Dopaminergic pathway imbalance in the neurobiology of depression

Rafael de Araújo Barreto

BSc in Veterinary Medicine
&
MSc in Veterinary Medicine of the Tropics

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School of Biomedical Sciences and Pharmacy

University of Newcastle

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Statement of originality

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____________________________________
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Acknowledgement of Authorship

I hereby certify that the work embodied in this thesis contains a published paper/s/scholarly work of which I am a joint author. I have included as part of my thesis a written statement, endorsed by my supervisor, attesting to my contribution to the joint publication.

____________________________________
Rafael de Araújo Barreto
# Table of Contents

Table of Contents .......................................................................................................................... 4  
List of Abbreviations ..................................................................................................................... 8  
Abstract ........................................................................................................................................ 11  
Publications .................................................................................................................................... 14  
CHAPTER 1 .................................................................................................................................... 15  
INTRODUCTION ............................................................................................................................. 15  
1.1. The neurobiology of depression ............................................................................................ 16  
1.1.1. Depression: Overview ........................................................................................................ 16  
1.1.2 Stress and stressors .............................................................................................................. 18  
1.1.3 The stress system and stress response .............................................................................. 20  
1.1.3.1. The Behavioural Stress Response ................................................................................ 21  
1.1.3.2. The Autonomic Stress Response .................................................................................. 21  
1.1.3.3. The Neuroendocrine Stress Response ......................................................................... 22  
1.1.4. Stress and the brain ........................................................................................................... 24  
1.1.5. Hypotheses of depression .................................................................................................. 25  
1.1.5.1. The monoamine hypothesis of depression ................................................................. 25  
1.1.5.2. The neuroendocrine hypothesis of depression ............................................................ 28  
1.1.5.3. The inflammatory/cytokine hypothesis of depression ................................................... 30  
1.1.5.4. The neurotrophic factor hypothesis of depression ...................................................... 32  
1.1.6. The dopamine system ........................................................................................................ 35  
1.1.7. Dopamine and depression .................................................................................................. 37  
1.1.8. The medial prefrontal cortex and stress ........................................................................... 40  
1.1.9. The mesoprefrontal dopamine pathway and depression ................................................... 44  
1.1.10. Stress and the nucleus accumbens .................................................................................... 45  
1.1.11. The mesoaccumbal dopamine pathway and depression ................................................ 45  
1.1.12. Depression and genes ...................................................................................................... 47  
1.1.13. Animal models of stress in depression research .............................................................. 49  
1.1.14. Restraint stress ................................................................................................................ 51  
1.2. AIM AND HYPOTHESIS ...................................................................................................... 55  
1.3. REFERENCES ....................................................................................................................... 59  
CHAPTER 2 ..................................................................................................................................... 68  
Genome-wide gene expression analysis of infralimbic (IL) mPFC after sub-chronic stress:  
Methods and effects of time ........................................................................................................... 68  
OVERVIEW ..................................................................................................................................... 69  
2.1. INTRODUCTION .................................................................................................................. 70
2.2. AIMS ............................................................................................................................... 74

2.3. MATERIAL AND METHODS .................................................................................. 75

2.3.1. Animals .................................................................................................................... 75

2.3.2. Experimental Procedures ..................................................................................... 75

2.3.3. Tissue preparation and RNA extraction .................................................................. 76

2.3.4. Gene expression microarray data processing ....................................................... 77

2.3.5. Pathway analysis ..................................................................................................... 77

2.3.5.1. Database for Annotation, Visualization, and Integrated Discovery (DAVID) ....... 77

2.3.5.2. Functional profiling by gene set enrichment analysis (GSEA) ......................... 78

2.4. RESULTS ....................................................................................................................... 80

2.4.1 Macrodissection of IL mPFC .................................................................................. 80

2.4.2 Assessment of RNA Quality .................................................................................... 80

2.4.3. Analysis of gene expression profiles in the IL mPFC at 2h and 24h after the last restraint stress ................................................................................................................... 83

2.4.4. Analysis of over-represented KEGG pathways in the IL mPFC at 2h and 24h after the last restraint stress as assessed by DAVID ................................................................. 88

2.4.5. Analysis of pathway enrichment in the IL mPFC of rats at 2h and 24h after the last restraint stress as assessed by GSEA .............................................................................. 91

2.5. DISCUSSION ............................................................................................................... 95

2.6. CONCLUSIONS .......................................................................................................... 104

2.7 REFERENCES .............................................................................................................. 105

CHAPTER 3 ............................................................................................................................ 119

Fluoxetine prevents development of an early stress-related molecular signature in the rat infralimbic medial prefrontal cortex. Implications for depression? ................................. 119

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CHAPTER 4 ............................................................................................................................ 143

Genome-wide gene expression analysis of the nucleus accumbens (NAc) after sub-chronic stress and the effects of stress and antidepressant treatment on the BDNF signalling pathway ................................................................................................. 143

OVERVIEW ......................................................................................................................... 144

4.1. INTRODUCTION ........................................................................................................ 145

4.2. AIMS ............................................................................................................................. 148

