Targeting neuroinflammation in Parkinson's disease

by Sarah Thomas Broome

Thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

under the supervision of Alessandro Castorina

University of Technology Sydney
Faculty of Science

May 2022

Certificate of Original Authorship

I, *Sarah Thomas Broome* declare that this thesis, is submitted in fulfilment of the requirements for the award of *Doctor of Philosophy*, in the *Faculty of Science* at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

This research is supported by the Australian Government Research Training Program.

Production Note:

Signature: Signature removed prior to publication.

Date: 15/01/2022

Acknowledgments

First and foremost, I am extremely grateful to my supervisor, Associate Professor Alessandro Castorina, for taking a chance on me as a second-year transfer student and providing invaluable advice, unfaltering support and patience during my PhD study. It has truly been an honour and privilege to be your student and part of the LCMN. This achievement was made possible because of you.

I would also like to express my sincere gratitude to Professor Hui Chen, Dr Jeremy Chan, Ms Fiona Ryan, Dr Ghaith Al-Badri and past LCMN members for their insightful suggestions, technical support, lab chats and coffee breaks.

Finally, I would like to thank my friends and family for their unwavering and unconditional support. To my friends who were always there to drag me out of the lab and give me a break. To my grandparents, Yvette and David Broome, who are unfailingly generous and kind and my inspiration for becoming a neuroscientist. To my brother, Daniel, for his exceptional music suggestions, sense of humour and expert editing skills and advice. To my father, Brian, for his generosity, chauffeur services, attempts at science jokes and foundation, and my mother, Marisa, who is my ultimate inspiration and guidepost for everything.

Impact of Covid-19 pandemic

Unfortunately, I commenced my PhD in a lab group that was not able to create a productive and supportive environment to complete my research and after one year I changed supervisors and projects. The data in my thesis is the product of the research done in the Laboratory of Cellular and Molecular Neuroscience (LCMN).

During the two remaining years of my PhD candidature the Covid-19 pandemic caused several disruptions. From March to June 2020 the lab was closed, as per government health order. As such no lab work could be conducted during this time. The next lockdown, from the end of June to October 2021 resulted in significant disruptions accessing and learning new techniques including immunohistochemistry and microscopy. During these lockdowns I focused on developing my writing skills, with previously collected data from the LCMN, as well as analysing and curating data I had already collected. The second lockdown resulted in a dramatic downscale of experiments planned for microscopy due to lack of access and technical support. Despite these obstacles, the resulting body of work, obtained within two years amid two lockdowns was possible due to the support of my supervisor and sacrifice of my family who did everything possible to keep our household virus free.

Table of Contents

Certificate of original authorship……..……....**ii**

Acknowledgements ……..……..**iii**

Impact Statement: Covid-19 pandemic ……...**iv**

Table of Contents ……..**v**

List of Figures ……..……**vii**

List of Tables……**viii**

List of Abbreviations ……..……..……..…**xi**

Publications ..…….…….…..**xii**

Presentations…….…….……..…**xiv**

Awards and Scholarships …….…….…….**xvi**

Professional contributions ..…….……..……**xvii**

Abstract ……..……..……**xix**

Chapter 1: General Introduction ……**1**

1.1 Parkinson's disease……..**2**

1.2 Neuroinflammation ……..……....**14**

1.3 Attempts to target neuroinflammation ……..**20**

1.4 Dopamine: an *immune transmitter* ……..……**23**

1.5 Buspirone ……**62**

1.6 PACAP and VIP ……..**66**

1.7 Aims and hypothesis ..……..……..**69**

Chapter 2: Assessing the anti-inflammatory activity of the anxiolytic drug buspirone using CRISPR-Cas9 gene editing in LPS-stimulated BV2 microglial cells……**72**

Chapter 3: Neurotoxicity of rotenone in the central nervous system of C57BL/6 mice ……..…..**122**

Chapter 4: The anxiolytic drug buspirone prevents rotenone-induced toxicity in a mouse model of Parkinson's disease ……**165**

Chapter 5: PACAP and VIP mitigate rotenone-induced inflammation in BV2 microglial cells ……...**218**

Chapter 6: General discussion ……..……..……..**249**

6.1 Summary of main findings ……..……..……..**250**

6.2 Challenges modelling Parkinson's disease ……..……**253**

6.3 Limitations of repurposing buspirone for Parkinson's disease ……..……**257**

6.4 Potential of PACAP and VIP as therapeutic targets ……..……**263**

6.5 Conclusions ……..……**265**

Bibliography ……..……..……**267**

List of Figures

Figure 1.1 Dopaminergic pathways and regions affected in Parkinson's disease ..**3**

Figure 1.2 Pathological mechanisms of neurodegeneration in Parkinson's disease……..**5**

