-striatal-thalamic-cortical (CSTC) circuitry appears to be hyperactive in this disorder (Insel, 1992). The direct and indirect pathways of the CSTC circuit balance each other in the control of complex motor sequences because of activation or suppression of the thalamo-cortical pathway (Alexander & Crutcher, 1990), but in patients with OCD, there may be an imbalance of the two pathways (Saxena *et al.*, 1998).

CSTC pathways are modulated by serotonergic (5-HT) neurons from the raphae nuclei and dopaminergic (DA) neurons from the substantia nigra. There are functional connections in the pathways of these two neurotransmitters, for example, stimulation of 5-HT₂ receptors in the substantia nigra, as well as on

presynaptic DA projections in the frontal cortex and striatum, results in decreased synthesis and release of DA (Wright & Hewlett, 1994; Kapur & Remington, 1996). DA activates the direct pathway by stimulation of D₁ receptors, while stimulation of D₂ receptors inhibits the indirect pathway (Gerfen *et al.*, 1990). It is therefore possible that abnormalities in serotonergic and dopaminergic neurotransmission mediate OCD symptomology. OCD could perhaps also be related to a hyperglutamatergic condition of the prefrontal cortex, since SPECT, PET and fMRI studies show increased elevated prefrontal cortical blood flow, activation and metabolism in OCD patients (review by Carlsson, 2000). This could possibly be explained by an overactive, overambitious cortex since OCD patients seem to 'think too much' (Osborn 1998) and positive feedback to the CSTC pathways (Saxena *et al.*, 1998).

Pharmacotherapy of OCD has focused mainly on two strategies. The first successful treatment of OCD was with clomipramine, a serotonergic tricyclic, suggesting involvement of the 5-HT system in OCD (Fernandez-Cordoba & Lopez-Ibor, 1967). Selective serotonin reuptake inhibitors (SSRIs) are effective in treating this disorder (Greist *et al.*, 1995), but only 40-60% of OCD patients respond to this treatment. More recent studies have concentrated on combining SSRIs with other agents (Jenike *et al.*, 1996). Dopamine antagonists, eg. risperidone and haloperidol, in combination with SSRIs have proved to be helpful in the treatment of refractory cases (McDougle *et al.*, 1994; McDougle *et al.*, 2000). Inositol, a key metabolic precursor in the phosphatidylinositol (PI) second

messenger cycle distal to 5-HT₂ receptors, has also been shown to be effective in OCD (Fux *et al.*, 1996). Inositol upregulates striatal D₂ and 5-HT₂ receptors through its effects on subcellular signaling, which is not dependent on changes in neurotransmitter levels (Einat *et al.*, 1999; Harvey *et al.*, 2002). This drug also has anxiolytic properties and rats injected chronically showed increased time spent in the open arms of the elevated plus-maze (Cohen *et al.*, 1997).

Two types of animal models have commonly been used to study OCD: drug induced and environmentally induced stereotypical behaviour. Stereotypies are reminiscent of the compulsions of OCD and can be induced by DA agonists, and this behaviour can be blocked by DA antagonists in primates (Ridley *et al.*, 1979), inhibition of DA synthesis (Stolk & Rech, 1970) or destruction of DA-containing neurons (Creese & Iversen, 1973). Similarly, rats injected with quinpirole (D₂/D₃ agonist) exhibit compulsive checking of specific places in an open field and clomipramine partially attenuates this behaviour (Szetchman *et al.*, 1998). Although the compulsive checking in rats is drug induced, the neurobiology of this behaviour can be related to OCD in terms of the involvement of both the dopamine and serotonin systems. Canine acral lick dermatitis (ALD) is another example of an animal model of OCD as this condition has been shown to respond to clomipramine, fluoxetine and citalopram (SSRIs) (Rapoport *et al.*, 1992; Stein *et al.*, 1992; Stein *et al.*, 1998). The neurobiology of ALD is similar to that of OCD since these animals respond more robustly to SSRI treatment than to noradrenergic agents and the symptoms can be related to grooming

disorder (Rapoport *et al.*, 1992). Similarly in equine weaving, there was improvement after treatment with paroxetine, a SSRI. Weaving is a spontaneous stereotypy and since it responds to SSRI treatment, this condition can be related to OCD (Nurnberg *et al.*, 1997). Haloperidol (D_2 antagonist) but not clozapine (D_4 antagonist) reduced spontaneous stereotypies in bank voles (Kennes *et al.*, 1988; Schoenecker & Heller, 2001), but citalopram only reduced stereotypies in female bank voles (Schoenecker & Heller, 2003). These studies show both face and construct validity as models of OCD in symptoms and involvement of dopamine and serotonin systems.

A deermice model of spontaneous stereotypy was previously characterised by Powell *et al.* (1999). Deermice that were reared in standard cages developed spontaneous stereotypies such as vertical jumping, backward somersaults and route tracing, while mice that were reared in an enriched environment developed these stereotypies at a lower rate and proportion; and it is thus possible to generate two populations of the same species: a non-stereotypical and a stereotypical group. Apomorphine induced stereotypies in a non-stereotypical population produced stereotypies that were topographically different from spontaneous stereotypies. Interestingly, there were no significant differences in the D_1 and D_2 receptor density of the striatum between these different populations, suggesting limited involvement of the DA system in spontaneous stereotypies (Powell *et al.*, 1999; Presti *et al.*, 2002). Deermice as an animal model for OCD, therefore has both face and construct validity, as the

somersaulting behaviour can be related to compulsions in OCD patients and this behaviour is only partially mediated by the dopamine system.

The aim of this study was therefore to further characterise the model in its suitability to study OCD. We addressed the question as to whether drugs that have been successfully used in the treatment in patients with OCD, can reduce spontaneous stereotypical behaviour in deermice. We also examined NMDA receptor function in the frontal cortex to gain some insight into whether these drugs may affect the CSTC circuit and specifically thalamic glutamatergic output. Mice were treated with risperidone, a $D_2/5\text{-HT}_2$ antagonist, citalopram, a SSRI and inositol, a metabolic precursor to the PI second messenger cycle.

4.2 Materials and methods

4.2.1 Animals

40 Adult deermice, *Peromyscus maniculatis bairdii*, (breeding pairs purchased from *Peromyscus* Genetic Stock Center, University of South Carolina) were raised and housed under standard laboratory conditions with a 12/12h light/dark cycle, standard caging (29x18x13cm) with two same sex mice per cage, food and water available ad libitum. The mice were randomly divided into four groups comprising 6 females and 4 males each.

4.2.2 Behaviour

Intra-peritoneal injections of various treatments were given daily, from week 1, between 13:00 and 14:00. The three treated groups received citalopram (1mg/kg, Stein *et al.*, 1998), risperidone (0,1mg/kg, Rosengarten & Quartermain, 2002) and inositol (1,2g/kg, Harvey *et al.*, 2001) respectively and the control group was injected with an equal volume of saline. All drugs were dissolved in saline and the injected volumes were 0,1ml except for inositol, which was 0,2ml. Risperidone was obtained from Janssen Pharmaceutica (South Africa), citalopram hydrobromide from Lundbeck (Denmark) and myo-inositol was purchased from Sigma. The behaviour of the mice was recorded for 9 weeks, 5 minutes at a time, either in the morning before 11am or in the afternoon after

4pm, for a total of 15 minutes per week. A video camera mounted on a tripod was used for the recordings so that a top view of the cages was obtained. The first week of recordings served as baseline (week 0) and treatment started in week 1. A 5sec interval scoring system was used and the absence or presence of two forms of stereotypical behaviour, backward somersaults (backflipping) and route tracing (patterned running), were noted. Raters that were blind to the medication status of the mice noted the behaviour. Summed ratings for each week of the trial were compared within the groups. Inter-rater reliability was $r=0,9$.

4.2.3 Receptor binding

After 8 weeks of treatment, the mice were decapitated and the brains removed and snap frozen in liquid nitrogen and stored at -80°C until radioligand binding assays were performed for NMDA receptor characterisation in the frontal cortices.

4.2.4 Statistical analyses

Analyses of the behavioural data were done using ANOVA with repeated measures. A Mann-Whitney U test was done on values for males in week 8 between all groups compared to control. Data for receptor binding were analysed using Kruskal-Wallis tests.

4.3 Results

4.3.1 Behaviour

Fig. 4.1 shows that somersaults increased during the 8 week period in saline treated mice. In contrast, this increase was absent in all the groups receiving drug treatment. This effect is more noticeable in males (Fig. 4.2.). The ANOVA showed no significant differences in the percentage of somersaults observed in any of the groups or between groups during the 8 week trial $p > 0.05$ (Fig. 4.1.) and there was also no significant difference between the control group and treatment groups when data for males and females are analyzed separately (Fig. 4.2. & 4.3.). A significant difference was found between all groups compared to the saline group when a Mann-Whitney U test was performed on data for males in week 8 only, with the saline group having a much higher value ($p < 0.0001$). No statistical analysis was done on the data for route tracing, since there were only 1 or 2 animals per group that showed this behaviour. The data shows that risperidone decreased route tracing behaviour during week 1 and the behaviour disappeared by week 8, while it increased or stayed more constant during some weeks in the other groups (Fig. 4.4.).

