Hippocampal Expression of the RNA binding proteins Hu C/D:

a stereological analysis in demented patients' post mortem brain tissue

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Introduction

Hu C/D is a RNA-binding protein and has been associated with neuronal development and maintenance. *Hu* binds to target mRNA and increases its stability. This increases the quantity of protein transcribed.

Hu C/D is exclusively expressed within neurons. In the hippocampus, an area of the brain located in the temporal lobe and associated with memory, initial analysis has shown that there is a significant reduction in *Hu* expression in a specific region, Cornu Ammonis (CA) 1 in patients who had been clinically diagnosed with dementia.

This study examined the hippocampal expression of *Hu C/D* in post-mortem tissue using a stereological technique, comparing the CA1 and CA2 regions. We investigated the differences between groups of controls, Vascular dementia (VaD), Alzheimer's Disease (AD) and a cohort of post stroke survivors (CogFAST): Post Stroke Non Demented (PSND) and Post Stroke Demented (PSD).

We hypothesis that there will be a significant reduction in the number of *Hu* positive cells in the CA1 region in cases of dementia compared to controls and PSD.

Methods

1. 40 cases were selected for analysis, 8 cases per group. Paraffin wax embedded sections were cut into fifteen serial 30 µm thick sections.
2. Three sections from each case were stained using standard immunohistochemical technique with an anti *Hu C/D* antibody and counterstained with haemotoxylin *(Figure 1)*. Slides were selected so that every 5th slide in the series was used allowing for correcting sampling of the tissue.
3. Using Stereologer 2000 software, the area of interest was mapped out at low magnification. Within this area a grid frame was placed to sample approximately 40 frames in the CA1 and 25 frames in CA2 allowing for uniform random sampling.
4. Neuronal cells were counted at x40 magnification using a dissector probe. Cell density was selected as a parameter because the total hippocampus tissue could not be sampled as tissue blocks are required for diagnostic purposes.

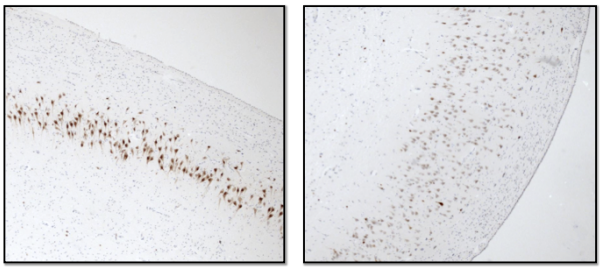

Figure 1: Hu C/D Staining in CA2 (A) and CA1 (B). Magnification x5

Results

Significant differences between the density of Hu C/D stained cells in CA1 and CA2 subfield in each condition (*Figure 2*).

However there was no significant difference between any condition in either region (*Figures 3A and B*). Although in the CA1 region, the VaD group appears to show this group has a greatly reduced density of Hu C/D positive cells compared to other groups.

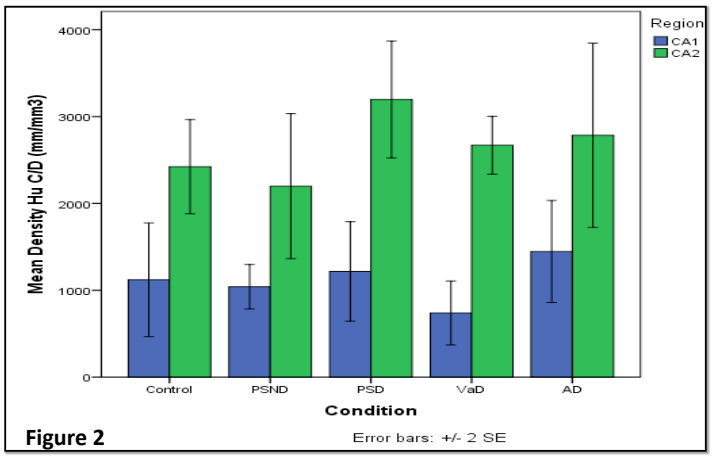

Figure 2

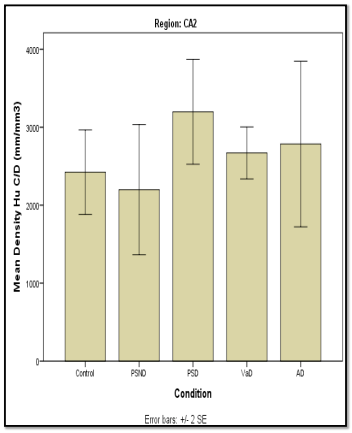

Figure 3A

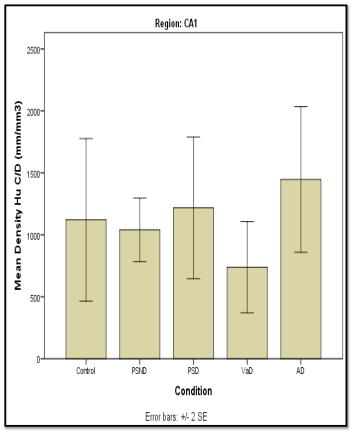

Figure 3B

Discussion

Significant decreases in *Hu* stained neurons in the CA1 compared to the CA2 in all conditions. Suggests significant regional and cellular differences of this protein within the hippocampus. Contrary to the hypothesis there was no reduction in density of Hu stained cells in clinically demented cases in the CA1 compared to controls or PSND. Previous studies have shown that the CA1 is specifically vulnerable to damage caused by ischemia and Alzheimer type pathology (Amyloid plaques and hyperphosphorylated tau). *Hu* has been suggested to be involved in cellular maintenance and maybe used as a possible marker for vulnerable cells. **However, this data would suggest that CA1 subfields in all these conditions are affected to some degree.**

Issues with this study could be:

- Number of cases studied (n=8) in each condition, however time did not allow for further analysis.
- Effect is not disease specific, seen by large spread of data in each group
- Differential staining seen at high magnification, therefore some cases may have weaker stained cells than others which is difficult to analyse.
- The analysis technique is operator subjective and future work may be to analyze total protein concentration via Western Blot.

Future work would be to analyse the expression of *Hu* in younger cases to identify if loss of *Hu* expression in CA1 is age specific.

References
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