4.3. MATERIAL AND METHODS .................................................................................. 149

4.3.1. Animals .................................................................................................................... 149

4.3.2. Experimental Procedures ..................................................................................... 149

4.3.2.1. Stress treatment ............................................................................................... 149
4.3.2.2. Chronic fluoxetine treatment ................................................................. 150
4.3.3. Tissue preparation and RNA extraction .................................................. 150
4.3.4. Gene expression microarray data processing ........................................... 151
4.3.5. Real-time quantitative polymerase chain reaction (RT-qPCR) .................. 152
4.3.6 Pathway Analysis .................................................................................... 153
4.3.6.1. Database for Annotation, Visualization, and Integrated Discovery (DAVID) 153
4.3.6.2. Functional profiling by gene set enrichment analysis (GSEA) ............. 154
4.4 RESULTS ..................................................................................................... 156
4.4.1. Macrodissection of NAc ......................................................................... 156
4.4.2. Genome-wide gene expression analysis .................................................. 156
4.4.3. Pathways associated with stress-induced gene expression change as determined by gene set enrichment analysis (GSEA) .............................................. 157
4.4.4. The effects of stress and fluoxetine on BDNF signalling-related gene expression in the NAc, as determined by real-time quantitative polymerase chain reaction (RT-qPCR) .......................................................... 160
4.5. DISCUSSION .............................................................................................. 162
4.6. CONCLUSION ............................................................................................. 167
4.7. REFERENCES ............................................................................................... 168

CHAPTER 5 ....................................................................................................... 172
Gene expression analysis of ventral tegmental area (VTA) dopamine neurons: the effects of stress and antidepressant treatment on BDNF signalling......................................................... 172

OVERVIEW ....................................................................................................... 173
5.1. INTRODUCTION .......................................................................................... 174
5.2. AIMS .......................................................................................................... 178
5.3 MATERIAL AND METHODS ....................................................................... 179
5.3.1 Animals .................................................................................................... 179
5.3.2 Experimental Procedures ......................................................................... 179
5.3.2.1 Stress treatment .................................................................................. 179
5.3.2.2. Chronic fluoxetine treatment ............................................................. 180
5.3.3 Tissue preparation ................................................................................... 180
5.3.4 Immuno-laser-microdissection of rat midbrain dopamine neurons ........... 181
5.3.4.1. Immunolabelling .............................................................................. 181
5.3.4.2. Laser capture microdissection (LCM) .............................................. 182
5.3.5. Real-time quantitative polymerase chain reaction (RT-qPCR) ............... 182
5.4. RESULTS .................................................................................................... 184
5.4.1. Laser microdissection of immuno-identified VTA dopamine neurons ...... 184
5.4.2. Effects of stress and fluoxetine antidepressant treatment on gene expression in VTA dopamine neurons ............................................................................ 185
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5. DISCUSSION</td>
<td>187</td>
</tr>
<tr>
<td>5.6. CONCLUSION</td>
<td>194</td>
</tr>
<tr>
<td>5.7. REFERENCES</td>
<td>195</td>
</tr>
<tr>
<td>CHAPTER 6</td>
<td>199</td>
</tr>
<tr>
<td>General Discussion</td>
<td>199</td>
</tr>
<tr>
<td>6.1 GENERAL DISCUSSION</td>
<td>200</td>
</tr>
<tr>
<td>6.2. FUTURE DIRECTIONS</td>
<td>212</td>
</tr>
<tr>
<td>6.3. REFERENCES</td>
<td>214</td>
</tr>
</tbody>
</table>
List of Abbreviations

5-HT - serotonin
5-HT T - serotonin transporter
ACTH - adrenocorticotropic hormone
AD - adrenaline
AGRF - Australian Genomics Research Facility
AMPAR - $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ANS - autonomic nervous system
AVP - arginine-vasopressine
BDNF - brain-derived neurotrophic factor
cDNA - complementary DNA
COMT - catecholamine-O-methyltransferase
CRFR1 - CRH receptor type 1
CRH - corticotropin-release-hormone
CSF - cerebrospinal fluid
D1 - dopamine receptor type 1
D2 - dopamine receptor type 2
D3 - dopamine receptor type 3
D4 - dopamine receptor type 4
D5 - dopamine receptor type 5
DA - dopamine
DAT - dopamine transporter
DAVID - database for annotation, visualization, and integrated discovery
DEPC - diethyl pyrocarbonate
DNA - deoxyribonucleic acid
DSM-IV-TR - diagnostic and statistical manual IV text revision
ECT - electro-convulsive shock therapy
ES - enrichment score
FDR - false discovery rate
fMRI - functional magnetic resonance imaging
GAPDH - glyceraldehyde-3-phosphate dehydrogenase
gDNA - genomic DNA
GPCR - G-protein coupled receptors
GR - glucocorticoid receptors
GRE - glucocorticoid-response elements
GSEA - gene set enrichment analysis
HPA - hypothalamic-pituitary-adrenal
HVA - homovanillic acid
IFN-γ - interferon gamma
IL - infralimbic
IL-2 - interleukin 2
IL-6 - interleukin 6
IL mPFC - infralimbic medial prefrontal cortex
KEGG - Kyoto encyclopedia of genes and genomes
LCM - laser capture microdissection
L-DOPA - L-3, 4-dihydroxyphenylalanine
MAO - monoamine oxidase
MAOi - monoamine oxidase inhibitor
MDD - major depressive disorder
mPFC - medial prefrontal cortex
MR - mineralocorticoid receptor
mRNA - messenger RNA
NA - noradrenaline
NAc - nucleus accumbens
NaCl - sodium chloride
NCBI - national center for biotechnology information
NES - normalized enrichment score
NMDAR - N-methyl-D-aspartate receptors
PBS - physiological buffer solution
PET - positron emission tomography
PFC - prefrontal cortex
PL - prelimbic
PL mPFC - prelimbic mPFC
PVN - hypothalamic paraventricular nucleus
qPCR - quantitative polymerase chain reaction
RIN - RNA integrity number
RNA - ribonucleic acid
Abstract