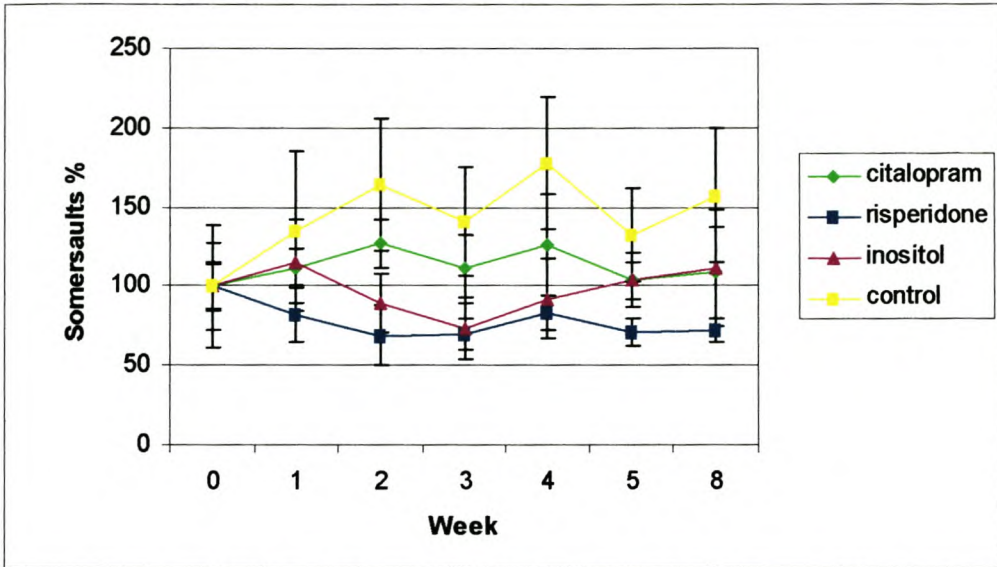


Fig. 4.1. Effect of treatment on number of somersaults during an 8 week trial. There were no significant differences between groups ($p > 0.05$). Values are shown as means \pm SEM.

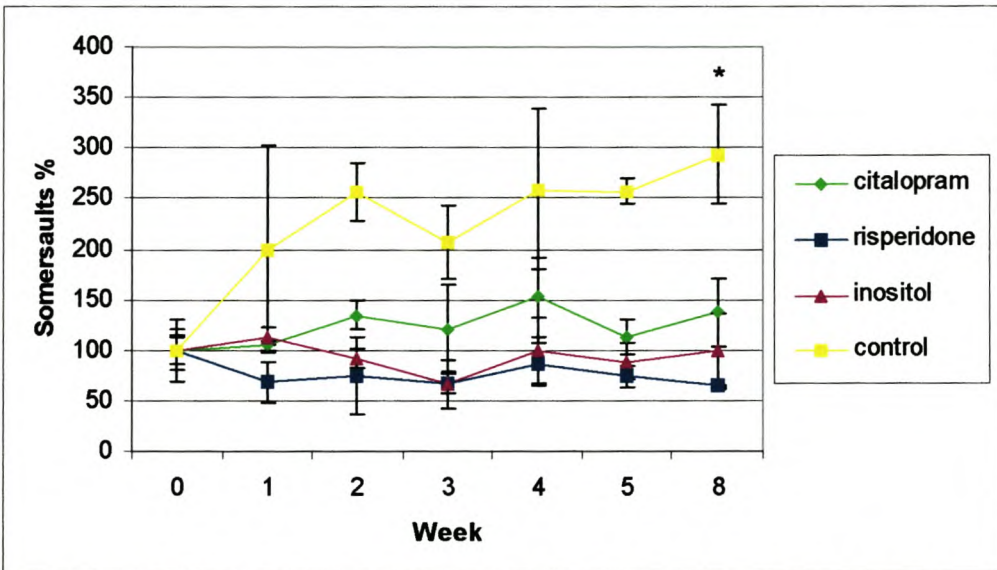


Fig. 4.2. Effect of treatment on number of somersaults during an 8 week trial for male deermice. There is a significant difference between control and treatment groups at week 8 ($p < 0.0001$). Values are shown as means \pm SEM.

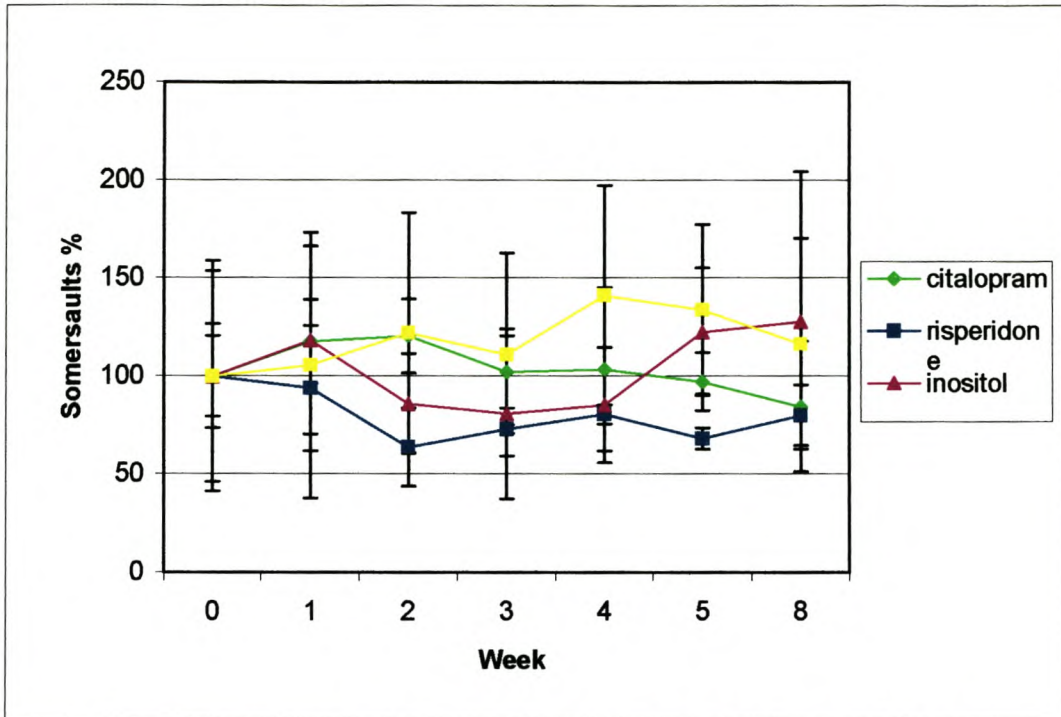


Fig.4.3. Effect of treatment on number of somersaults during an 8 week trial for female deermice. No significant differences were found between groups ($p < 0.05$). Values are shown as means \pm SEM.

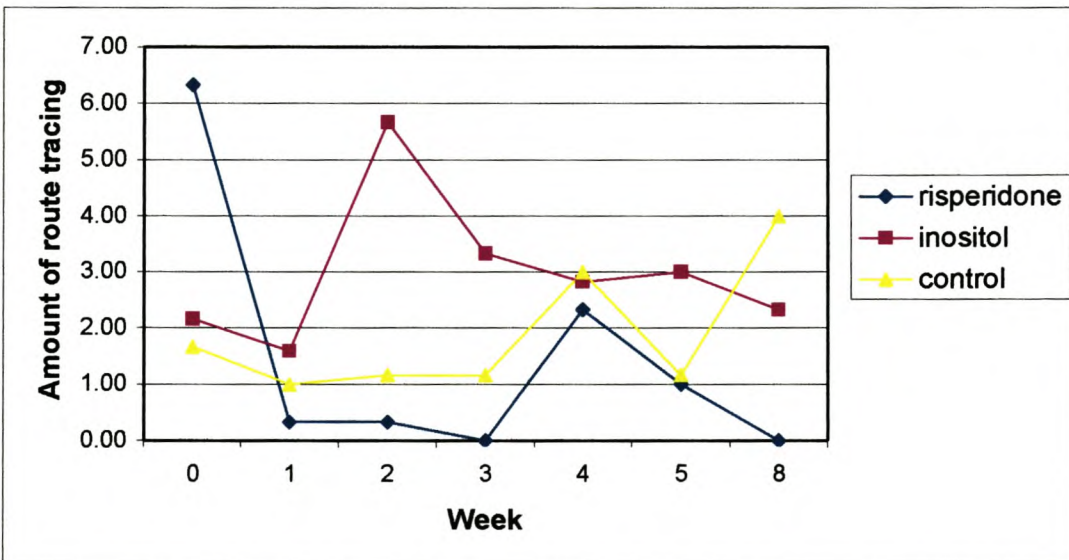


Fig. 4.4. Effect of treatment on route tracing behaviour during an 8 week trial. Values are shown as means.

4.3.2 Receptor binding

In all 3 treatment groups, there is a numerical increase in density of NMDA receptors in the frontal cortex, although statistical significance was not reached, $p=0.811$ (Fig. 4.5.). The affinity of NMDA receptors was increased by treatment with risperidone (Fig. 4.6.), but again statistical significance was not reached ($p=0.759$).