Figure 1.3 Therapies for Parkinson's disease……**7**

Figure 1.4 Mechanisms of Parkinson's disease mimetics ……**11**

Figure 1.5 Microglia phenotypes…….……**16**

Figure 1.6 Overview of neuroinflammation observed in Parkinson's disease…....**19**

Figure 1.7 Buspirone's known pharmacological targets……....**65**

Figure 1.8 Summary of peptide interactions with receptors…….....**66**

Figure 1.9 Summary of broad immunomodulatory and neuroprotective functions mediated by PACAP and VIP…….....**69**

Figure 6.1 Summary of main findings of thesis……....**250**

Figure 6.2 Inducible and conditional knockout models to isolate neuronal and glial functions of the D3 receptor ……..…**258**

Figure 6.2 Schematic diagram illustrating main findings of thesis……..…**266**

List of Tables

Table 1 Summary of the main genetic mutations associated with PD……....**6**

Table 2 Characteristics of dopamine receptor subtypes……...**8**

List of Abbreviations

5-HT	Serotonin/5-hydroxytrptamine
5HT1a	Serotonin receptor 1a
5HT2a	Serotonin receptor 2a
6-OHDA	6-hydroxydopamine
A1	Classically activated astrocytes
A2	Alternatively activated astrocytes
ADNP	Activity-dependent neuroprotective protein
Arg1	Arginase-1
BBB	Blood brain barrier
BDNF	Brain derived neurotrophic factor
BW	Body weight
CNS	Central nervous system
COMT	Catechol-O-methyltransferase
CRISPR	Clustered regularly interspaced short palindromic repeats
CSF	Cerebrospinal fluid
D2R	Dopamine-2-receptor
D3R	Dopamine-3receptor
D4R	Dopamine-4-receptor
DBS	Deep brain stimulation
DR	Dopamine receptor
Drd3	Gene encoding the dopamine-3-receptor
DRT	Dopamine replacement therapy
FDA	Food and Drug Administration (United States of America)
Fizz1	Found in inflammatory zone/Retnla

GABA	Gamma-aminobutyric acid
GFAP	Glial fibrillary acidic protein
GIT	Gastrointestinal tract
Htr1a	Gene encoding serotonin receptor 1a
IBD	Irritable bowel syndrome
IFN-γ	Interferon-gamma
IL-1β	Interleukin-1 beta
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-10	Interleukin-10
iPSCs	Induced pluripotent stem cells
iNOS	Inducible nitric oxide synthase
KO	Knockout
L-Dopa	Levodopa
LPS	Lipopolysaccharide
Mθ	Resting microglia
M1	Classically activated microglia
M2	Alternatively activated microglia
MAO	Monoamine oxidase
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MPP+	1-methyl-4-phenylpyridinium
NMDA	N-methyl-D-aspartic acid
NMS	Non-motor symptoms
NO	Nitric oxide
NSAIDs	Non-steroidal anti-inflammatory agents

PACAP	Pituitary adenylate cyclase-activating polypeptide
PD	Parkinson’s disease
PET	Positron emission tomography
ROS	Reactive oxygen species
SNpc	*Substantia nigra pars compacta*
TGF-β	Transforming growth factor beta
TH	Tyrosine hydroxylase
TNF-α	Tumour necrosis factor-alpha
VIP	Vasoactive intestinal peptide

Publications

Publications arising from thesis

Submitted:

Thomas Broome, S. and Castorina, A. **Neurotoxicity of Rotenone in the Central Nervous System of C57BL/6 mice**. *Scientific reports*. Submitted on: 18/11/2021. Current status: Editors invited 12/01/2022

Published:

Thomas Broome, S., & Castorina, A. (2022). **The Anxiolytic Drug Buspirone Prevents Rotenone-Induced Toxicity in a Mouse Model of Parkinson's Disease**. *International journal of molecular sciences*, *23*(3), 1845. https://doi.org/10.3390/ijms23031845

Broome, S. T., Musumeci, G., & Castorina, A. (2022). **PACAP and VIP Mitigate Rotenone-Induced Inflammation in BV-2 Microglial Cells**. *Journal of molecular neuroscience : MN*, 10.1007/s12031-022-01968-1. Advance online publication. https://doi.org/10.1007/s12031-022-01968-1

Thomas Broome, S., Fisher, T., Faiz, A., Keay, K. A., Musumeci, G., Al-Badri, G., & Castorina, A. (2021). **Assessing the Anti-Inflammatory Activity of the Anxiolytic Drug Buspirone Using CRISPR-Cas9 Gene Editing in LPS-Stimulated BV-2 Microglial Cells**. *Cells*, *10*(6), 1312. https://doi.org/10.3390/cells10061312

Thomas Broome, S., Louangaphay, K., Keay, K. A., Leggio, G. M., Musumeci, G., & Castorina, A. (2020). **Dopamine: an immune transmitter**. *Neural regeneration research*, *15*(12), 2173–2185. https://doi.org/10.4103/1673-5374.284976

Published conference abstracts:

Thomas Broome, S. and Castorina, A. (2021). **Repurposing the anxiolytic drug buspirone to counteract inflammation in cellular and animal models of Parkinson's disease**. Life Sciences, Medicine, and Biomedicine (ISSN 2600-7207).

