Neurobiological alterations in the rat medial prefrontal cortex following exposure to chronic psychological stress

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DECLARATION

This thesis contains no material which has been accepted for the award of any other Degree or Diploma in any University or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying subject to the provisions of the Copyright Act 1968.

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ABSTRACT

Persistent exposure to stressful events can produce serious disorders of cognitive function and mood state. Globally, depression affects between 5 and 20% of the world's population, and represents a large burden of disease. Current treatments are not effective for all people with depression. Research efforts directed towards understanding the neurobiology of stress and depression are increasing in an attempt to better understand, treat, and possibly even prevent mood-related psychopathology. One of the first steps is understanding the response of the prefrontal cortex, part of the brain responsible for the control of stress and emotional responses, to chronic stress. With this in mind, the experiments in this thesis were aimed at elucidating cellular changes in the rat medial prefrontal cortex after exposure to chronic psychological stress.

The experiments described in Chapter Two aimed to examine the phenotype of chronically activated neurons in the forebrain following exposure to chronic stress. The medial prefrontal cortex was the only area examined to display a difference in levels of ΔFosB, a marker used for functional imaging of chronic neuronal activation. It was found that these cells were glutamatergic pyramidal projection neurons.

The results of Chapter Two, along with emerging evidence that stress activates the neuroimmune system (microglial cells), and that these cells appear to be able to alter neuronal connectivity, led us to investigate how exposure to chronic stress affects microglial activity in the medial prefrontal cortex in Chapter Three. It was found that chronic psychological stress increased microglial cell immunoreactivity, as well as local neuronal activity. In this study, stress also impaired performance on a working memory task, a cognitive function that is prefrontal cortex dependent. Administration of minocycline hydrochloride, a tetracycline antibiotic which is known to inhibit microglial activation,

reversed the effects of stress on microglial cells. Additionally, minocycline administration reduced the impact of stress on neuronal activation and working memory performance. This suggests that microglia mediate the effects of stress on prefrontal cortex neuronal function and prefrontal cortex dependent behaviour.

In Chapter Four, following on from the results of the previous two studies, we investigated how exposure to chronic stress alters microglial cell morphology. This is an important issue, as experience-dependent changes to microglial cells are only just starting to be elucidated, and form is closely related to function for these cells. Cells from animals exposed to chronic psychological stress were digitally reconstructed and analysed for morphological characteristics. It was found that microglial cells in the medial prefrontal cortex of animals exposed to chronic psychological stress had increased ramification (branching) without an increase in the overall size of the cell. This was associated with an increase in the structural protein β1-integrin, which has been implicated in microglial ramification. These effects were reversed in animals who were administered minocycline. Increased ramification of microglia may be the morphological representation through which microglia exert their effects following exposure to chronic psychological stress.

Overall, the experiments presented in this thesis have contributed to our knowledge of how neurons and microglial cells in the medial prefrontal cortex respond to chronic stress, and how some of the effects of stress may be mediated by a neuroinflammatory response evoked by microglia. These results form an important contribution to further understanding the neurobiology of the stress response, and may have implications for the future development of efficacious pharmacotherapies for stress-induced psychopathologies.

LIST OF ABBREVIATIONS

HPA	Hypothalamic-pituitary-adrenal
IEG	Immediate-early gene
mRNA	Messenger ribonucleic acid
LPS	Lipopolysaccharide
mPFC	Medial prefrontal cortex
PFC	Prefrontal cortex
IL	Infralimbic cortex
PrL	Prelimbic cortex
ACd	Dorsal anterior cingulate cortex
GABA	ɣ-Aminobutyric acid
BNST	Bed nucleus of the stria terminalis
TNF-α	Tumor necrosis factor-α
CNS	Central nervous system
IL-1β	Interleukin-1β
Iba-1	Ionized calcium-binding adaptor protein-1
SSRI	Selective serotonin reuptake inhibitor
SNRI	Serotonin-noradrenaline reuptake inhibitor
NO	Nitric oxide
NMDA	N-methyl-D-aspartic acid