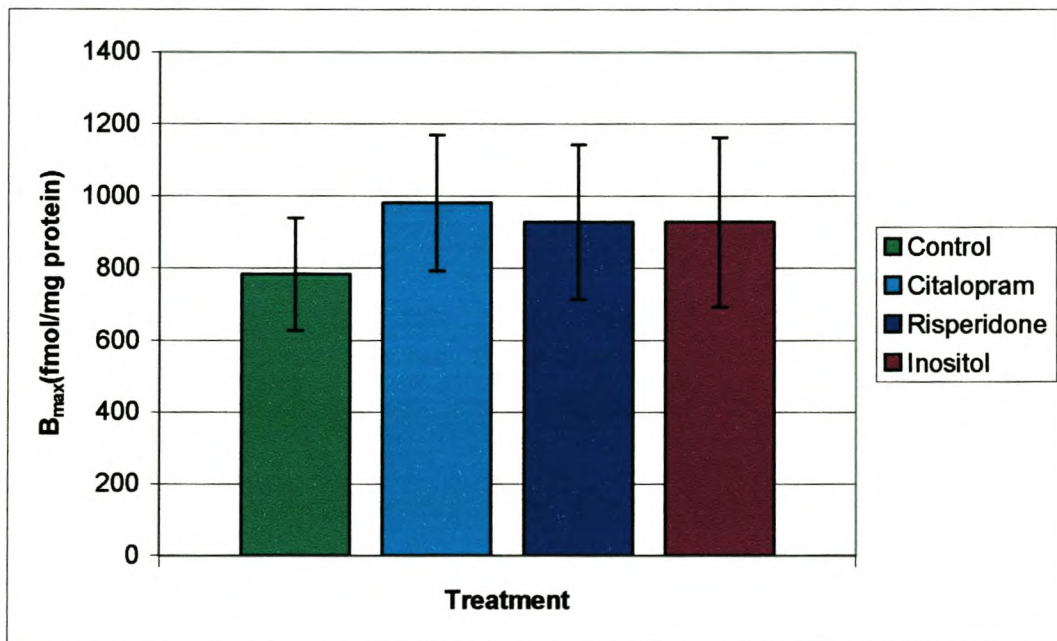


Fig. 4.5. Effect of treatment on NMDA receptor density in the frontal cortex compared to control. There were no significant differences between groups ($p>0.05$). Values are shown as means \pm SEM.

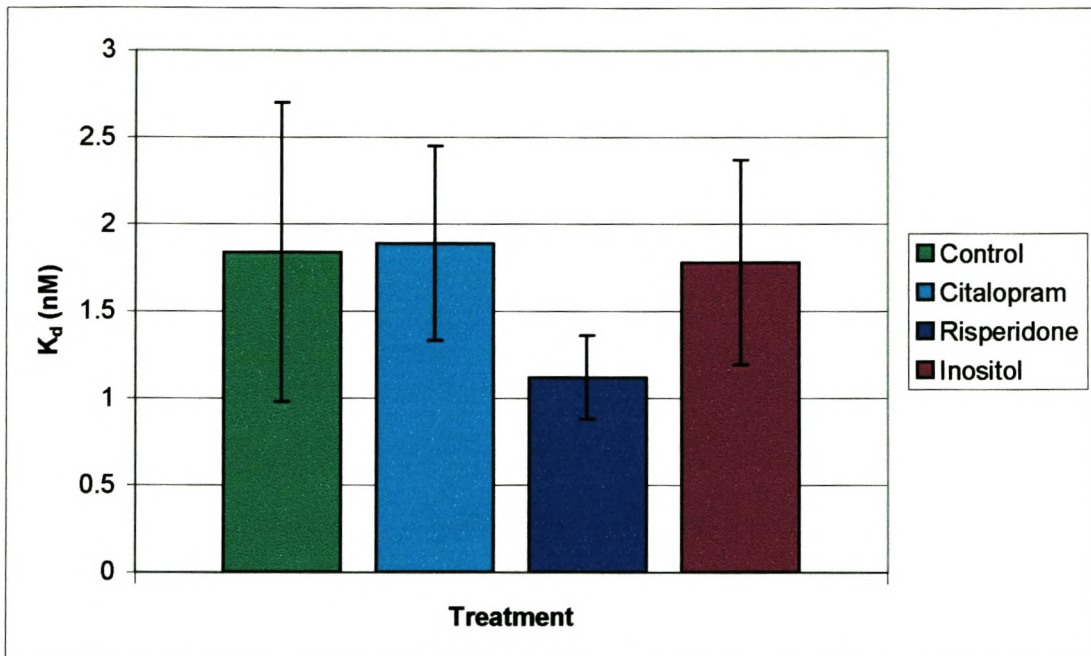


Fig.4.6. Effect of treatment on NMDA receptor affinity in the frontal cortex compared to control. There were no significant differences between groups ($p > 0.05$). Values are shown as means \pm SEM.

4.4 Discussion

4.4.1 Behaviour

The data for male mice clearly show how stereotypical behaviour in the control group increased over the 8 week period, while the behaviour in treated groups did not increase to the same extent. Stereotypes were not significantly reduced in any of the treatment groups when compared to their baseline values.

This is not surprising as it is known that in clinical populations, drug treatment is only partially effective in OCD patients (Piccinelli *et al.*, 1995) and only 40-60% of patients respond to SSRI monotherapy (Goodman, 1996). In contrast, animal studies by Schoenecker & Heller (2001) have shown that chronic treatment with citalopram in bank voles also did not reduce spontaneous stereotypical behaviour under undisturbed conditions, but only after acute stress. Interestingly, while the suppressive effect of drugs was not apparent in our female group of animals, citalopram treatment reduced stereotypical behaviour during peak activity levels in female bank voles, but not in males (Schoenecker & Heller, 2003). This discrepancy may be species related as dogs with ALD also respond to chronic treatment with clomipramine, fluoxetine and citalopram (SSRIs) (Rapoport *et al.*, 1992; Stein *et al.*, 1992; Stein *et al.*, 1998).

While SSRI's are commonly used to address stereotypic behaviour, risperidone appeared to be the most effective of the 3 drugs we used as the number of somersaults never rised above baseline levels. Dopamine antagonists have been shown to be effective reducers of stereotypies. Haloperidol (D₂ antagonist) at a concentration of 0,2mg/kg reduced spontaneous stereotypies in bank voles under undisturbed conditions (Kennes & De Rycke, 1988; Schoenecker & Heller, 2001).

Inositol administration has been demonstrated to be useful in treatment of OCD (Fux *et al.*, 1996). In our model, inositol stabilized the degree of stereotypic behaviour and prevented any further increases as seen in control animals. Chronic inositol treatment did not reduce stereotypical behaviour in guinea pigs (Harvey *et al.*, 2001).

4.4.2 Receptor binding

It is possible that all treatments reduced the hyperglutamatergic state in the frontal cortex that may be associated with a hyperactive CSTC circuit (review by Carlsson, 2000). This decrease would cause an increase in receptor numbers, and an increase, although not significant, in receptor affinity was evident in risperidone group. A previous study with deermice showed that there was no significant difference in the densities of striatal D₁ and D₂ receptors in stereotyping and non-stereotyping populations (Powell *et al.*, 1999), suggesting

that these receptors may not be essential in manifesting stereotypical behaviour. We chose to focus on the NMDA receptors in the frontal cortex as this could be used as an indication of thalamic output and the hyperglutamatergic state in the frontal cortex.

Inositol induced a significant increase in D₂ receptor density and a marginal increase in 5-HT₂ receptor density in the striata of guinea pigs (Harvey *et al.*, 2001). The mechanism by which inositol upregulates striatal D₂ and 5-HT₂ receptors is not dependent on changes in neurotransmitter levels (Einat *et al.*, 1999), but on its effects on subcellular signaling (Harvey *et al.*, 2002). The upregulation of 5-HT₂ receptors on presynaptic DA projections in the striatum would cause inhibition of DA release and subsequent upregulation of postsynaptic DA receptors (Harvey *et al.*, 2001). Inositol also reverses desensitization of 5-HT₂ receptors (Rahman & Neuman, 1993).

4.4.3 Conclusion

Since we had no significant reduction in stereotypies after drug treatment, it is difficult to make any conclusions on the involvement of the DA and 5-HT neurotransmitter systems, and their effect on glutamatergic output, in stereotypical behaviour of deermice. The suppression of increase in stereotypical behaviour seen in the control group could however be an indication that both dopamine and serotonin are involved.

Experimental stress (handling and injections) could explain why there was no significant reduction in the amount of somersaults in the treatment groups. Although somersaults were not reduced, it stayed constant in all treatment groups while it increased throughout the trial in saline injected animals. Measuring behaviour of female mice during peak activity levels could yield significant data, as in the case of bank voles (Schoenecker & Heller, 2003). It would also be necessary to distinguish between low and high stereotypers and divide the treatment group accordingly in order to observe the effects of the drugs. Furthermore, if activity levels are significantly higher during the dark phase, the effects of the drugs could be more evident when behaviour is observed during this phase.