Other publications

Jansen, M. I., **Thomas Broome, S.,** & Castorina, A. (2022). **Exploring the Pro-Phagocytic and Anti-Inflammatory Functions of PACAP and VIP in Microglia: Implications for Multiple Sclerosis**. *International Journal of Molecular Sciences*, *23*(9), 4788.

Jansen, M+.; **Thomas Broome, S**+.; Castorina, A. (2021). **Targeting the neurological comorbidities of multiple sclerosis: the beneficial effects of VIP and PACAP neuropeptides**. *J. Integr. Neurosci*
(+ denotes equal contribution first authors)

Karunia, J.; Niaz, A.; Mandwie, M.; **Thomas Broome, S**.; Keay, K.A.; Waschek, J.A.; Al-Badri, G.; Castorina, A. (2021). **PACAP and VIP Modulate LPS-induced Microglial Activation and Trigger Distinct Phenotypic Changes in Murine BV2 Microglial Cells**. *Int. J. Mol. Sci,* 22, 10947. doi: https://doi.org/10.3390/ijms222010947

Thomas Broome, S.; Musumeci, G.; Castorina, A. (2021). **Doxycycline and Minocycline Act as Positive Allosteric Modulators of the PAC1 Receptor and Induce Plasminogen Activators in RT4 Schwann Cells**. *Appl. Sci*. 11, 7673. https://doi.org/10.3390/app11167673

Conference presentations

Invited oral presentations:

Thomas Broome, S. and Castorina, A. Repurposing the anxiolytic drug buspirone to counteract inflammation in cellular and animal models of Parkinson's disease, *University of Sydney, Conjoint Neuroscience Meeting* (2021)

Thomas Broome, S. My career journey and doctoral research, *Students of Brain Research* (2021)

Thomas Broome, S. and Castorina, A. Targeting neuroinflammation in Parkinson's disease, *Australasian Neuroscience Society, Early Career Researcher Webinar Series* (2020)

Oral presentations:

Thomas Broome, S. and Castorina, A. Repurposing the anxiolytic drug buspirone to counteract inflammation in cellular and animal models of Parkinson's disease, *International Society of Neuroimmunology Congress* (2021)

Thomas Broome, S. and Castorina, A. Assessing the anti-inflammatory activity of Buspirone, *Australian Society of Medical Research NSW Scientific Meeting* (2021*), First place*

Thomas Broome, S. and Castorina, A. Aspirin for Parkinson's disease, *Australian Society of Medical Research, Victoria Student Symposium*, Flash talk (2021), *Second place*

Thomas Broome, S. and Castorina, A. Repurposing buspirone for Parkinson's disease, *Australasian Neuroscience Society*, Flask talk (2020), *Finalist*

Thomas Broome, S. Aspirin for Parkinson's disease, University of Technology Sydney, *3-minute-thesis competition* (2020)

Thomas Broome, S. Aspirin for Parkinson's disease, *BioMed Link Conference*, University of Melbourne, Australia, 3-minute-thesis (2019), *First place*

Poster presentations:

Thomas Broome, S. and Castorina, A. *Students of Brain Research Symposium* (2021), *Second place*

Thomas Broome, S. and Castorina, A. Repurposing the anxiolytic drug buspirone to counteract inflammation in cellular and animal models of Parkinson's disease, *Graduate Research Symposium*, Taylor's University, Malaysia (2021)

Thomas Broome, S. and Castorina, A. Targeting neuroinflammation in Parkinson's disease, *European Molecular Biology Laboratory (EMBL) PhD Symposium* (2020)

Thomas Broome, S. and Castorina, A. Targeting neuroinflammation in Parkinson's disease, *BioMed Link Conference*, University of Melbourne (2019)

Awards and Scholarships

Paper of the Month (Jan-Feb 2022)

Thomas Broome, S., & Castorina, A. (2022). **The Anxiolytic Drug Buspirone Prevents Rotenone-Induced Toxicity in a Mouse Model of Parkinson's Disease**. *International journal of molecular sciences*, *23*(3), 1845. https://doi.org/10.3390/ijms23031845

School of Life Sciences, University of Technology Sydney

Vice Chancellor's Conference Award (2021)

Travel fund to attend the 15th International Society of Neuroimmunology Congress, November 2021.

