

Examining the Effects of Serotonin on Medium Spiny Neurons (MSNs)

By: Aishwarya Belhe, Daniel Tam, Lorene Lanier

Background

- Serotonin is a key hormone for our feelings of happiness and is also associated with depression.
- Pregnant woman with chronic depression are prescribed selective serotonin reuptake inhibitors (SSRIs).
- MSNs are the predominant constituent neurons of the striatum. In rodents, MSNs constitute about 95% of all striatal neurons and have thousands of synaptic contacts proceeding from neurons distributed across several cortical areas.
- The effects of SSRIs on the developing embryo, especially on its developing Medium Spiny Neurons (MSNs), are under examined.
- This knowledge might better inform us about the safety regarding the consumption of SSRIs by pregnant women.

Hypothesis: Serotonin will affect the development of MSNs.

Methods

1. Dissect embryonic mouse brain (Fig. 1)
2. Allow MSNs to grow 19 days in-vitro
3. Fix
4. Prepare coverslips by incubating overnight with immunofluorescent primary antibody DARPP-32, a protein expressed in MSNs
5. Visualize with epifluorescent microscope at 20x objective
6. Quantify dendritic arborization using Sholl Analysis

Figure 1: Schematic representation of the preparation of cell cultures

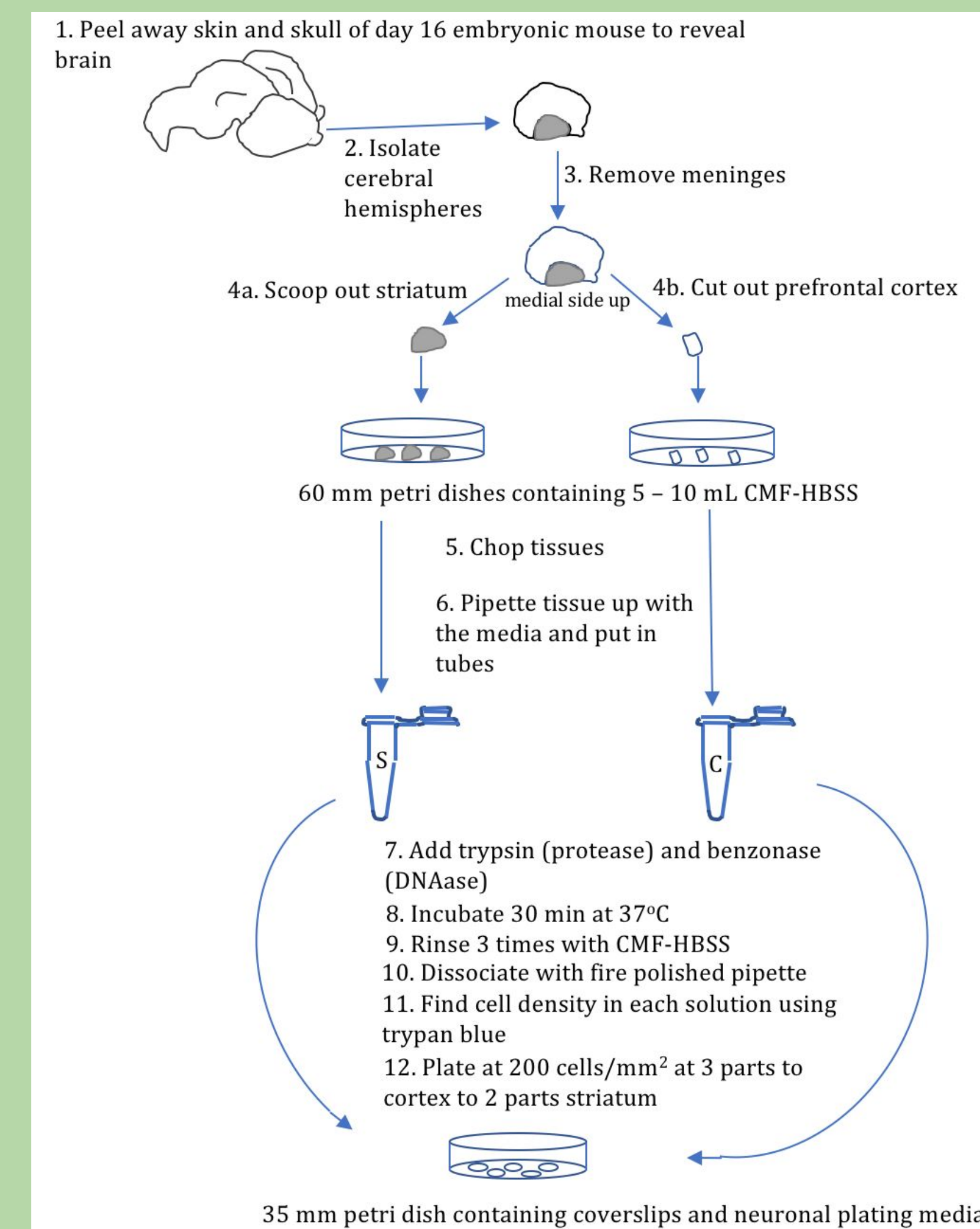
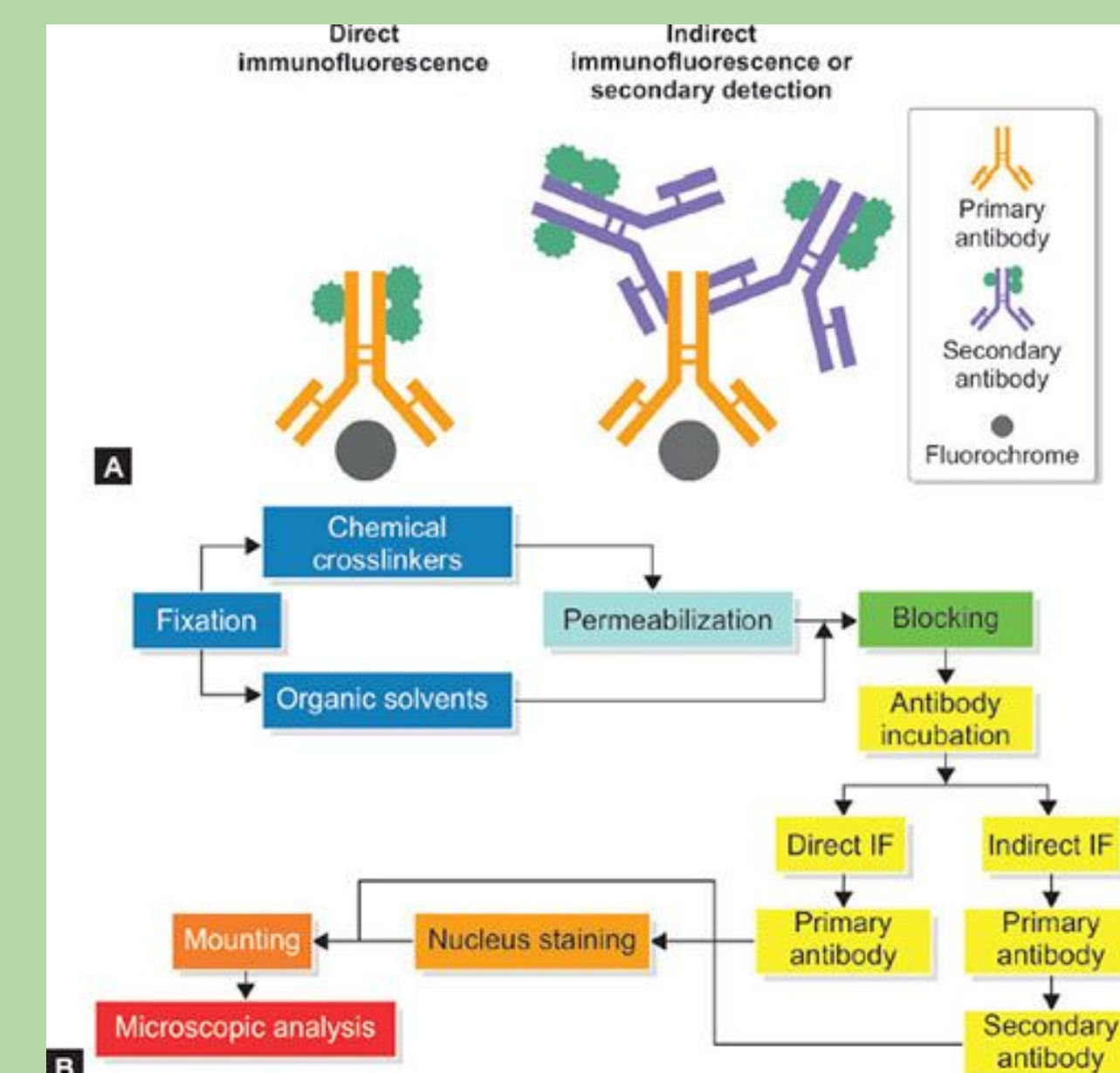
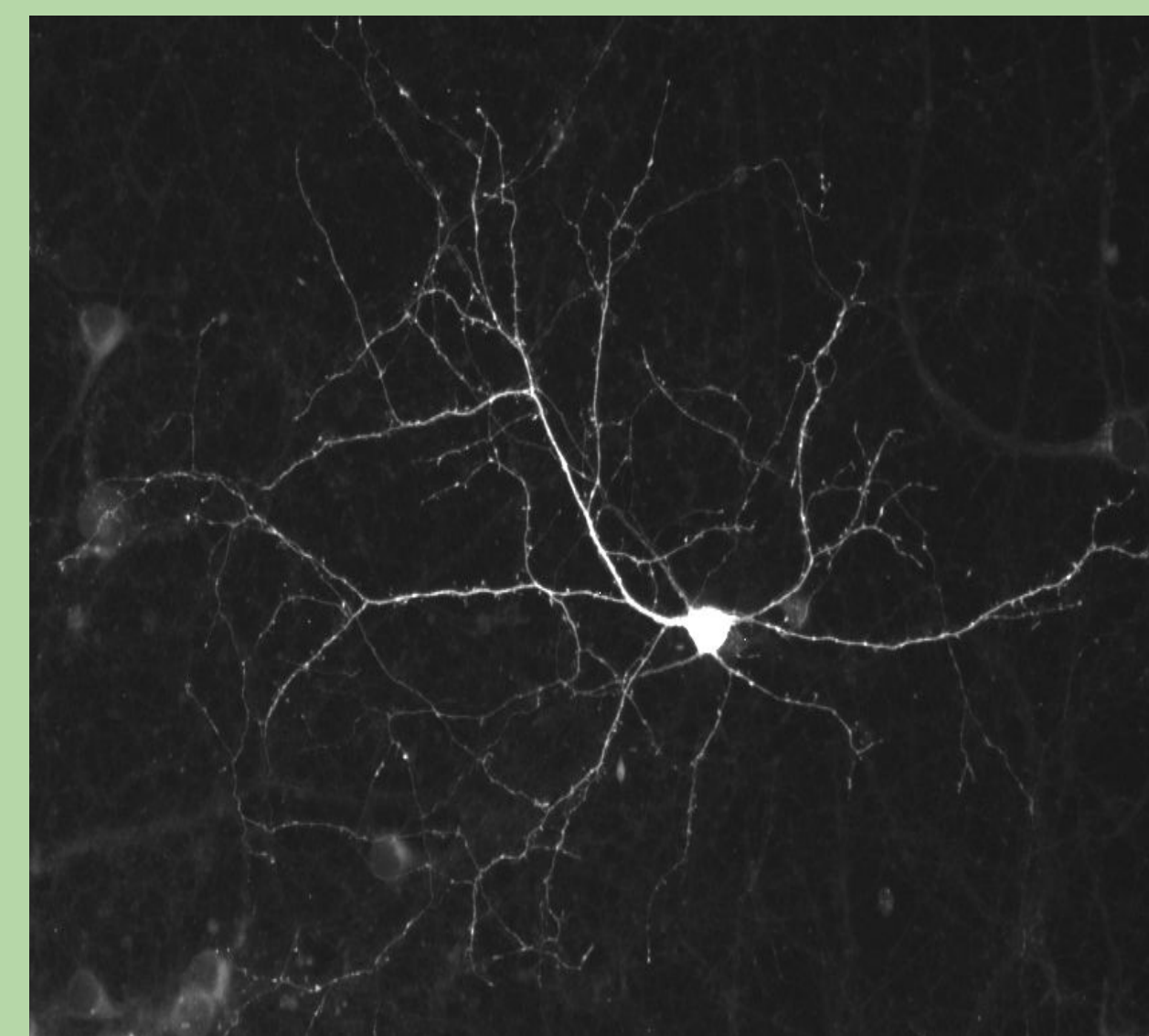


