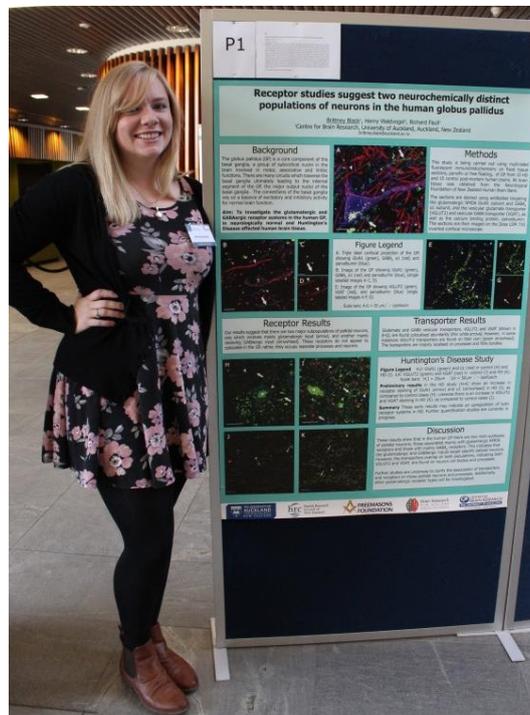


UNIVERSITY OF AUCKLAND

Characterising Brain Cells in the Human Globus Pallidus in Huntington's and Parkinson's Disease



Freemasons Foundation
RESEARCH IMPACT REPORT

September 2016



Thank You

We would like to thank the Freemasons Foundation for their continued support of the Centre for Brain Research and for funding Brittney Black to carry on her PhD research into the human brain. The impact of your generosity allows us to explore the mysteries of the human brain and try to understand how it is affected in devastating neurodegenerative diseases: Huntington's and Parkinson's. On behalf of the Centre for Brain Research and the University of Auckland, thank you for all your incredible support.

Financial Input

In April 2015, the Freemasons Foundation activated a doctoral scholarship for Brittney Black with the Centre for Brain Research. The generous \$126,000 award will be paid as doctoral fees and a \$25,000 stipend over the entire four-year project, allowing our doctoral candidate to delve into this exciting frontier of research.

Summary of Outcomes and Impact

Brittney's research on the post-mortem human brain has revealed novel subpopulations of neurons in the globus pallidus: one population that receives mostly excitatory inputs and one that receives primarily inhibitory inputs. Please see full explanation below.

This exciting new finding could reinvent the way we think about the basal ganglia circuitry of the human brain. The basal ganglia are situated at the base of the forebrain and are associated with a variety of functions including control of voluntary motor movements, procedural learning, routine behaviours or "habits", eye movements, cognition and emotion. Better knowledge of these pathways are crucial for understanding what goes wrong in diseases, such as Huntington's and Parkinson's, and how we might improve treatments or find preventions.

Over the winter Brittney has been able to package her preliminary findings into a scientific poster and presented it at four different conferences in the USA and New Zealand.

Research Activity and Outcomes

The region of interest for Brittney's doctoral research project is a part of the human brain called the globus pallidus. It is a structure, deep within the brain, that is involved in the regulation of voluntary movement. If the globus pallidus is damaged, it can result in movement disorders, as its regulatory function will be impaired. A past doctoral student has already quantified cell loss in Huntington's Disease in the globus pallidus, but Brittney's work is to focus on neurochemical characterisation of neurons (specialised cells within the brain) in the region. Neurochemistry is broadly a term meaning all the neurotransmitters (brain chemicals that communicate information between neurons and our body), neuropeptides (signalling molecules used by neurons), and other molecules that influence the function of a neuron. Therefore, this kind of characterisation puts together many of the molecules that neurons use to talk to each other to get a fuller picture of how these neurons actually work and/or malfunction.

These qualitative studies on neurons of the globus pallidus are currently underway, via immunohistochemistry experiments. Immunohistochemistry is the process of detecting antigens (proteins), in the tissue section by utilizing antibodies and their binding capability to specific targets. In the past few months, Brittney has got the glutamate receptor antibodies to continually work on the human tissue samples. Glutamate receptors are proteins on the surface of neurons that receive chemical messages, specifically via glutamate neurotransmitters, sent from neighbouring neurons. Glutamate is highly abundant in the human body, but it is also the brain's main excitatory neurotransmitter, which means it activates/excites the brain's neurons causing them to signal and transmit messages. It is also the precursor for GABA, the brain's main inhibitory neurotransmitter, which will also be explored in this PhD. Inhibitory neurotransmitters are responsible for balancing the excitation, meaning they "depress" the neurons, preventing them from firing. Neurotransmission (the overall communication of the neurons) relies on a balance of excitation and inhibition. Glutamate receptors are important for neural communication, memory formation and learning. They are also implicated in many neurological disorders and neurodegenerative diseases.