The 8 week trial might have been insufficient to induce significant differences in receptor density and affinity, and further studies with higher dosages and investigation of striatal serotonin and dopamine receptor activity could result in interesting observations of the specific involvement of different neurotransmitter pathways in spontaneous stereotypical behaviour. Acute infusion of NMDA and D₁ receptor antagonists into the striatum of deermice attenuated stereotypical jumping (Presti *et al.*, 2003). It has also been shown that activating 5-HT_{2A} receptors in the frontal cortex are involved in releasing glutamate from thalamocortical afferents (Marek *et al.*, 2001). It is therefore important to do studies to target specific receptor populations such as D₁, D₂ and 5-HT₂ in the

striatum, as well as 5-HT₂ in the frontal cortex, by injecting different drugs into these areas.

Evidence in previous literature and some of our results indicate that deermice can be employed as an animal model for OCD. Although predictive validity of this model still needs to be investigated, this model has good face and construct validity.

Chapter 5:

General Conclusions

The aim of our study was to develop two different models of psychiatric disorders. In the first model we used adult rats and chronically injected them with corticotropin-releasing factor (CRF) in the basolateral amygdala (BLA). Patients with certain psychiatric disorders show hypothalamic-pituitary-adrenal (HPA) axis abnormalities like a blunted ACTH response to CRF injection and increased or decreased basal cortisol levels. Most patients with post traumatic stress disorder (PTSD), obsessive compulsive disorder (OCD) and Major Depression also have increased levels of CRF in their cerebrospinal fluid. Since the amygdala is involved in the behavioural and endocrine response to stress, we investigated whether elevation of CRF levels in this region could be a factor in the development of symptoms characteristic of these psychiatric disorders.

We injected rats with CRF for 5 days and subsequently monitored their behaviour and stress response as well as the hippocampal serotonin (5-HT)_{1A} receptor density and affinity. The CRF injected rats did not show an increase in stress related behaviour when tested on the elevated plus-maze or open field. Unilateral injections instead increased activity and induced a more relaxed state in CRF-injected rats compared to control rats. This result is in contrast to what other authors, also injecting CRF in the BLA, found. There are, however, a few studies that reported increased activity and a relaxed state after intracerebroventricular injection of CRF in rats, that correspond to our results. We are therefore not certain of the effect of different concentrations of CRF on

the behaviour of rats and future studies should include the administration of a wider concentration range of CRF for a longer time period.

There was no difference in the hippocampal 5-HT_{1A} receptor density or affinity of CRF-injected and control rats. This indicates that the elevation of CRF in the BLA did not alter 5-HT neurotransmission in the hippocampus. This result is supported by the fact that we did not see an increase in anxiety in the rats, since alteration in 5-HT transmission is associated with anxiety.

We found evidence that the chronic CRF injections could cause decreased basal corticosterone levels in the rats. It is also evident that the mechanism causing decreased corticosterone is independent of downregulation of pituitary glucocorticoid receptors, since we found no alteration in basal ACTH levels or ACTH/corticosterone levels after stress. Our results indicate that decreased basal cortisol levels, as observed in PTSD patients, may be caused by chronically elevated levels of CRF in the BLA, the exact mechanism, however, is not known. We can speculate that CRF somehow caused adrenal insufficiency in the rats, but this needs to be further investigated. If this result can be repeated, elevated CRF in the amygdala of rats can be used to model some of the symptoms of PTSD.

The second study used deermice showing spontaneous stereotypical behaviour as a potential model for OCD. From previous literature it is evident that deermice

can be useful as a model for OCD in terms of face validity, since the spontaneous stereotypical behaviour elicited by the mice is reminiscent of compulsions in OCD patients. It has also been shown that these stereotypies are topographically different to drug induced stereotypies in the same species. Therefore, we investigated whether using a model with spontaneous stereotypies is indeed more relevant to OCD than a model with drug induced stereotypies.

In order to validate our model in terms of predictive validity, we injected adult deermice daily for 8 weeks with risperidone, citalopram or inositol to see what the effects of these drugs would be on stereotypical somersaulting behaviour. We found that all three drugs suppressed an increase in stereotypies seen in the saline injected group. Together with this result, there was a trend towards increased receptor densities (NMDA in the frontal cortex) with all 3 treatments as well as increased affinity in the risperidone group. The increase in stereotypies towards week 8 of the trial, as observed in the control group, was presumably due to stress from handling and injections. Our results show some evidence for the involvement of both dopamine and serotonin in the development of spontaneous stereotypies. However, some of the limitations of the study included the short duration of treatment, small group numbers and excessive stress experienced by the animals. Therefore, to further validate this model, future studies should focus on these shortfalls and perhaps include the central injection of D₁ and D₂ antagonists as well as 5-HT agonists in the striatum.

Chapter 6:

References

Alexander G E, Crutcher M D, Functional architecture of basal ganglia circuits – neural substrates of parallel processing. *Trends Neurosci* 1990; 13: 266-271.

Altemus M, Pigott T, Kalogeras K T, Demitrack M, Dubbert B, Murphy D L, Gold P W, Abnormalities in the regulation of vasopressin and corticotropin releasing factor secretion in obsessive-compulsive disorder. *Arch Gen Psychiatry* 1992; 49: 9-20.

Barnes N M, Sharp T, A review of central 5-HT receptors and their function. *Neuropharmacology* 1999, 38: 1083-1152.

Berksen G, Abnormal stereotyped motor acts. 1967. In: Zubin J, Hunt H F (Eds.), *Comparative Psychopathology*. Grune & Stratton: New York.

Bickerdike M J, Wright I K, Marsden C A, Social isolation attenuates rat forebrain 5-HT release induced by KCL stimulation and exposure to novel environment. *Behav Pharmacol* 1993; 4: 231-236.

Blier P, De Montigny C, Modification of 5-HT neuron properties by sustained administration of the 5-HT_{1A} agonist gepirone: Electrophysiological studies in the rat brain. *Synapse* 1987; 1: 470-480.

Blier P, De Montigny C, Chaput Y, Modification of the serotonin system by the antidepressant treatments: Implications for the therapeutic response in major depression. *J Clin Psychopharmacol* 1987; 7: 24S-35S.

Boscarino J A, Posttraumatic stress disorder, exposure to combat, and lower plasma cortisol among Vietnam veterans: Findings and clinical implications. *J Clin Consult Psychol* 1996; 64:191-201.

Bremner J, Licinio J, Darness A, Elevated CSF corticotropin-releasing factor concentrations in post-traumatic stress disorder. *Am J Psychiatry* 1997; 154: 624-629.

Bremner J D, Vythilingam M, Anderson C, Vermetten E, McGlashan T, Heninger G, Rasmusson A, Southwick S M, Charney D S, Assessment of the hypothalamic-pituitary-adrenal axis over a 24-hour diurnal period and in response to neuroendocrine challenges in women with and without childhood sexual abuse and posttraumatic stress disorder. *Biol Psychiatry* 2003; 54: 710-718.

Britton D R, Koob G F, Rivier J, Intraventricular corticotropin releasing factor enhances behavioural effects of novelty. *Life Sci* 1982; 31: 363-367.

Buwalda B, De Boer S F, Van Kalkeren A A, Koolhaas J M, Physiological and behavioral effects of chronic intracerebroventricular infusion of corticotropin releasing factor in the rat. *Psychoneuroendocrinology* 1997; 22: 297-309.

Carlsson M L, On the role of cortical glutamate in obsessive-compulsive disorder and attention-deficit hyperactivity disorder, two phenomologically antiethical conditions. *Acta Psychiatr Scand* 2000;102:401-13.

Chalmers D T, Lovenberg T W, Souza E B D, Localization of novel corticotropin-releasing factor receptor (CRF2) mRNA expression to specific subcortical nuclei in rat brain: comparison with CRF1 receptor mRNA expression. *J Neurosci* 1995; 15: 6340-6350.

Charney D S, Bremner J D, The neurobiology of anxiety disorders. 1999. In: Charney D S, Nestler E J, Bunney B S (Eds), *Neurobiology of Mental Illness*. Oxford University Press, New York, Oxford.

Cheetam S C, Crompton M R, Katona C L E, Horton R W, Brain 5-HT₁ binding sites in depressed suicides. *Psychopharmacology (Berl)* 1990; 102: 544-548.

Cohen H, Kotler M, Kaplan Z, Matar M, Kofman O, Belmaker R G, Inositol has behavioral effects with adaptation after chronic administration. *J Neural Trans* 1997; 104: 299-305.

Cole R A L, Sawchenko P E, Neurotransmitter regulation of cellular activation and neuropeptide gene expression in the paraventricular nucleus of the hypothalamus. *J Neurosci* 2002, 22: 959-969.

Creese I, Iversen SD. Blockage of amphetamine induced motor stimulation and stereotypy in the adult rat following neonatal treatment with 6-hydroxydopamine. *Brain Res* 1973;55:369-382.

Crossman A R, Neary D (Eds.), *Neuroanatomy*. 1995. Churchill Livingstone.

Cummings S, Elde R, Ells J, Lindall A, Corticotropin-releasing factor immunoreactivity is widely distributed within the central nervous system of the rat: an immunohistochemical study. *J Neurosci* 1983; 3: 1355-1368.

Curzon G, Stereotyped and other motor responses of 5-HT receptor activation. 1990. In: Cooper S J, Dourish C T (Eds.), *Neurobiology of stereotyped behaviour*. Clarendon Press, Oxford.

Dallman, M F, Stress Update: Adaptation of the hypothalamic-pituitary-adrenal axis to chronic stress. *Trends Endocrinol Metab* 1993; 4: 62-69.