Figure 2: The process of immunofluorescence



The steps followed in order to prepare the neuronal cell cultures are shown in Figure 1. Four treatment groups were prepared - control, dopamine (1uM), serotonin (1uM) and serotonin (10uM). Dopamine was added as a positive control since previous studies have shown an increase in dendritic branching on treatment with dopamine. Once these cultures are prepared, they are subjected to treatment with primary and secondary antibodies. The process is shown in Figure 2. The cells are treated with primary antibodies in order to detect the primary antigen in the cell culture. The secondary antibodies have fluorophores attached to them, which aid in the visualization of these neurons, as shown in Figure 3. This image is then traced - a step required for Sholl Analysis.

Figure 3: Image of a treated neuron using IF microscopy



Analysis and Results

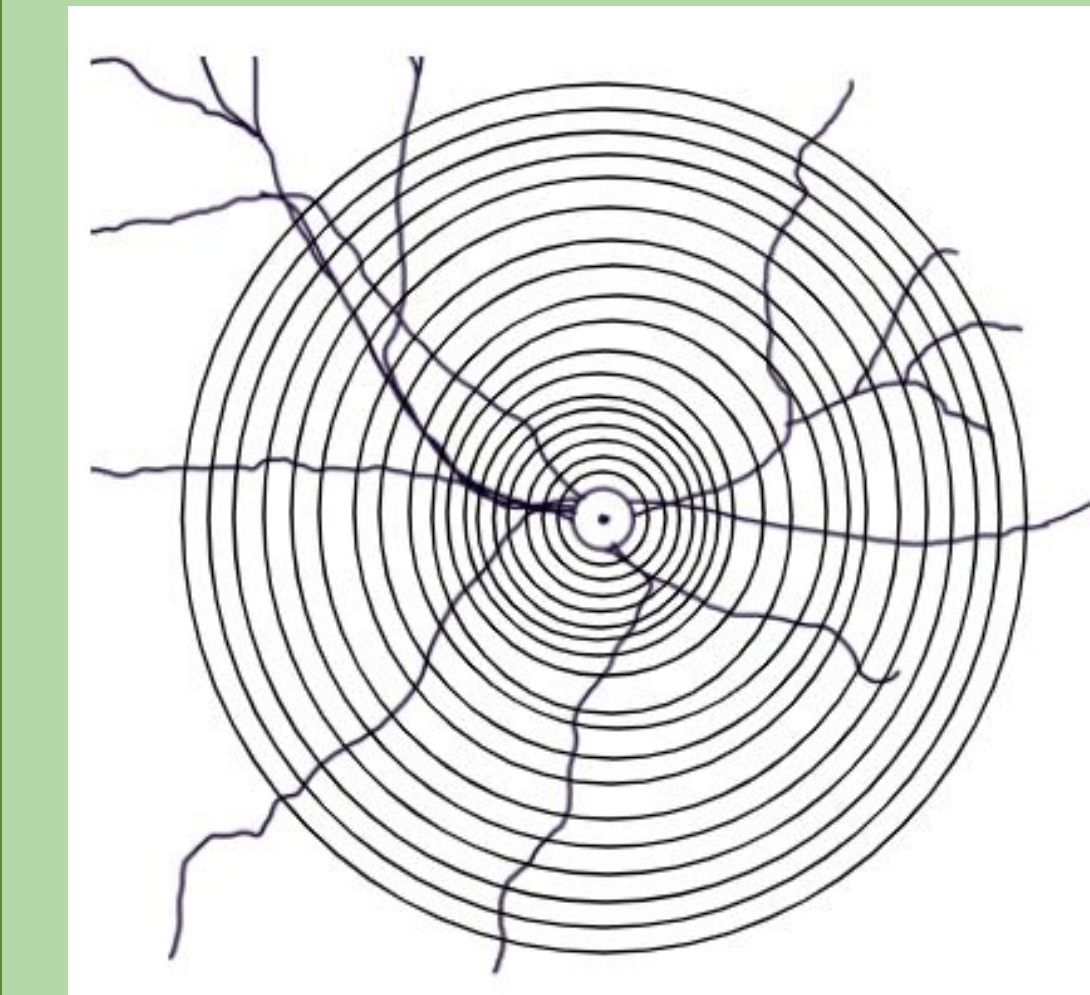


Figure 4: Concentric circles overlaid on the traced neurons for Sholl Analysis

Using a method called Sholl analysis to quantify the morphological characteristics of neurons, concentric circles radiating from soma are used to evaluate the complexity of the dendritic arbor. The number of crossings of each dendrite at a certain distance from the soma is the plotted, as shown below. This analysis indicated an increase in the density of dendrites with increase in serotonin concentration. However, the positive control didn't show expected results, speaking to the significance of our results.

