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# INVOLVEMENT OF MICROGLIA ACTIVATION IN THE DEVELOPMENT OF CNS DISEASES

GIOVANNI PIETROGRANDE

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School of Biomedical Sciences and Pharmacy

Faculty of Health and Medicine

University of Newcastle

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## Statement

I, Giovanni Pietrogrande, hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision.

The thesis contains published scholarly work of which I am a co-author. For each such work a written statement, endorsed by the other authors, attesting to my contribution to the joint work has been included.

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# Publications

- Chronic stress induced disturbances in Laminin: A significant contributor to modulating microglial pro-inflammatory tone?
  **Pietrogrande G**, Mabotuwana N, Zhao Z, Abdolhoseini M, Johnson SJ, Nilsson M, Walker FR.
  Brain Behav Immun. 2018 Feb;68:23-33. doi: 10.1016/j.bbi.2017.09.012. Epub 2017 Sep 22.

- Low Oxygen Post Conditioning as an efficient non-pharmacological strategy to promote motor function after stroke.
  **Pietrogrande G**, Zalewska K, Zhao Z, Abdolhoseini M, Johnson SJ, Nilsson M, Walker FR.
  Submitted to Translational Stroke Research

- Low Oxygen Post Conditioning prevents thalamic secondary neuronal loss caused by excitotoxicity in a murine model of cortical stroke.
  **Pietrogrande G**, Zalewska K, Zhao Z, Abdolhoseini M, Johnson SJ, Nilsson M, Walker FR.
  To be submitted to Scientific reports after approval from all the authors

# Additional publications

- Sustained administration of corticosterone at stress-like levels after stroke suppressed glial reactivity at sites of thalamic secondary neurodegeneration.
  Zalewska K, **Pietrogrande G**, Ong LK, Abdolhoseini M, Kluge M, Johnson SJ, Walker FR, Nilsson M.
  Brain Behav Immun. 2017 Nov 21. pii: S0889-1591(17)30515-9. doi: 10.1016/j.bbi.2017.11.014.

- Growth hormone improves cognitive function after experimental stroke
  Ong LK, Chow WZ, TeBay C, Kluge M**, Pietrogrande G**, Zalewska Z, Crock P, Åberg ND, Bivard A, Johnson SJ, Walker FW, Nilsson M, Isgaard J.
  Stroke, manustript accepted

- Normobaric post-conditioning hypoxia improves stroke-induced cognitive impairment through improved glymphatic flow

  Zhao Z, Ong LK, **Pietrogrande G**, Ottersen O, Johnson SJ, Nilsson M, Walker FR.
  Stroke, manuscript under review

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- Delayed normobaric hypoxic exposure rescued neuron loss and reduced Amyloid beta accumulation at thalamic secondary neurodegeneration post-stroke
  Zhao Z, Ong LK, **Pietrogrande G**, Johnson SJ, Nilsson M, Walker FR
  Stroke, in preparation

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# Abbreviations

CNS: central nervous system

MS: multiple sclerosis

DAMP: damage associated molecular pattern

TLR: toll-like receptor

HOSC: organotipic hippocampal slice culture

NMDA: N-methyl-D-aspartate

ROS: reactive oxygen species

LPS: lipopolysaccharides

IL1β: interleukin 1 beta

TNF-α: tumor necrosis factor -α

SND: secondary neurodegeneration

Iba1: ionized calcium-binding adapter molecule 1

iNOS: inducible nitric oxide synthase

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IFN-γ: interferon gamma

IRF: Interferon regulatory factor

ISRE: interferon (IFN)-stimulated response element

GTP: guanosine-5'-triphosphate

ECM: extracellular matrix

FAK: focal adhesion kinase

HPA: hypothalamic–pituitary–adrenal

GC: glucocorticoid

MCAO: middle cerebral artery occlusion

LOPC: low oxygen post conditioning

tPA: tissue plasminogen activator

WD: wallerian degeneration

SND: secondary neurodegeneration

NMDAR: N-methyl-D-aspartate receptor

PSD95: post synaptic density protein 95

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# Abstract

Microglia are the resident immune cells within the brain, however, in the last 20 years it has become clearer that their function is more complex than ordinary macrophages and goes well beyond guarding CNS from pathogens. In fact microglia are also responsible for maintaining the homeostatic balance within the brain. Factors that can modulate this balance can be of differing natures: for instance they can include subtle and prolonged factors like hormonal changes driven by stress; alternatively they can be dramatic like an ischemic injury; or prolonged and sustained as during the processes of secondary neurodegeneration associated with stroke. During my PhD I focused my attention on all these aspects, which can be considered at the opposite poles in the spectrum of events threatening homeostasis. In this thesis I describe the current knowledge about these CNS pathologies and how they are linked to inflammation in general and microglia activation in particular. Only by analysing microglia in such different conditions it is possible to fully appreciate the different shades that characterize microglia activation, and especially how different tuning of inflammation can have very different consequences for the CNS.

There is already quite an extensive literature that describes the role of microglia in chronic stress and in stroke. These studies show the pivotal importance of microglia in these pathologies and especially the importance of microglia activation as modulator of inflammation. Thus, I decided to take an innovative approach and investigate aspects that are crucial to fully understand the role of microglia but that have not been yet characterised

More specifically, in the first study I investigated the status of microglia activation in a murine model of chronic stress. Higher levels of basal inflammation correlate in a unique

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way with development of depression in humans but the leading mechanism is still largely uncharacterised. Using *in vivo* and *in vitro* models, I found that the extracellular matrix (ECM) is dysregulated after chronic stress and influences microglia activation. This finding makes ECM a potential key player in the development of mood disorders.

The focus of the second study was the role of microglia activation in the post-acute phase after stroke, which is a leading cause of disability worldwide (1). In this context, the reports about microglia activation are mixed, since they can have both a positive and a negative influence on the outcome. Using a novel intervention, low oxygen post conditioning (LOPC), I analysed the correlation between its neuroprotective effect and microglia activation, and I validated LOPC therapeutic potential in improving motor function.

In the third study, I extended my previous findings to the stroke-associated neurodegenerative event called secondary neurodegeneration (SND). SND develops in areas distal but connected to the infarct site and is characterised by progressive neuronal loss concomitant to glial activation. Microglia activation is inextricably linked to SND; however microglial involvement in SND and its contribution are still largely not understood. Using LOPC as intervention and analysing the temporal evolution of microglia activation in the site of SND, I concluded that LOPC can ameliorate neuronal loss and promote the return of microglia to a basal state in later stages.

In conclusion, in this thesis I present novel observations into microglia involvement in the development of CNS pathologies and provide useful insights in the mechanism leading to microglia activation, with important implications for future research.

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