Now extensive characterisation of these glutamate receptors is on-going. Images of this can be seen below.

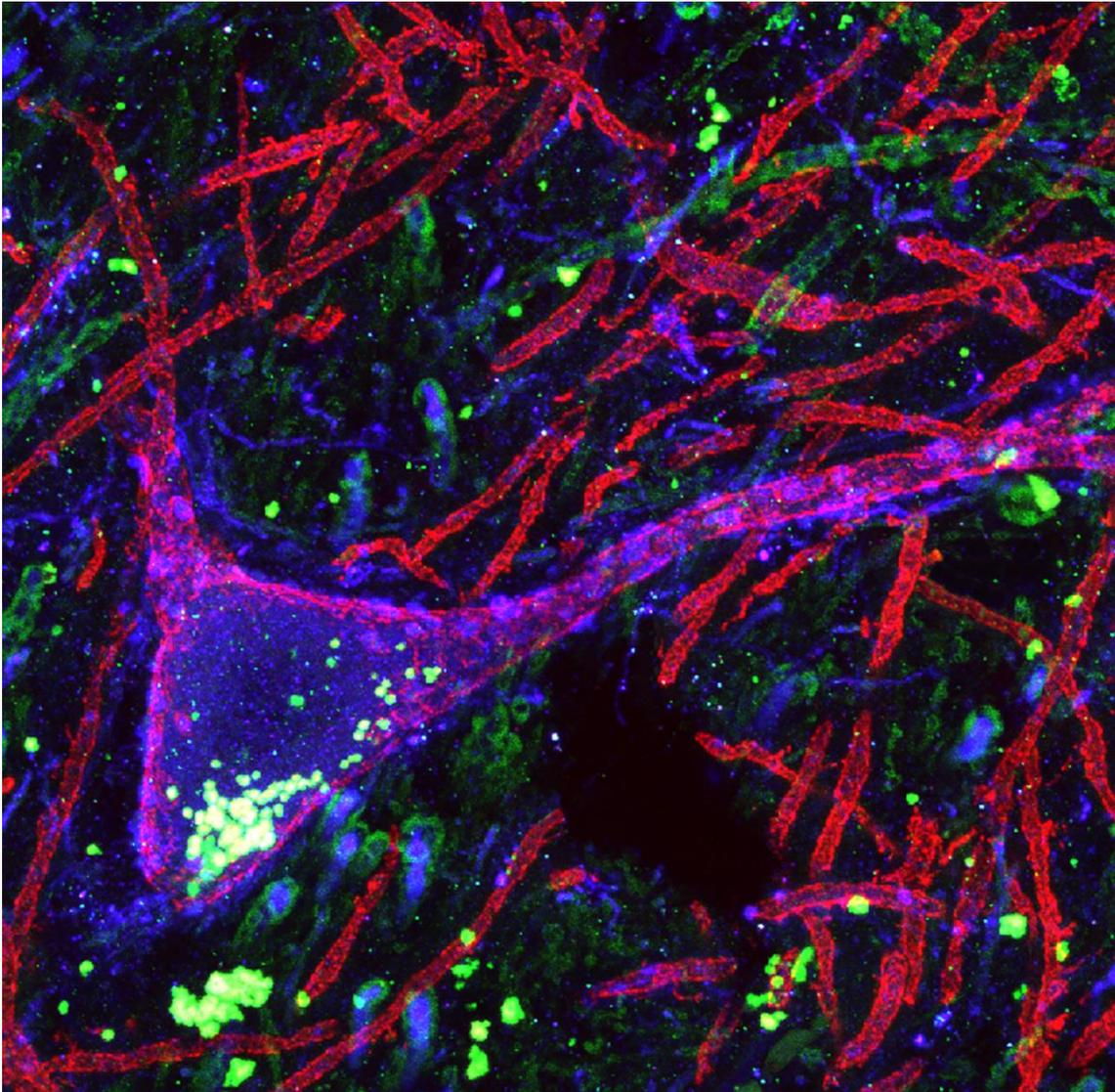


Figure 1 Fluorescent immunohistochemistry image showing a 3D stack of neurons and process in the human globus pallidus. **Green** – Glutamate NMDA1 Receptor. **Red** – GABA_A α 1 Receptor. **Blue** – Parvalbumin (calcium binding protein, which acts as a neuron marker)

What is amazing about these findings is that the excitatory Glutamate (**green**) receptor subunits are found on completely separate neurons and processes to the inhibitory GABA (**red**) receptor subunits used in this study. If they were found in the same location, the **red** and **green** signal would show up as **yellow**, however this does not happen.

