The Origins of Inhibitory Interneurons in the Human Cerebral Cortex

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Introduction

- Inhibitory Interneurons are a type of neuron that control the excitability of neural networks by sending chemical messages with a neurotransmitter called GABA. In the cerebral cortex they play a vital role in controlling our thought processes . Through the study of interneurons during brain development, and the differences between humans and mice, we can gain a greater understanding of brain evolution and diseases such as epilepsy, autism and schizophrenia.
- In mice, GABA interneurons migrate from an area of the brain called the ganglionic eminence (GE), to the outer layer of the brain called the cerebral cortex during brain development. However, in humans it is still up for debate as to whether they are made in the GE, the cortex or both.
- Stem cells from the GE or cortex of human foetal brains (provided with the appropriate ethical permission www.hdbr.org) were grown to differentiate into neurons. We then tagged them with fluorescent markers using immunocytochemistry to see whether they were interneurons. The fluorescent markers (antibodies) bind to specific proteins on the surface of these cells called antigens. In my project I used GABA antibodies, which will bind to the cells if GABA is present (as well as others such as B-tubulin, calretinin and COUPTFII).

Method

- We prepared cell samples from three different regions of human fetal brain (GE, anterior cortex, and posterior cortex) at 8-12 Post Conceptional Weeks. These neural stem cells were isolated and expanded using the neurosphere method, then plated in differentiation media for 8 days to grow into neurons.
- Next we used a common laboratory technique called Immunocytochemistry (ICC) that is used to visualize the localization of a specific protein or antigen in cells. This uses a specific primary antibody that binds to it. The primary antibody allows visualization of the protein under a fluorescence microscope when it is bound by a secondary antibody that has a fluorescent capability (seen in Figure 1). ICC allows us to evaluate whether or not these cells express the antigen in question.
- Cells were labelled for b-tubulin (a marker for all post-mitotic neurons) which fluoresced green and either GABA (interneuron neurotransmitter) calretinin (a marker for some interneurons) or COUP-TFII (another marker for some interneurons) all of which fluoresced red.
- Once visualized, we counted double labelled cells (orange/yellow) for the antibody markers to see what percentage of interneurons were present in each of the samples.

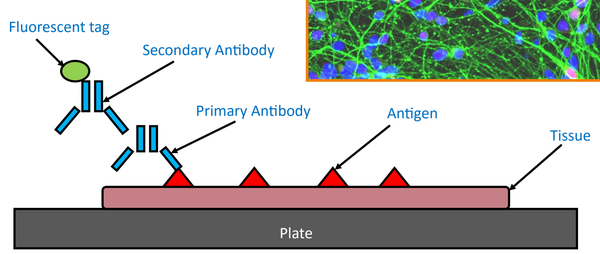


Figure 1: Immunolabeling used in ICC
This image was made based on the image from https://en.wikipedia.org/wiki/Immunolabeling made by Jakodak - Created using the open source Dia software Previously published: No prior publication.

Aims

- **See if GABA interneurons are made in the cortex of human developing brains.**
- **Differentiate between the anterior (front) and posterior (back) parts of the cortex producing these neurones if they're present.**

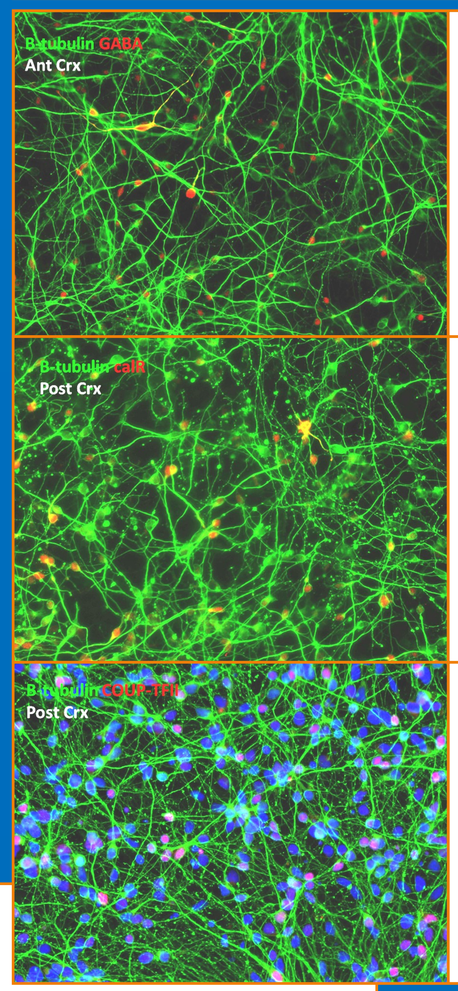
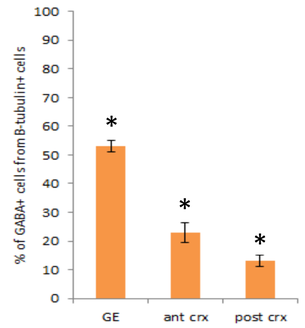


Figure 2:
The green cells show the presence of B-tubulin, a marker showing all post mitotic neurones present. Those in red show GABA, the interneuron transmitter. Cells that appear orange/yellow show which post mitotic cells are interneurons.

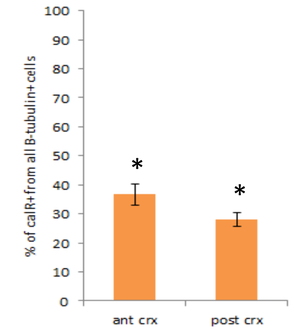
Figure 3:
The green cells again how the presence of B-tubulin. Those in red show calretinin (calR), a marker for calcium binding protein that identifies many Interneurons.

Figure 4: The blue cells represent the total cell count in the sample. Those in red show COUP-TFII, the transcription factor for interneurons derived from the GE in mouse. This shows that in humans, some of these interneurons are born in the cortex.

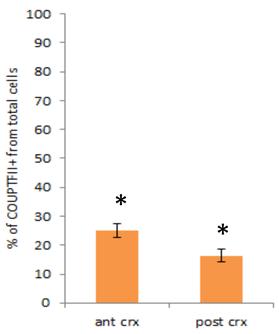
Results



- **Figure 5:** As expected, the highest percentage of b-tubulin cells expressing GABA was found in cultures derived from the GE (53 %) the source of GABA interneurons in mice. However, GABA positive neurons were found in the cortex, 23±4% of b-tubulin+ cells expressed GABA in cultures from the anterior cortex, significantly higher than cultures from the posterior cortex (13±2%).



- **Figure 6:** A significantly higher number of calretinin+ neurons were observed in culture from the anterior cortex than from the posterior cortex; we calculated 37±4% and 28±2% of b-tubulin+ cells expressed calretinin in culture from the anterior and posterior regions, respectively.



- **Figure 7:** We next proceeded to test the immunoreactivity for COUP-TFII. When comparing anteriorly and posteriorly derived cortical cultures, COUP-TFII was expressed in 25±2% and 16±2% of the total number of cells, respectively.

*: Shows results were statistically significant $p<0.05$

Conclusion

- All these findings provide additional evidence that the human cortex is capable of making its own interneurons. In addition, our findings suggest that the anterior cortex might be considered as the favoured region for making these interneurons. The anterior cortex develops into the prefrontal cortex which is most highly evolved part of the brain crucial to higher cognitive processes.

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