Vulnerability of interneuron populations in the prefrontal cortex of a mouse model of Lewy Body Dementia

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Aims

- Investigate specific population of inhibitory interneurons in the prefrontal cortex
- Compare the population of these interneurons in mutant and control mice in specific areas of the prefrontal cortex

Introduction

Lewy Body Dementia (LBD) is a common form of neurodegeneration which is believed to arise from mutations in a protein called α-synuclein. Previous experiments has shown that a type of nerve cell called interneurons , crucial in cognitive function are believed to have a failure in transmitting inhibitory signals in the diseased state. This suggests that there might be a change in interneuron population in cognitive areas of the brain with LBD.

It is also known that the prefrontal cortex plays a crucial role in cognition, the focus area of this project. Therefore, study of inhibitory interneurons in the prefrontal cortex will deepen our understanding to the changes if any occurred in patients with Lewy Body Dementia.

The effects of mutant α-synuclein can be studied in genetically engineered mice that over-expresses the mutant protein in its brain. Prefrontal cortices of the mutant mice and control mice were obtained and stained using immunofluorescence histochemistry. Three different types of interneuron expressing three different protein (calretinin, calbindin & antiparvalbumin) were identified with specific antibodies. A fluorescent marker was used for tagging perineuronal nets, specialized extracellular structures that are found around certain interneurons.

Method

Sections of frontal cortex were made from mouse brain 40µm thick using a freezing microtome.

Next we used immunohistochemistry (IHC) a common tissue staining technique to stain the brain cells. The sections were incubated with primary antibody which binds a specific antigen (parvalbumin, calbindin or calretinin, see figure 1) and a biotinylated lectin which binds perineuronal nets. The addition of green fluorescent secondary antibodies that bind to the primary antibody, along with a red fluorescent tag that binds to the lectin, allowed interneurons and perineuronal nets to visualized under the fluorescence microscope.

Images of stained PFC sections were captured under a fluorescence microscope . The number of each type of interneuron (parvalbumin, calbindin or calretinin expressing with or without perineuronal nets) were observed in specific functional areas of the prefrontal cortex, and any differences in the cell populations in mutant sections compared to control sections were looked for

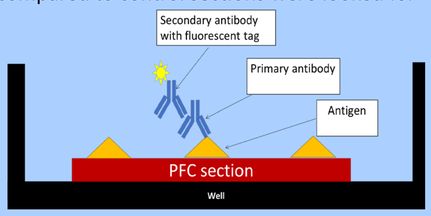

Figure 1 shows the diagrammatic representation of IHC. Image was adapted from https;//en.wikipedia.org/wiki/ Immunolabelling made by Jakodak .

Acknowledgements

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Results

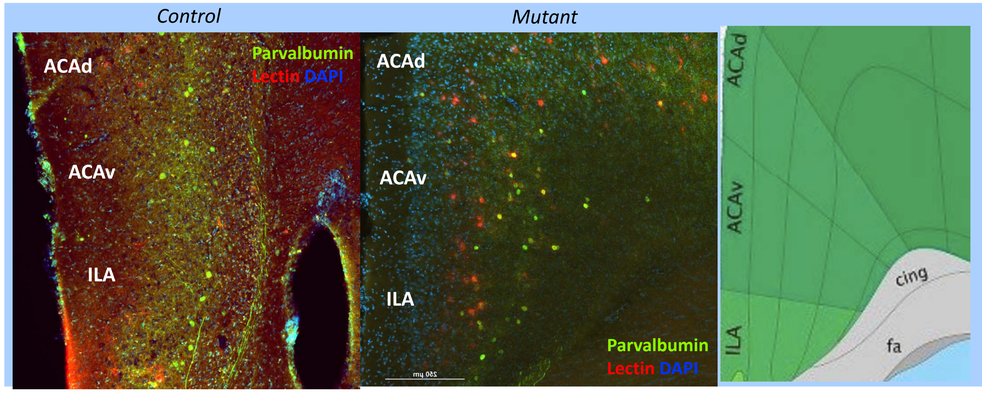

Figure 2: Images on the left show control and mutant PFC slices. Image on the right shows the area of our research from Allen Brain Atlas. DAPI is a background stain. There are no significant differences in the population of parvalbumin expressing cells. However, parvalbumin cells express more in the deep layer of the infralimbic are (ILA) and anterior cingulate (ACAd/ACAv). Lectin stains for perineuronal nets which are surprisingly not always associated with parvalbumin expressing cells.