This divide reveals subpopulations of neurons in the globus pallidus: one population that receives mostly excitatory inputs and one that receives primarily inhibitory inputs. This exciting new finding could reinvent the way we think about the basal ganglia circuitry of the human brain.

Research Outputs

This year Brittney has been able to take her research on the road, presenting it as a poster to scientific audiences at four conferences. She attended the Hereditary Disease Foundation's Huntington Disease conference in Boston, Massachusetts. This was a very niche specific conference, allowing clinicians and researchers to hear about the latest findings and trials for the disease. While in Boston, Brittney was also able to meet up with Professor Ann Graybiel, Department of Brain and Cognitive Sciences at MIT and go for a lab visit there and to talk about research. Following the conference, Brittney was also fortunate enough to visit the New York Brain Bank in Columbia, NY and meet with the director and pathologist Professor Jean Paul Vonsattel. Vonsattel is well known in the HD field, as he came up with the pathological grading system of Huntington's Disease.

The second conference was the Association of Pacific Rim Universities' Brain and Mind Research conference hosted at the Centre for Brain Research. This conference focused mainly on olfaction and brain plasticity, but also general brain research as well.

Next was the Australasian Winter Conference on Brain Research in Queenstown, New Zealand. The AWCBR meeting is a key event for Australasian researchers to collaborate and learn about the current neuroscience research going on in our own country.

Finally Brittney attended, and organised, the student-led conference HealthX hosted at the Faculty of Medical and Health Sciences of the University of Auckland. It is a forum where students can get together and present their research, in order to network and engage with fellow scientists, promote research and scientific thinking and improve their communication skills. From this, Brittney's poster has been one of those selected to represent the Faculty at the university-wide postgraduate competition, Exposure.

An image of the poster is shown on the next page.

Receptor studies suggest two neurochemically distinct populations of neurons in the human globus pallidus

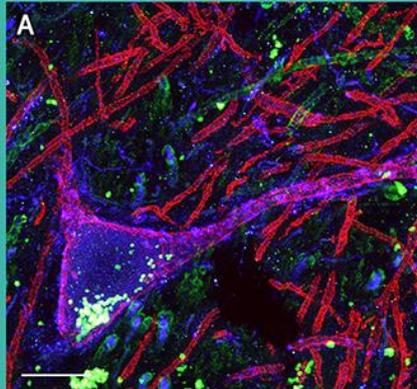
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Background

The globus pallidus (GP) is a core component of the basal ganglia, a group of subcortical nuclei in the brain involved in motor, associative and limbic functions. There are many circuits which traverse the basal ganglia ultimately leading to the internal segment of the GP, the major output nuclei of the basal ganglia. The connections of the basal ganglia rely on a balance of excitatory and inhibitory activity for normal brain function.

Aim: To investigate the glutamatergic and GABAergic receptor systems in the human globus pallidus, in neurologically normal and Huntington's Disease affected human brain



Methods

This study is being carried out using multi-label fluorescent immunohistochemistry on fixed tissue sections, paraffin or free floating, of GP from 10 HD and 10 control post-mortem human brains. All brain tissue was obtained from the Neurological Foundation of New Zealand Human Brain Bank.

The sections are stained using antibodies targeting the glutamatergic NMDA GluN1 subunit and GABA_A α1 subunit, and the vesicular glutamate transporter (VGLUT2) and vesicular GABA transporter (VGAT), as well as the calcium binding protein, parvalbumin. The sections are then imaged on the Zeiss LSM 710 inverted confocal microscope.

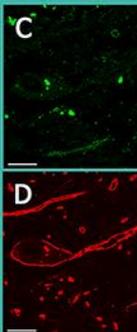
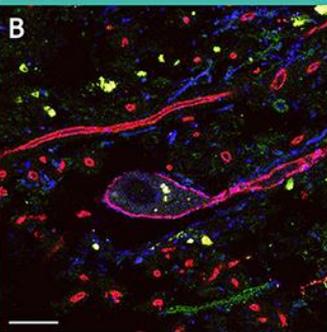


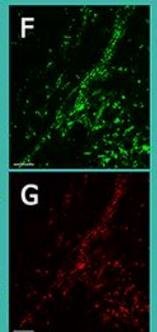
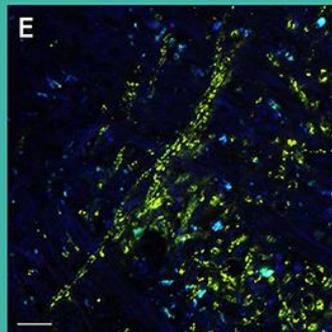
Figure Legend

A: Triple label confocal projection of the GPI showing GluN1 (green), GABA_A α1 (red) and parvalbumin (blue).