Davis M, The role of the amygdala in fear-potentiated startle: implications for animal models of anxiety. *Trends Pharmacol* 1992; 13: 35-41.

De Bellis M D, Chrousos G P, Dorn L D, Hypothalamic pituitary adrenal axis dysregulation in sexually abused girls. *J Clin Endocrinol Metab* 1994; 78:249-255.

De Kloet E R, Steroids, stability and stress. *Front Neuroendocrinol* 1995; 16:416-425.

De Kloet E R, Reul J M, Feedback action and tonic influence of glucocorticoids on brain function: a concept arising from the heterogeneity of brain receptor systems. *Psychoneuroendocrinology* 1987; 12: 83-105.

De Kloet E R, Vreugdenhil E, Oitzl M S, Joels M, Brain corticosteroid receptor balance in health and disease. *Endocrine Rev* 1998; 19:269-301.

De Montigny C, Blier P, Effects of antidepressant treatment on 5-HT neurotransmission. 1992. In: Marsden C A, Heal D J (Eds.), *Central serotonin receptors and psychotropic drugs*. Oxford: Blackwell Scientific Publications.

De Souza E B, Insel T R, Perrin M H, Rivier J, Vale W, Kuhar M J, Corticotropin-releasing factor receptors are widely distributed within the rat central nervous system: an autoradiographic study. *J Neurosci* 1985; 5: 3189-3203.

De Vry J, 5-HT_{1A} receptor antagonists: Recent developments and controversial issues. *Psychopharmacology (Berl)* 1995; 121: 1-26.

Delgado P L, Charney D S, Price L H, Aghajanian G K, Landis H, Heninger C R, Serotonin function and the mechanism of antidepressant action. *Arch Gen Psychiatry* 1990; 47: 411-418.

Dioro D, Viau V, Meany M J, The role of the medial prefrontal cortex (cingulate cortex) in the regulation of hypothalamic-pituitary-adrenal responses to stress. *J Neurosci* 1993; 13: 3839-3947.

Dunn A J, Berridge C W, Physiological and behavioural responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety of stress responses? *Brain Res Rev* 1990; 15: 71-100.

Dupont R L, Rice D P, Miller L S, Economic costs of anxiety disorders. *Anxiety* 1996; 2: 167-172.

Einat H, Belmaker R H, Kopilov M, Klein E, Gazawi H, Ben-Shachar D, Rat brain monoamines after acute and chronic myo-inositol treatment. *Eur Neuropsychopharmacol* 1999;10:27-30.

Ellison G, Nielsen E B, Lyon M, Animal model of psychosis; hallucinatory behaviours in monkeys during the late stage of continuous amphetamine intoxication. *J Psychiatric Res* 1980; 16: 13-22.

Escorihuela R M, Fernandez-Teruel A, Gil L, Aguilar R, Tobena A, Driscoll P, Inbred Roman high- and low-avoidance rats: differences in anxiety, novelty-seeking, and shuttlebox behaviors. *Physiol Behav* 1999; 67:19-26.

Feighner J P, Boyer W F, Serotonin-1A anxiolytics: an overview. *Psychopathology* 1989; 22(Suppl. 1): 21-26.

Feldman S, Weidenfeld J, The excitatory effects of the amygdala on the hypothalamo-pituitary-adrenocortical responses are mediated by hypothalamic norepinephrine, serotonin, and CRF-41. *Brain Res Bull* 1998; 45: 389-393.

Fernandez-Cordoba E, Lopez-Ibor A J, La monoclormipramina en enfermos psiquiatricos resistentes a otros tratamientos. *Acta Luso-Esp Neurol Psiquiatr Ciene Afines* 1967;26:119-147.

Fossey M D, Lydiard B R, Ballenger J C, Laraia M T, Bissette G, Nemeroff C B, Cerebrospinal fluid corticotropin releasing factor concentrations in patients with anxiety disorders and normal comparison subjects. *Biol Psychiatry* 1996; 39: 703-707.

Foster A C, Wong E H F, The novel anticonvulsant MK-801 binds to the activated state of the N-methyl-D-aspartate receptor in rat brain. *Br J Pharmac* 1987; 91:403-9.

Fux M, Levine J, Aviv A, Belmaker RH, Inositol treatment of obsessive compulsive disorder. *Am J Psychiatry* 1996;153:1219-1221.

Gehris T L, Kathol R G, Black D W, Noyes R Jr, Urinary free cortisol levels in obsessive-compulsive disorder. *Psychiat Res* 1990, 32:151-158.

Gerfen C R, Engber T M, Mahan L C, Susel Z, Chase T N, Monsma FJ(Jr), Sibley DR, D₁ and D₂ receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* 1990;250:1429-1432.

Gerson S C, Baldasserini R J, Motor effects of serotonin in the central nervous system. *Life Sci* 1980; 27: 1435-1451.

Goenjian A K, Yehuda R, Pynoos R S, Basal cortisol and dexamethasone suppression of cortisol among adolescents after the 1988 earthquake in Armenia.

Am J Psychiatry 1996; 153: 929-934.

Goodman W K, Neuropharmacology of obsessive-compulsive disorder. In: Jenicke M A, chairperson. Recent developments in neurobiology of obsessive-compulsive disorder. J Clin Psychiatry 1996; 57: 492-503

Graeff F G, Viana M B, Mora P O, Dual role of 5-HT in defense and anxiety. Neurosci Biobehav Rev 1997;21: 791-799.

Graybiel A M, The basal ganglia. Current Biology 2000; 10: R509-R511.

Greenwood-Van Meerveld B, Gibson M, Gunter W, Shepard J, Foreman R, Myers D, Stereotaxic delivery of corticosterone to the amygdala modulates colonic sensitivity in rats. Brain Res 2001; 893: 135-142.

Greist J H, Jefferson J W, Kobak K A, Katzelnick D J, Serlin R C, Efficacy and tolerability of serotonin transport inhibitors in obsessive-compulsive disorder. Arch Gen Psychiatry 1995;52:53-60.

Griebel G, Perrault G, Sanger D J, Characterization of the behavioural profile of the non-peptide CRF receptor antagonist CP-154,526 in anxiety models in rodents. *Psychopharmacology* 1998; 138: 55-66.

Groenink L, Pattij T, De Jongh R, Van der Gugten J, Oosing R S, Dirks A, Olivier B, 5-HT_{1A} receptor knockout mice and mice overexpressing corticotropin-releasing hormone in models of anxiety. *Eur J Pharmacol* 2003; 463: 185-197.

Habib K E, Gold P W, Chrousos G P, Neuroendocrinology of stress. *Endocrinol Metab Clin North Am* 2001; 30: 695-728.

Harvey B H, Brink C B, Seedat S, Stein D J, Defining the neuromolecular action of myo-inositol. Application to obsessive-compulsive disorder. *Prog Neuropsychopharmacol Biol Psychiatry* 2002;26:21-32.

Harvey B H, Scheepers A, Brand L, Stein D J, Chronic inositol increases striatal D2 receptors but does not modify dexamphetamine-induced motor behaviour. Relevance to obsessive-compulsive disorder. *Pharmacol Biochem Behav* 2001;68:245-253.

Hauber W, Involvement of basal ganglia transmitter systems in movement initiation. *Prog Neurobiol* 1998; 56: 507-40.

Hauger R L, Irwin M R, Lorang M, Aguilera G, Brown M R, High intracerebral levels of CRH result in CRH receptor downregulation in the amygdala and neuroimmune desensitization. *Brain Res* 1993; 616: 283-292.

Heinrichs S C, Menzaghi F, Pich E M, Baldwin H A, Rassnick S, Britton D T, Koob G F, Anti-stress action of a corticotropin-releasing factor antagonist on behavioral reactivity to stressors of varying type and intensity. *Neuropsychopharmacol* 1994; 11: 179-186.

Heim C, Ehlert U, Hellhammer D H, The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. *Psychoneuroendocrinology* 2000; 25: 1-35.

Holsboer F, Gerken A, Von Bardeleben U, Grimm W, Beyer H, Muller O A, Stalla G K, Human corticotropin-releasing hormone in depression. *Biol Psychiatry* 1986; 21:601-611.

Honkaniemi J, Pelto-Huikko M, Rechardt L, Isola J, Lamni A, Fuxe K, Gustafsson J, Wikstrom A, Hokfelt T, Colocalization of peptide and glucocorticoid receptor immunoreactivities in rat central amygdaloid nucleus, *Neuroendocrinology* 1992; 55: 451-459.

Insel T R, Toward a Neuroanatomy of Obsessive-Compulsive Disorder. *Arch Gen Psychiatry* 1992;49:739-744.

Isaacson R L, Gispen W H, Neuropeptides and the issue of stereotypy in behaviour. 1990. In: Cooper S J, Dourish C T (Eds.), *Neurobiology of stereotyped behaviour*. Clarendon Press, Oxford.

Jackson A, Etgen A M, Estrogen modulates 5-HT_{1A} agonist inhibition of lordosis behavior but not binding of [³H]-8-OH-DPAT. *Pharmacol Biochem Behav* 2001; 68: 221-227.

Jacobson L, Sapolsky R, The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. *Endocr Rev* 1991; 12: 118-134.

