Repetitive stressors at various lifetime periods differentially affect the HPA axis, neuronal neurotrophic factors and behavioural responses

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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.

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ABSTRACT

Early adverse life events appear to increase the susceptibility of developing psychiatric disorders later in life. The molecular mechanisms involved in the development of pathological behaviour remain unclear. Dysregulation of the hypothalamic-pituitary-adrenal axis and alterations in neurotrophic factors have been implicated. Previous studies have demonstrated that maternal separation (MS) and time-dependent sensitization (TDS) stress result in the development of behavioural and neuroendocrine abnormalities (Daniels et al., 2004; Uys et al., 2006). Whilst definite hormonal changes were apparent, the behavioural alterations were not comprehensive. The aim of the present study was therefore to combine the MS and TDS stress models to investigate whether the combination would yield stronger behavioural abnormalities. To attempt to elucidate the underlying mechanisms that may precipitate the proposed behavioural abnormalities, we collected trunk blood for ACTH and corticosterone determinations, and brain tissue (frontal cortex, dorsal and ventral hippocampus) for neurotrophin (brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) and neurotrophin-3 (NT-3)) measurements. Adult behavioural responses were evaluated using the elevated plus maze and the open field tests.

No behavioural abnormalities were found in these two tests. However, tendency for decreased locomotor activity was observed in the combination stressor group. The maternally deprived animals spent less time in the inner zone of the open field. We suggest that when both the stress paradigms are applied in combination that later stressors would ameliorate the detrimental effects of earlier stressors upon the brain, resulting in normal behavioural responses, since maternal deprivation and TDS individually have been shown to result in definite behavioural alterations.

Subjecting animals to MS led to significant decreases in both ACTH and corticosterone levels, when compared to controls. Rats exposed to TDS, on the other hand, did not yield any significant HPA axis abnormalities. Interestingly, the combination of MS and

TDS yielded results comparable to the MS group. It therefore appeared as though TDS normalized the endocrine irregularities imposed by maternal deprivation in the animals exposed to both stress paradigms.

Brain neurotrophic factors were significantly up-regulated in the combination stressor group when compared to the non-stressed controls. These included NGF ($p=0.007$) and NT-3 ($p=0.05$) in the dorsal hippocampus; BDNF ($p=0.014$), NGF ($p=0.006$) and NT-3 ($p=0.001$) in the ventral hippocampus and NT-3 ($p=0.001$) in the frontal cortex. This up-regulation was more pronounced in the combination group when compared to both the maternal separation and the TDS groups. The increased growth factor levels may represent a compensatory mechanism by the brain to neutralize the damaging effects of repetitive stress exposure. As such the lack of behavioural abnormalities observed in our experiments may mirror the extent of this compensation.

This is the first study to report on the combined effects of maternal separation and TDS. In contrast to expectation, our results showed that repetitive exposure to stress in the course of a lifespan does not necessarily lead to exaggerated effects on the individuals' behaviour and/or neurochemistry. Instead, our data suggested that multiple exposures to stress could have beneficial outcomes as stressors may mitigate some of the effects of the early life stressors.

ABSTRAK

Vroeë lewens gebeure wat nadelig van aard is, blyk asof dit kan lei tot die ontwikkeling van psigiatriese versteurings later in die volwasse lewenstydperk. Die molekulêre meganismes betrokke in die ontwikkeling van patalogiese gedrag is tot hede nog onduidelik. Dit is al vasgestel dat die disregulering van die HPA as en die veranderinge in neurotropiese faktore betrokke is in die proses. Vorige studies het al die rol van die verwydering van die ma van haar kinders en tyd-afhanklike sensitisasie stress modelle in die ontwikkeling van gedrags en neuroendokriene abnormalitiete gedemonstreer (Daniels et al., 2004; Uys et al., 2006). In hierdie studies was die neuroendokriene afwykings baie duidelik, terwyl die gedrags versteurings nie heeltemal betroubaar was nie. Die doel van die huidige studie was dus om die gevolge van die verwydering van die moeder van haar pasgeborenes en die tyd-afhanklike sensitisasie modelle te kombineer om vas te stel of daar meer duidelike gedragsafwykings na vore sal kom. Om dus te bepaal wat die onderliggende meganismes van die voorgestelde gedrags versteurings is, is rotte se bloed opgevang vir adrenokortikotrofiese hormoon (‘ACTH’) en kortikosteroon bepaling, brein weefsel (dorsale en ventrale hippokampus sowel as die frontale korteks) geneem vir neurotrofiese konsentrasie bepalinge (‘brain-derived’ neurotrofiese factor (‘BDNF’), ‘nerve growth factor’ (‘NGF’) en ‘neurotrophin-3’ (‘NT-3’)). Volwasse gedrag is geevalueer op die ‘elevated plus maze’ en die ‘open field’ toetse.