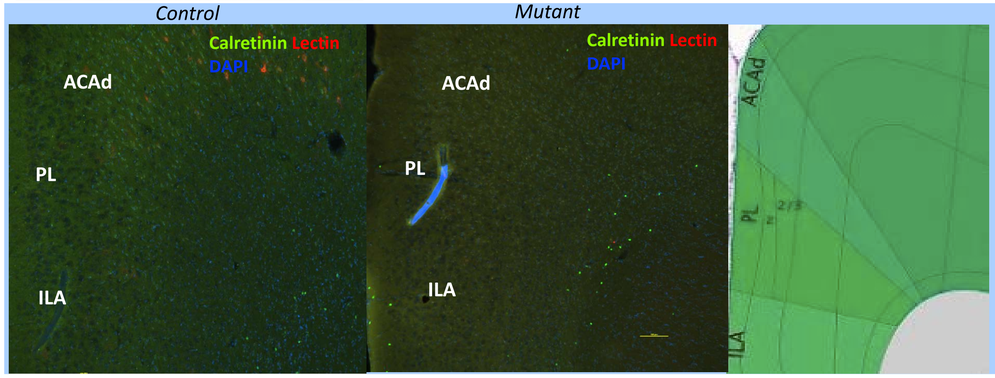

Figure 3: Images on the left show control and mutant PFC slices. Image on the right shows the area of our research from Allen Brain Atlas. DAPI is a background stain.
There are no significant differences in the population of calretinin binding cells. However, a few calretinin cells express in layer 6 of the infralimbic area (ILA), prelimbic area (PL) and anterior cingulate (ACAd). Calretinin cells are scarcely present in the superficial layers.

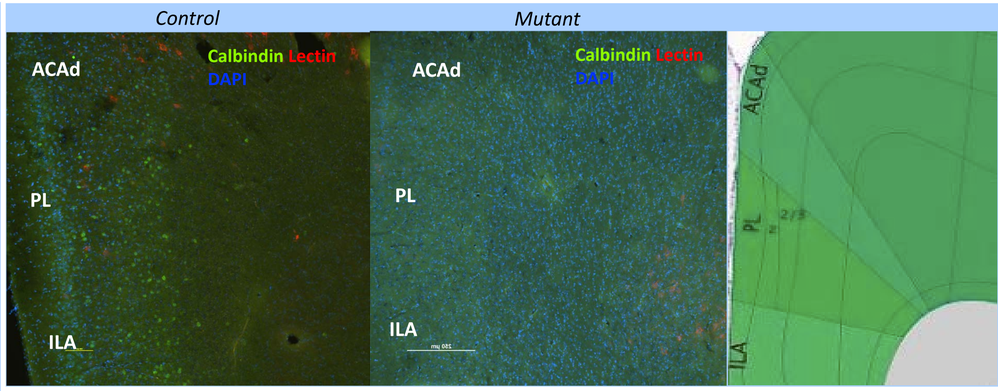

Figure 4: Images on the left show control and mutant PFC slices. Image on the right shows the area of our research from Allen Brain Atlas. DAPI is a background stain.
There are no significant differences in the population of calbindin binding cells. However, it was observed that the number of calbindin cells decrease towards the posterior of the PFC. The control image shows a section from the anterior . The mutant image shows a section from the posterior.

Conclusion

Our observations show that there is no significant difference in the interneuron populations in mutant and control mice in the cognitive areas of the PFC. However, we found that parvalbumin expressing interneurons which normally have perineuronal nets around them show otherwise. Many perineuronal nets are not associated with the types of cells that we have investigated. Calretinin cells tend to outline in the deep layers of the motor and cognitive areas. It was observed that calbindin cells appear more in the anterior of PFC than posterior.