Jenike M A, Rauch S L, Cummings J L, Savage C R, Goodman W K, Recent Developments in Neurobiology of Obsessive-Compulsive Disorder. *J Clin Psychiatry* 1996;57:492-503.

Jolkkonen J, Lepola U, Bisette G, Nemeroff C B, Riekkinen, CSF corticotropin-releasing factor is not affected in panic disorder. *Biol Psychiatry* 1993; 33: 136-138.

Kagamiishi Y, Yamamoto T, Watanabe S, Hippocampal serotonergic system is involved in anxiety-like behavior induced by corticotropin-releasing factor. *Brain Research* 2003; 991: 212-221.

Kalen P, Rosegren E, Lindvall O, Bjorklund A, Hippocampal noradrenaline and serotonin release over 24 hours as measured by the dialysis technique in freely moving rats: Correlation to behavioural activity state, effect of handling and tail pinch. *Eur J Neurosci* 1989; 1: 181-188.

Kalin N H, Behavioural and endocrine studies of corticotropin-releasing hormone in primates. 1990. In: De Souza E B, Nemeroff C B (Eds). *Corticotropin-releasing factor: Basic and clinical studies of a neuropeptide*. Boca Raton, CRC Press, Inc.

Kapur S, Remington G, Serotonin-dopamine interactions and its relevance to schizophrenia. *Am J Psychiatry* 1996;153:466-476.

Katoaka Y, Shibata K; Miyazaki A, Inoue Y, Tominga K, Koizumi S, Ueki S, Niwa M, Involvement of the dorsal hippocampus in mediation of the antianxiety action of tandospirone, a 5-hydroxytryptamine_{1A} agonist anxiolytic, *Neuropharmacology* 1991; 30: 475-480.

Keck B J, Lakoski J M, Regional heterogeneity of serotonin_{1A} receptor inactivation and turnover in the ageing female rat brain following EEDQ. *Neuropharmacol* 2000; 39: 1237-1246

Kennes D, De Rycke P H, The influence of the performance of stereotypies on plasma corticosterone and eosinophil levels in bank voles, *Clethrionomys glareolus*. 1988. In: Unselm J, van Putten G, Seeb K, Ekesbo I (Eds.), *Proceedings of the international congress on applied ethology in Farm animals*. Darmstadt: KTBL.

Kennes D, Ödberg F O, Bouquet Y, De Rycke P H, Changes in naloxone and haloperidol effects during the development of captivity-induced jumping stereotypy in bank voles. *Eur J Pharmacol* 1988;153:19-24

Kessler R C, McGonagle D A, Zhao S, Nelson C B, Hughes M, Eshleman S, Wittchen H U, Kendler K S, Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. *Arch Gen Psychiatry* 1994; 51: 8-19

Koob G F, Heinrichs S C, A role for corticotropin releasing factor and urocortin in behavioral responses to stressors. *Brain Res* 1999; 848: 141-152.

Koob G F, Heinrichs S C, Pich E M, Mensaghi F, Baldwin H, Miczek K, Britton K T, The role of corticotropin-releasing factor in behavioural responses to stress. Ciba Foundation symposium 1993; 172: 277-289, discussion 290-5.

Koob G F, Markou A, Weiss F, Schulteis G, Opponent process and drug dependence: neurobiological mechanisms. Seminars Neurosci 1993; 5: 351-358.

Korte S M, De Boer S F, A robust animal model of state anxiety: Fear-potentiated behaviour in the elevated-plus maze. Eur J Pharmacol 2003;463:163-175.

Le Doux J E, Information flow from sensation to emotion: plasticity in the neural computation of stimulus value. 1990. In: Gabriel M, Moore J (Eds.), Learning and Computational Neuroscience: Foundations of Adaptive Networks. Cambridge, Mass: MIT Press.

Levy A D, Van de Kar L D, Endocrine and receptor pharmacology of serotonergic axiolytics, antipsychotics and antidepressants. Life Sci 1992; 51: 83-94.

Liang K C, Lee E H Y, Intra-amygdala injections of corticotropin-releasing factor facilitate inhibitory avoidance learning and reduce exploratory behavior in rats. Psychopharmacology 1988; 96: 232-236.

Linthorst A C, Flachskamm C, Hopkins S J, Hoadley M E, Labeur M S, Holsboer F, Reul J M, Long-term intracerebroventricular infusion of corticotropin-releasing hormone alters neuroendocrine, neurochemical, autonomic, behavioral and cytokine responses to a systemic inflammatory challenge. *J Neurosci* 1997; 17: 4448-4460.

Lopez J F, Chalmers D T, Little K Y, Watson S J, Regulation of 5-HT_{1A} receptor, glucocorticoid and mineralocorticoid receptor in rat and human hippocampus: Implications for the neurobiology of depression. *Biol Psychiatry* 1998; 43: 543-573.

Lowry O H, Rosenbrough N J, Farr A L and Randall R J, Protein measurement with the Folin-phenol reagent. *J Biol Chem* 1951; 193:265-275

Lundkvist J, Chai Z, Teheranian R, Hasanvan H, Bartfai T, Jenck F, Widmer U, Moreau J-L, A non-peptidic corticotropin releasing factor receptor antagonist attenuates fever and exhibits anxiolytic-like activity. *Eur J Pharmacol* 1996; 309: 195-200.

Lyon M, Robbins T, The action of CNS stimulant drugs: a general theory concerning amphetamine effects. 1975. In: Essman W, Valzelli L (Eds.),

Current developments in Psychopharmacology, vol 2. New York: Spectrum Publications.

Makino S, Gold P W, Schulkin J, Corticosterone effects on corticotropin-releasing hormone mRNA in the central nucleus of the amygdala and the parvocellular region of the paraventricular nucleus of the hypothalamus. *Brain Res* 1994; 640: 105-112.

Marek G J, Wright R A, Gewirtz J C, Schoepp D D, A Major role for thalamocortical afferents in serotonergic hallucinogen receptor function in neocortex. *Neuroscience* 2001; 105: 113-126.

Marsden C A, The pharmacology of new anxiolytics acting on 5-HT neurons. *Postgrad Med J* 1990; 66(Suppl. 2): S2-S6.

Marshall R D, Blanco C, Prinz D, Liebowitz M R, Klein D F, Coplan J, A pilot study of noradrenergic and HPA axis functioning in PTSD vs. panic disorder. *Psychiatry Research* 2002; 110: 219-230.

Martijena I D, Calvo N, Volosin M, Molina V A, Prior exposure to a brief restraint session facilitates the occurrence of fear in response to a conflict situation: behavioral and neurochemical correlates. *Brain Research* 1997; 752: 136-142.

Mason G J, Stereotypies: a critical review. *Anim Behav* 1991; 41: 1015-1037.

Mason J W, Ciller E L, Kosten T R, Ostroff R B, Podd, L, Urinary free-cortisol levels in post-traumatic stress disorder patients. *J Nerv Ment Dis* 1986; 174:145-159.

Matsuzaki I, Takamatsu Y, Moroji T, The effects of intracerebroventricularly injected corticotropin-releasing factor on the central nervous system: behavioural and biochemical studies. *Neuropeptides* 1989; 13: 147-155.

McDougle C J, Goodman W K, Leckman, Lee N C, Heninger G R, Price L H, Haloperidol addition in fluvoxamine-refractory obsessive compulsive disorder: A double-blind, placebo-controlled study in patients with and without tics. *Arch Gen Psychiatry* 1994;51:302-8.

McDougle C J, Kresh L E, Posey D J, Repetitive thoughts and behavior in pervasive developmental disorders: Treatment with serotonin reuptake inhibitors. *J Autism Dev Disord* 2000;30:427-435.

McEwen B S, De Kloet E R, Rostene W H, Adrenal steroid receptors and actions in the nervous system. *Physiol Rev* 1986; 66: 1121-1150.

Meany M J, Aitken D H, [³H]Dexamethasone binding in rat frontal cortex. *Brain Res* 1985; 328: 176-180.

Meijer O C, De Kloet E R, Corticosterone and serotonergic neurotransmission in the hippocampus: Functional implications of central corticosteroid receptor diversity. *Cri Rev Neurobiol* 1998; 12: 1-20.

Micallef J, Blin O, *Neurobiology and Clinical Pharmacology of Obsessive-Compulsive Disorder*. *Clin Neuropharmacol* 2001;24:191-207.

Monteleone P, Catapano F, Tortorella A, Di Martino S, Maj J, Plasma melatonin and cortisol circadian patterns in patients with obsessive-compulsive disorder before and after fluoxetine treatment. *Psychoneuroendocrinology* 1995; 20: 763-770.

Montgomery A J, Bench C J, Young A H, Hammers A, Gunn R N, Bhagwagar Z, Grasby P M, PET measurement of the influence of corticosteroids on serotonin-1A receptor number. *Biol Psychiatry* 2001; 50: 668-676.

Morgan J T, Curran T, Stimulus-transcription coupling in the nervous system: Involvement of the inducible proto-onco-gene *fos* and *jun*. *Annu Rev Neurosci* 1991; 14: 421-451.

Morimoto M, Morita N, Ozawa H, Yokoyama K, Kawata M, Distribution of glucocorticoid receptor immunoreactivity and mRNA in the rat brain: an immunohistochemical and in situ hybridization study. *Neurosci Res* 1996; 26: 235-269.