B: Image of the GP showing GluN1 (green), GABA_A α1 (red) and parvalbumin (blue), (single labeled images in C, D).

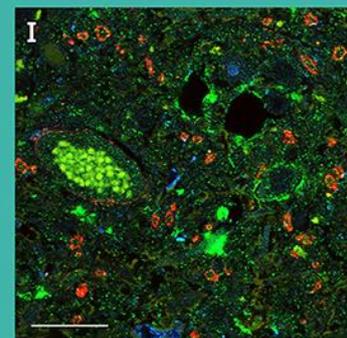
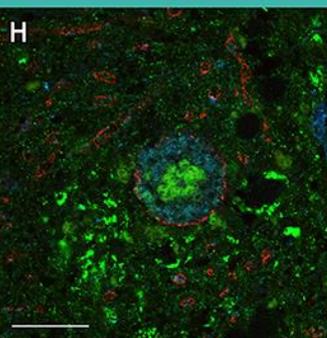
E: Image of the GP showing VGLUT2 (green), VGAT (red), and parvalbumin (blue) (single labeled images in F, G).

Scale bars: A-G = 20 μm; * - Lipofuscin



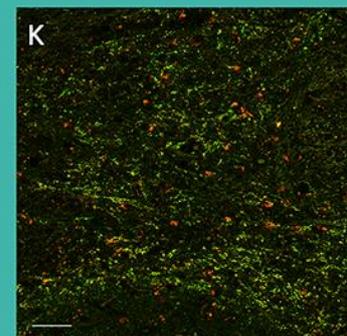
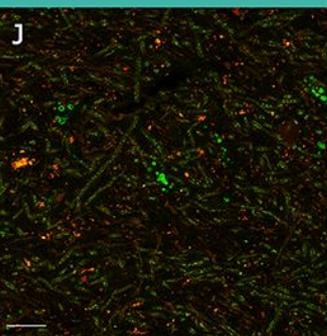
Receptor Results

Our results suggest that there are two major subpopulations of pallidal neurons, one which receives mainly glutamatergic input (arrow), and another mainly receiving GABAergic input (arrowhead). These receptors do not appear to colocalise in the GP.



Transporter Results

The respective transporters, VGLUT2 and VGAT (shown in E, F, G) appear to colocalise abundantly, although some VGLUT2-positive transporters do not colocalise.



Huntington's Disease Study

Figure Legend H,I: GluN1 (green) and α1 (red) in control (H) and HD (I). J,K: VGLUT2 (green) and VGAT (red) in control (J) and HD (K).
Scale bars: H,I = 20μm J,K = 50μm * - lipofuscin

Preliminary results in the HD study (H-K) show an increase in receptor staining of GluN1 (arrow) and α1 (arrowhead) in HD (I), as compared to control cases (H). Likewise there is an increase in VGLUT2 and VGAT staining in HD (K), as compared to control cases (J).

Summary These early results may indicate an upregulation of both receptor systems in HD. Further quantifications studies are currently in progress.

Discussion

These results show that in the human GP there are two main subtypes of pallidal neurons; those associated mainly with glutamatergic NMDA receptors and those with mainly GABA_A receptors. This indicates that the glutamatergic and GABAergic inputs target specific pallidal neurons. However, the transporters overlap on both populations, indicating both VGLUT2 and VGAT, are found on neuron cell bodies and processes.

Further studies are underway to clarify the association of transporters and receptors on these pallidal neurons and processes.

Future Impact

At the Centre for Brain Research, we have the very unique opportunity to study human brain tissue, generously bequeathed from patients after death to identify the pattern of disease progression throughout the brain. Furthermore, the control cases (brains from pathologically normal individuals) allow us to continue unravelling the mysteries of the brain itself!

Remarkably, Brittney's preliminary studies suggest that there are distinct neuronal populations in the globus pallidus, one that receives mainly glutamatergic input and another that receives mainly GABAergic input. Identifying these populations allow us to further understand function, and the wider circuitry of the brain, which is basically a map of how neurons of different brain regions link up with one another. Knowing this information will also allow us to see what is different in diseased states, whether it is a potential cause or effect of the disease.

Additionally, quantification studies are in progress that will let us know whether the glutamatergic receptors are in fact unregulated in the globus pallidus of Huntington's Disease. Therefore, these glutamatergic systems could be new pharmacological targets for treatments of motor dysfunctions in HD.

We never know when we will stumble upon the puzzle piece which could be key for preventing or even slowing the progression of these neurodegenerative diseases, and this is why our research on the human brain is so critical. We could not do this research without continued support to the Centre for Brain Research from generous donors, namely the Freemasons Foundation.

We look forward to providing a progress report in March 2017.

FOR MORE INFORMATION PLEASE CONTACT:

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