It is generally accepted that susceptibility to depression is influenced by a combination of biological and environmental factors such as genetics and stress. However, the molecular mechanisms underlying this disease remain incompletely defined. Moreover, it is not even unequivocally clear which part or parts of the brain is/are responsible for the manifestation of depression and its symptoms. However, recent studies have indicated that the midbrain dopamine system modulates depression-like behavior in stress-based animal models of depression. In the brain of mammals, dopamine neurons of the ventral tegmental area in the midbrain give rise to two major dopaminergic pathways, the mesocortical and mesolimbic pathways. These pathways innervate a number of brain regions, including the medial prefrontal cortex (mPFC; mesocortical or mesoprefrontal pathway) and the nucleus accumbens (NAc; mesolimbic or mesoaccumbal pathway). Importantly, molecular disturbances in both of these brain regions are believed to influence the development of psychiatric disorders such as schizophrenia, addiction and depression. The work presented in this thesis is based on the hypothesis that stress differentially induces molecular alterations in these dopaminergic pathways, creating a functional imbalance which, we propose, contributes to the pathogenesis of depression.

The studies which comprise the first three research chapters sought to establish how stress alters the gene expression profile in the target areas of the two dopaminergic pathways. The stress paradigm used was based on previous work that had clearly demonstrated stress had to be administered in a repeated and chronic fashion to induce depression-like behaviours in animal models of depression. It was proposed that by using a sub-chronic stress paradigm, where depression-like behaviours are not observed,
it might be possible to identify an early molecular signature for the pathogenesis of the disease. Firstly, by screening the whole rat genome using microarray technology it was revealed that a number of genes involved in neurotrophin signalling had altered expression in the infralimbic mPFC (IL mPFC). This finding is completely in accord with both the neurotrophic factor and neuroplasticity hypotheses of depression. Indeed, chronic pretreatment with a clinically used antidepressant (i.e., fluoxetine) prevented some of the changes observed, especially in regards to the expression of ntrk2, the gene that encodes for the receptor of the brain-derived neurotrophic factor (BDNF). These results add to the mounting evidence that neurotrophins may play a role on the pathophysiology of depression and the beneficial effects of antidepressants. In addition, it supports the notion that the mPFC is an important brain area involved in depression. Interestingly, the stress related effects on BDNF signalling seemed to be localized to the IL mPFC, as these alterations were not seen in the NAc. Therefore, at least as it applies to a pre-depressive state, these findings support our notion of dopaminergic pathway imbalance in the etiology of depression.

The last chapter comprises a study whereby expression of genes involved in dopamine neurotransmission and neurotrophic factor signalling was evaluated specifically in VTA dopamine neurons obtained from sub-chronically stressed and control animals. Consistent with a role for neurotrophic factors in depression, the results showed that the expression of the gene that encodes for BDNF was increased by stress and, importantly, that antidepressant treatment could prevent this alteration.

The findings presented in this thesis demonstrate that stress causes alteration in cellular neurotrophic mechanisms that might have consequences for neuroplastic phenomena and neuronal activity. Importantly, they reveal that these alterations can be observed after a relatively short period of exposure to stress, and that they occur only in
a mesocortical dopaminergic pathway, as these changes were not observed in a mesolimbic pathway target region. The present findings support the mounting evidence that VTA dopamine neurons and the forebrain regions they target could play a crucial role in the pathogenesis of depression, perhaps by undergoing neuroplastic changes that differentially alter both dopaminergic pathways responsivity to stress, creating an imbalance that favours the development of depressive symptoms.
Publications

The following publications have arisen from data presented in the present thesis:

Journal Articles


Conference Abstracts


Rafael A. Barreto, Frederick R. Walker, Peter R. Dunkley, Trevor A. Day and Douglas W. Smith. Stress induces alteration of the neurotrophin signalling pathway in the rat infralimbic medial prefrontal cortex that is reversed by fluoxetine. 41st Annual meeting of the Society for Neuroscience (SfN), Washington D.C., 2011.