Murray C J L, Lopez A D (Eds.). 1996. Summary: The global burden of disease: a comprehensive assessment of mortality and disability from diseases, injuries, and risk factors in 1990 and projected to 2020. Cambridge, MA: Harvard University Press.

Narrow W E, One-year prevalence of mental disorder, excluding substance use disorders, in the U S: NIMH ECA prospective data. Population estimates based on U S Census estimated residential population age 18 and over on July 1, 1998. Unpublished.

Nemeroff C B, Krishnan K R, Reed C, Leder R, Beam C, Dunnick N R, Adrenal gland enlargement in major depression. *Arch Gen Psychiatry* 1993; 49: 384-387.

Nemeroff C B, Widerlov E, Bissette G, Walleus H, Karlsson I, Eklund K, Kilts C D, Loosen P T, Vale W, Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. *Science* 1984; 226: 1342-1344.

Nurnberg H G, Keith S J, Paxton D M, Consideration of the Relevance of Ethological Animal Models for Human Repetitive Behavioral Spectrum Disorders. *Biol Psychiatry* 1997;41:226-229.

Nutt D J, Anxiety and its therapy: Today and tomorrow. 1991. In: Briley M, File S E (Eds.), *New concepts in anxiety*. London: Macmillan Press.

Okuyama S, Chaki S, Kawashima N, Yoshiko S, Ogawa S-I, Nakazato A, Kumagai T, Okubo T, Tomisawa K, Receptor binding, behavioral and electrophysiological profiles of non-peptide corticotropin-releasing factor subtype 1 receptor antagonist CRA 1000 and CRF 1001. *J Pharmacol Exp Ther* 1999; 289: 926-935.

Olivier B, Molewijk E, van Oorschot R, Van der Poel G, Sethof T, Van der Heyden J, Mos J, New animal models of anxiety. *Eur Neuropsychopharmacol* 1994; 4: 93-102.

Olivier B, Pattij T, Wood S J, Oosting R, Sarnyai Z, Toth M, The 5-HT(1A) receptor knockout mouse and anxiety. *Behav Pharmacol* 2001; 12: 439-450.

Osborn I (Ed.), 1998. *Tormenting thoughts and secret rituals. The hidden epidemic of obsessive-compulsive disorder*. Random House Inc., New York.

Overall K L, Natural animal models of human psychiatric conditions: Assessment of mechanism and validity. *Progr Neuropsychopharmacol Biol Psychiat* 2000; 24:727-776.

Owens M J, Nemeroff C B, Physiology and pharmacology of corticotropin-releasing factor. *Pharmacol Rev* 1991; 43: 425-473.

Owens M J, Nemeroff C B, Role of serotonin in the pathophysiology of depression: focus on the serotonin transporter. *Clin Chem* 1994; 40: 288-295.

Paxinos G, Watson C (eds). *The rat brain on stereotaxic co-ordinates*, 2nd ed. Academic Press, Harcourt Brace Jovanovich Publishers, San Diego, USA 1986.

Pazos A, Palacios J M, Quantitative autoradiographic mapping of serotonin receptors in the rat brain: Serotonin-1 receptors. *Brain Res* 1985; 346: 205-230.

Pecknold J C, Matas M, Howarth B G, Ross C, Swinson R, Vezeau C, Ungar W, Evaluation of buspirone as an antianxiety agent: buspirone and diazepam versus placebo. *Can J Psychiatry* 1989; 34: 766-771.

Pellow S, Chopin P, File S E, Briley M, Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Meth* 1985;14:149-167.

Piccinelli M, Pini S, Bellantuono C, Wilkinson G, Efficacy of drug treatment in obsessive compulsive disorder. A meta-analytic review. *Br J Psychiatry* 1995; 166: 424-443.

Pietersen C Y. 2001. M.Sc. Thesis. The role of corticotropin –releasing factor in Anxiety disorders. University of Stellenbosch, Faculty of Health Sciences, Department of Medical Physiology.

Plotsky P M, Owens M J, Nemeroff C B, Psychoneuroendocrinology of depression. Hypothalamic-pituitary-adrenal axis. *Psychiat Clin North Am* 1998; 21, 293-307.

Post R M, Gold P, Rubinow D R, Ballenger J C, Bunney W E, Goodwin F K, Peptides in cerebrospinal fluid of neuropsychiatric patients: an approach to central nervous system peptide function. *Life Sciences* 1982; 31: 1-15.

Powell S B, Newman H A, Pendergast J F, Lewis M H, A Rodent Model of Spontaneous Stereotypy: Initial Characterization of Developmental, Environmental, and Neurobiological Factors. *Phys Behav* 1999;66:355-363.

Presti M F, Mikes H M, Lewis M H, Selective blockade of spontaneous motor stereotypy via intrastriatal pharmacological manipulation. *Pharmacol Biochem Behav* 2003; 74: 833-839.

Presti M F, Powell S B, Lewis M H, Dissociation between spontaneously emitted and apomorphine-induced stereotypy in *Peromyscus maniculatus bairdii*. *Phys Behav* 2002;75:347-353.

Prewitt C M, Herman J P, Lesion of the central nucleus of the amygdala decreases basal CRH mRNA expression and stress-induced ACTH release. *Ann NY Acad Sci* 1994; 746: 438-440.

Prut L, Belzung C, The open field as a paradigm to measure the effects of drugs on anxiety –like behaviors: a review. *Eur J Pharmacol* 2003; 463:3-33.

Raghupathi R K, Mcgonigle P, Differential effects of three acute stressors on the serotonin 5-HT_{1A} receptor system in rat brain. *Neuroendocrinology* 1997; 65: 246-258.

Rahman S, Neuman R S, myo-Inositol reduces serotonin (5-HT₂) receptor induced homologous and heterologous desensitization. *Brain Res* 1993;631:349-351.

Rapoport J L, Ryland D H, Driete M, Drug treatment of canine acral lick: An animal model of obsessive-compulsive disorder. *Arch Gen Psychiatry* 1992;48:517-521.

Rassnick S, Heinrichs S C, Britton K T, Koob G F, Microinjection of a corticotropin-releasing factor antagonist into the central nucleus of the amygdala reverses anxiogenic-like effects of ethanol withdrawal. *Brain Res* 1993; 605: 25-32.

Regier D A, Narrow W E, Rae D S, The de facto mental and addictive disorders service system. Epidemiologic Catchment Area prospective 1-year prevalence rates of disorders and service. *Arch Gen Psychiatry* 1993; 50: 85-94.

Ridley R M, The psychology of perseverative and stereotyped behaviour. *Prog Neurobiol* 1994; 44: 221-231.

Ridley R F, Baker H F, Scraggs P R, The time course of the behavioral effects of amphetamine and their reversal by haloperidol in a primate species. *Biol Psychiatry* 1979;14:753-765

Rittenhouse P A, Bakkum E A, O'Connor P A, Carnes M, Bethea C L, Van de Kar L D, Comparison of neuroendocrine and behavioral effects of ipsapirone, a 5-HT_{1A} agonist, in the three stress paradigms: immobilization, forced swim and conditioned fear. *Brain Res* 1992; 580: 205-214.

Robbins T W, Mittelman G, O'Brien J, Winn P, The neurophysiological significance of stereotypy induced by stimulant drugs. 1990. In: Cooper S J, Dourish C T (Eds.), *Neurobiology of stereotyped behaviour*. Clarendon Press, Oxford.

Robbins T W, Sahakian B J, Behavioural and neurochemical determinants of drug-induced stereotypy. 1981. In: Clifford R F (Ed.), *Metabolic Disorders of the Nervous system*. Pitman, London.

Rodgers R J, Dalvi A, Anxiety, Defence and the Elevated Plus-maze. *Neurosci Biobehav Rev* 1997;21:801-810.

Rosengarten H, Quartermain D, The effect of chronic treatment with typical and atypical antipsychotics on working memory and jaw movements in three- and eighteen-month-old rats. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 2002; 26: 1047-1054.

Royce J R, On the Construct Validity of Open-Field Measures. *Psychological Bull* 1977; 84:1098-1106.

Rubin R T, Phillips J J, Sadow T F, McCracken J T, Adrenal gland volume in major depression: increase during depressive episode and decrease with successful treatment. *Arch Gen Psychiatry* 1995; 52: 213-218.

Rushen J, Lawrence A B, Terlouw E M C, The motivational basis of stereotypies. 1993. In: Lawrence A B, Rushen J (Eds.), *Stereotypic animal behaviour: fundamentals and applications to welfare*. Wallingford, England, UK; Tucson, AZ, USA: CAB International.

Sajdyk T J, Fitz S D, Goddard A W, Ball S G, Shekar A, Over-stimulation of the basolateral amygdala of rats results in social dysfunction which is reversed with d-cycloserine. *Biol Psychiatry* 2003; 53: 73S.

Sajdyk T J, Schober D A, Gehlert D R, Shekhar A, Role of corticotropin-releasing factor and urocortin within the basolateral amygdala of rats in anxiety and panic responses. *Behav Brain Res* 1999; 100: 207-215.

Sanders S K, Shekhar A, Regulation of anxiety by GABA_A receptors in the rat amygdala. *Pharm Biochem Behav* 1995; 52: 1-6.