Geen gedragsabnormalitiete is in hierdie twee gedrags toetse gemeet nie. Alhoewel, daar neigings getoon is van verlaagde lokomotoriese aktiwitiet in die kombinasie stres groep en die groep wat weerhou was van hul ma, is minder tyd in die binnenste sone van die ‘open field’ gespandeer. Ons stel voor dat wanneer die twee stres modelle in kombinasie aangewend word, dit ‘n antagonistiese uitwerking op die brein het. Dit bleik uit die feit dat die twee stresmodelle individueel definitiewe gedragsversteurings tot gevolg gehad het, maar dat dit geen noemenswaardige gedragsafwykings in kombinasie getoon het nie.

Rotte wat weerhou was van hul ma het betekenisvolle afnames in beide ‘ACTH’ en kortikosteroon vlakke getoon in vergelyking met die kontrole diere. Rotte wat blootgestel was aan net die tyd-afhanklike sensitisasie stres het nie betekenisvolle HPA as abnormaliteite getoon nie. Die kombinasie van die twee stresmodelle het soortgelyke resultate as die groep diere wat net van hul moeders weerhou was getoon. Dit wil voorkom asof die tyd-afhanklike sensitisasie stres die neuroendokriene abnormalitiete wat deur die verwydering van die ma veroorsaak was normaliseer in die groep wat aan beide stressors blootgestel was.

Brein neurotrofiese faktore was betekenisvol hoër in die kombinasie stress groep in vergelyking met die kontrole groep. Die volgende groeifaktore was betekenisvol hoer in vergelyking met die kontrole waardes: ‘NGF’ ($p=0.007$) en ‘NT-3’ ($p=0.05$) in die dorsale hippokampus; ‘BDNF’ ($p=0.014$), ‘NGF’ ($p=0.006$) en ‘NT-3’ ($p=0.001$) in die ventrale hippokampus en ‘NT-3’ ($p=0.001$) in die frontale korteks. Hierdie veranderinge in groeifaktore was duideliker in die kombinasie groep in vergelyking met die groeifaktor veranderinge in die twee individuele stres modelle. Die verhoging in neurotrofiese groeifaktore kan moontlik ‘n kompensatoriese meganisme van die brein om die nadelige effek van herhaalde stres te neutraliseer weerspieël. Hierdie kompensatoriese meganisme kan ook verantwoordelik wees vir die feit dat geen gedragsabnormalitiete in ons model sigbaar was nie.

Hierdie is die eerste studie om die gekombineerde effek van tyd-afhanklike sensitisasie saam met die verwydering van die ma te ondersoek. In teenstelling met ons verwagting, het ons resultate gewys dat wanneer ‘n individu blootgestel word aan herhaalde stressors gedurende sy lewensloop, dit heel moontlik nie lei tot groter gedrags- of neurochemiese afwykings nie. Ons data het eerder voorgestel dat herhaalde stres blootstelling voordelige uitkomste kan lei as die latere stressors die vorige stres se nadelige gevolge neutraliseer. Verder ondersteun die resultate die belangrike rol wat die genetiese

komponent in predisponering van die ontwikkeling van psigopatologie speel, want die blootstelling aan herhaalde stres alleenlik is nie genoegsaam nie.

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List of abbreviations

ACTH	Adrenocorticotrophic hormone
AMPA	Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
ANOVA	Analysis of variance
AVP	Arginine vasopressin
B	Behaviour
BDNF	Brain-derived neurotrophic factor
CBG	Corticosteroid binding globulin
CNS	Central nervous system
CRH/CRF	Corticotropin-releasing hormone/factor
D	Decapitation
DEX	Dexamethasone
DNA	Deoxyribonucleic acid
DHC	Dorsal hippocampus
DSM	Diagnostic and Statistical Manual of Mental Disorders
ECT	Electroconvulsive therapy
EDTA	Ethylenediamine tetraacetate
ELISA	Enzyme-linked immunosorbent assay
EPM	Elevated plus maze
ER	Endoplasmic reticulum
FC	Frontal cortex
GABA	Gamma-aminobutyric acid
GR	Glucocortcoid receptor
H	Handled
HPA axis	Hypothalamic-pituitary-adrenal axis
HRP	Horseradish peroxidase
5HT	5-hydroxytryptamine, serotonin
IRMA	Immunoradiometric assay
LG-ABN	Licking, grooming, arched back nursing

LNGFR	Low-affinity nerve growth factor receptor
LTP	Long-term potentiation
m/p Ab	monoclonal/polyclonal Antibodies
MR	Mineralocorticoid receptors
mRNA	messenger Ribonucleic acid
MS	Maternal separation
NGF	Nerve growth factor
NMDA	N-methyl-D-aspartate
NT-3/4/5/6/7	Neurotrophin-3/4/5/6/7
$P75^{NTR}$	Low-affinity neurotrophin receptor
PND	Postnatal day
PNS	Peripheral nervous system
PTSD	Posttraumatic stress disorder
PVN	Paraventricular nucleus
PMSF	Phenylmethylsulfonyl fluoride
RIA	Radioimmunoassay
RS	Re-stress
SEM	Standard error of the mean
SHRP	Stress-hyporesponsive period
SPSS	Statistical Package for Social Sciences
TDS	Time-dependent sensitisation
TGN	Trans-golgi network
Trk A/B/C	Tropomyosin related kinase A/B/C
VGCC	Voltage-gated calcium channel
VHC	Ventral hippocampus
W	Weaned

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