Second place poster (2021)

Students of Brain Research (SOBR) Symposium.

First place PhD Student Presentation (2021)

Australian Society of Medical Research (ASMR) NSW Annual Scientific Meeting.

Second place flash talk (2021)

Australian Society of Medical Research (ASMR) Victoria Student Symposium.

First Place Three-minute-thesis (2019)

BioMed Link Conference, University of Melbourne.

BioMed Link Conference Travel Award, Melbourne, Australia (2019)

Travel fund to attend the BioMed Link Conference in Melbourne.

UTS Research Excellence PhD Scholarship (July 2018 – December 2021)

Financial support during my PhD candidature.

Professional experience

Manuscript Peer Reviewer (Since 2021)

Neuroscience (IF 3.59), Frontiers in Molecular Neuroscience (IF 5.693), Scientific Reports (IF 4.379)

Deputy Convener Gala Dinner (2021) and Convener Annual Scientific Meeting (2022)

Australian Society of Medical Research, NSW Committee

NSW Representative (**2021-2022)**

Australasian Neuroscience Society, Student Committee

#ThisIsMyField Team member (2021)

Pint of Science Australia

Organising Committee and Translational Research Session Chair (2020)

EMBL Australia Postgraduate Symposium

EMBL Australia PhD Course, Hobart (2019)

European Molecular Biology Laboratory (EMBL) Australia

SOUL Award (2019-2021)

University of Technology Sydney

Parkinson's NSW Collaboration with UTS Business School (2019-2020)

Team Leader, Parkinson's NSW and University of Technology Sydney

Accomplish Award (2020)

University of Technology Sydney

Vice-President and Volunteers and Engagement Coordinator (2020)

batyr @ University of Technology Sydney

Postgraduate Student Representative, Research Committee (2019-2020)
University of Technology Sydney

Mentor (Since 2019)
Women's College, The University of Sydney, Australia

PROFESSIONAL MEMBERSHIPS

Students of Brain Research	From 2021
Australian Society of Medical Research	From 2020
Australasian Neuroscience Society	From 2020

Abstract

Parkinson's disease (PD) is a chronic neurodegenerative disease characterised by the progressive loss of midbrain dopaminergic neurons. No one knows how or why dopamine neurons are lost but several studies confirm the presence of neuroinflammation as a critical pathological component of the disease. As such, disease-modifying strategies that can reduce/halt neuroinflammation are likely to ameliorate PD progression and reduce severity.

Considerable evidence suggests that blockade of the dopamine D3 receptor (D3R) is neuroprotective and reduces inflammation in animal models of PD. However, to date there are no selective D3R antagonists in the market. Recently, computational analyses have demonstrated that buspirone, an FDA-approved anxiolytic drug with serotonin 1A (Htr1a) agonist activity, also functions as a potent D3R antagonist. Therefore, we aimed to test in cellular and animal models of PD if buspirone's D3R antagonism exerts anti-inflammatory and, therefore, neuroprotective activities.

In vitro, CRISPR-Cas9 gene deletion of the D3R and/or buspirone treatment in BV2 microglial cells attenuated microglial polarisation after LPS challenge. To determine if this result translated *in vivo,* we generated a rotenone mouse model of PD. Systemic administration of rotenone replicated several pathogenic and behavioural features of PD, including significant locomotor and exploratory impairments, dopaminergic degeneration, widespread alterations of mitochondrial function, increased oxidative stress, glial activation and heightened expression of inflammatory mediators in the midbrain and several extra-nigral CNS sites.

Accordingly, we used this model to assess the ability of buspirone to mitigate rotenone-induced toxicity. Buspirone prevented rotenone-induced behavioural deficits and mitigated dopaminergic degeneration. Drug treatment also prevented astrocyte and microglial activation, which was paralleled by a global downregulation of pro-inflammatory markers and the upregulation of anti-inflammatory markers and neurotrophic factors in several brain regions.

Interestingly, throughout our studies we also report disruptions in the expression levels of the neuroprotective and immune modulatory peptides pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) following rotenone intoxication, which were rescued by buspirone. This prompted us to test whether these neuropeptides elicited anti-inflammatory activities against rotenone toxicity in BV2 microglia. Both peptides reliably suppressed microglial activation, suggesting an indirect involvement in the anti-inflammatory machinery triggered by buspirone.

In conclusion, our findings indicate that buspirone mitigates rotenone-induced neurotoxicity and inflammation via the activation of multiple protective and anti-inflammatory pathways, perhaps including PACAP and VIP neuropeptides. Altogether, these findings support the notion that targeting the D3R, PACAP or VIP may be a promising therapeutic strategy to reduce inflammation in PD.