Saxena S, Brody A L, Schwartz J M, Baxter L R, Neuroimaging and frontal-subcortical circuitry in obsessive-compulsive disorder. *Br J Psych* 1998;173:26-37.

Schoenecker B, Heller K E, The involvement of dopamine (DA) and serotonin (5-HT) in stress-induced stereotypies in bank voles (*Clethrionomys glareolus*). *Applied Animal Behaviour Science* 2001;73:311-319.

Schoenecker B, Heller K E, Stimulation of serotonin (5-HT) activity reduces spontaneous stereotypies in female but not in male bank voles (*Clethrionomys glareolus*). Stereotyping female voles as a new animal model for human anxiety and mood disorders? *Applied Animal Behaviour Science* 2003;80:161-170.

Schulkin J, Gold P W, McEwen B S, Induction of corticotropin releasing hormone gene expression by glucocorticoids: implication for understanding the states of fear and anxiety and allostatic load. *Psychoneuroendocrinology* 1998; 23: 219-243.

Servant D, Role of corticotropin-releasing-factor in anxiety. *Biol Psychiatry* 1997; 42:156S.

Shepard J D, Barron K W, Myers D A, Corticosterone delivery to the amygdala increases corticotropin-releasing factor mRNA in the central amygdaloid nucleus and anxiety-like behavior. *Brain Res* 2000; 861: 288-295.

Shepard J D, Barron K W, Myers D A, Stereotaxic localization of corticosterone to the amygdala enhances hypothalamo-pituitary-adrenal responses to behavioral stress. *Brain Research* 2003; 963: 203-213.

Sherman J E, Kalin NH, The effects of ICV-CRH on novelty-induced behavior. *Pharmacol Biochem Behav* 1987; 26: 699-703.

Smith G W, Aubry J-M, Dellu F, Contrarino A, Bilezikjian L M, Gold L H, Chen R, Marchuk Y, Hauser C, Bentley C A, Sawchenko P E, Koob G F, Vale W, Lee K-F, Corticotropin releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response and aberrant neuroendocrine development. *Neuron* 1998; 20: 1093-1102.

Smith M A, Bisette G, Slotkin T A, Knight D L, Nemeroff C B, Release of corticotropin-releasing factor from rat brain regions in vitro. *Endocrinology* 1986; 118: 1997-2000.

Smith M A, Davidson J, Ritchie J C, Kudler H, Lipper S, Chappell P, Nemeroff C B, The corticotropin-releasing hormone test in patients with posttraumatic stress disorder. *Biol Psychiatry* 1989; 26: 349-355.

Song C, Early B, Leonard B E, Behavioral, neurochemical and immunological responses to CRF administration. Is CRF a mediator of stress? *Ann NY Acad Sci* 1995; 29: 77155-77172.

Stein D J, Hugo F J, Neuropsychiatric aspects of anxiety disorders. 2002. In: Hales R, Yudofsky S (Eds), *Textbook of Neuropsychiatric Disorders*. American Psychiatric Publishing, Washington D C.

Stein DJ , Mendelsohn I, Potocnik F, Van Kradenberg J, Wessels C, Use of the selective serotonin reuptake inhibitor citalopram in a possible animal analogue of obsessive-compulsive disorder. *Depress Anxiety* 1998;8:39-42.

Stein D J, Shoulberg N, Helton K, Hollander E, The Neuroethological Approach to Obsessive-Compulsive Disorder. *Compr Psychiatry* 1992;33:274-281.

Stein M B, Yehuda R, Koverola C, Hanna C, Enhanced dexamethasone suppression of plasma cortisol in a adult woman traumatized by childhood sexual abuse. *Biol Psychiatry* 1997; 42: 680-686.

Stolk J M, Rech R H, Antagonism of d-amphetamine by alpha-methyl-l-tyrosine: behavioral evidence for the participation of catecholamine stores and synthesis in the amphetamine stimulant response. *Neuropharmacol* 1970;9: 249-263.

Stout S C, Owens M J, Nemeroff C B, Regulation of corticotropin-releasing factor neuronal systems and hypothalamic-pituitary-adrenal axis activity by stress and chronic antidepressant treatment. *J Pharmacol Exp Ther* 2002; 300: 1085-1092.

Suda T, Yajima F, Tomori N, Demura H, Shizume K, In vitro study of immunoreactive corticotropin-releasing factor release from the rat hypothalamus. *Life Sci* 1985; 37: 1499-1505.

Sullivan R M, Gratton A, Prefrontal cortical regulation of hypothalamic-pituitary-adrenal function in the rat and implications for psychopathology: side matters. *Psychoneuroendocrinology* 2002; 27: 99-114.

Sutton R E, Koob G F, Le Moal M, Rivier J, Vale W, Corticotropin releasing factor produces behavioural activation in rats. *Nature* 1982; 297: 331-333.

Swiergel A H, Takahashi L K, Kalin N H, Attenuation of stress-induced behavior by antagonism of corticotropin-releasing factor receptors in the central amygdala in the rat. *Brain Res* 1993; 623: 229-234.

Szetchman H, Sulis W, Eilam D, Quinpirole induces compulsive checking behaviour in rats: A potential animal model of obsessive compulsive disorder (OCD). *Behav Neurosci* 1998;112:1475-1485.

Toates F, Multiple factors controlling behaviour: implications for stress and welfare. 2000. In: Moberg G P, Mench J S (Eds.), The biology of animal stress: basic principles and implications for animal welfare. Wallingford UK; New York, NY: CAB International.

Uryu K, Okumura T, Shibasaki T, Sakanaka M, Fine structure and possible origins of nerve fibers with corticotropin-releasing factor-like immunoreactivity in the rat central amygdaloid nucleus. *Brain Res* 1992; 577: 175-179.

Vale W J, Spiess J, Rivier C, Rivier J, Characterization of a 41-residue ovine hypothalamic peptide that stimulates the secretion of corticotropin and β -endorphin. *Science* 1981; 213: 1394-1396.

Van de Kar L D, Blair M L, Forebrain pathways mediating stress-induced hormone secretion. *Front Neuroendocrinol* 1999; 20: 1-48.

Vander A J, Sherman J H, Luciano D S (Eds), *Human Physiology*. Sixth Edition. McGraw-Hill, 1994.

Veith L, Acral lick dermatitis in the dog. *Canine Pract* 1986; 3: 15-22.

Vizi E S, Kiss J P, Neurochemistry and pharmacology of the major hippocampal transmitter systems: Synaptic and nonsynaptic interactions. *Hippocampus* 1998; 8: 566-607.

Walker D L, Toufexis D J, Davis M, Role of the bed nucleus of the stria terminalis versus the amygdala in fear, stress and anxiety. *Eur J Pharmacol* 2003; 463: 199-216.

Wong M L, Kling M S, Munson P J, Listwak, S, Licinio J, Prolo P, Karp B, McCutcheon I E, Geraciotti T D, DeBellis M D, Rice K C, Goldstein D S, Veldhuis J D, Chrousos G P, Oldfield E H, McCann S M, Gold P W, Pronounced and sustained central hypernoradrenergic function in major depression with melancholic features: relation to hypercortisolism and corticotropin-releasing hormone. *Proc Natl Acad Sci USA* 2000; 97:325-330.

Wood P L, Richard J W, Morphine and nigrostriatal function in the rat and mouse: the role of nigral and striatal opiate receptors. *Neuropharmacol* 1982; 21: 1305-1310.

Woodworth R S. 1918. *Dynamic Psychology*. Columbia University Press: New York.

Wright M, Hewlett WA, Neurobiology of obsessive compulsive disorder. *Comp Ther* 1994;20:95-100.

Wurbel H, Stauffacher M, Prevention of stereotypy in laboratory mice: effects on stress physiology and behaviour. *Physiol Behav* 1996; 59: 1163-1170.

Wurbel H, Stauffacher M, Age and weight at weaning affect corticosterone level and development of stereotypies in ICR-mice. *Anim Behav* 1997; 53: 891-900.

Wurbel H, Stauffacher M, vonHolst D, Stereotypies in laboratory mice: quantitative and qualitative description of the ontogeny of 'wire-gnawing' and 'jumping' in ICR and ICR-nu mice. *Ethology* 1996; 102: 371-385.

Yehuda R, Psychoneuroendocrinology of post-traumatic stress disorder. *Psychiatr Clin North Am* 1998; 21: 359-379.

Yehuda R, Boisoneau D, Mason J, Giller E L, Glucocorticoid receptor number and cortisol excretion in mood, anxiety and psychotic disorders. *Biol Psychiatry* 1993; 34: 18-25.

Yehuda R, Kabana B, Binder-Bryens K, Southwick S M, Mason J W, Giller E L, Low urinary cortisol excretion in Holocaust survivors with PTSD. *Am J Psychiatry* 1995; 152: 245-247.

Yehuda R, Lowy M T, Southwick S M, Shaffer D, Giller E L, Increased number of glucocorticoid receptor in posttraumatic stress disorder. *Am J Psychiatry* 1991; 148: 499-504.

Yehuda R, Southwick S M, Nussbaum G, Wahby V, Giller E L, Mason J W, Low urinary cortisol excretion in patients with post-traumatic stress disorder. *J Nerv Ment Dis* 1990; 178:366-369.