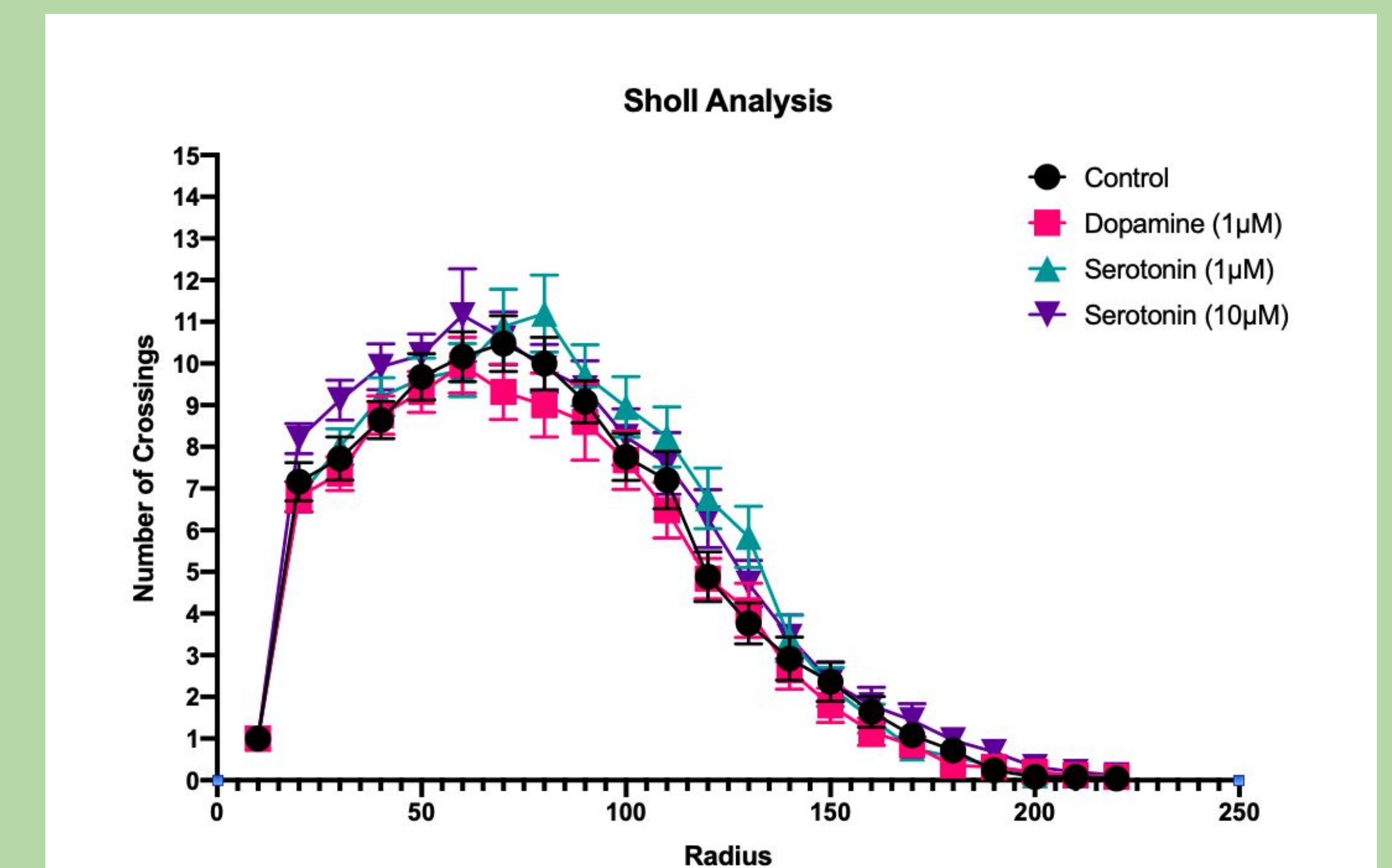


Figure 5: Graph obtained from Sholl Analysis

Conclusion and Future Directions

Increasing the amount of serotonin leads to an increase in the density of MSNs. This implores further study of these effects to build a holistic picture of the safety of antidepressants for pregnant women. In the future, further investigation of which receptors are involved in the mechanism of this increase can be studied.

References

- Mayo Clinic (2019). The most commonly prescribed type of antidepressant. Retrieved July 03, 2020, from <https://www.mayoclinic.org/diseases-conditions/depression/in-depth/ssris/art-20044825>
- Mondal, S. K. (2017). Manual of Histological Techniques (1st ed.). Jaypee Brothers Medical Publishers Pvt Ltd. https://doi.org/10.5005/jp/books/13001_20
- Penrod, R. D., Campagna, J., Panneck, T., Preese, L., & Lanier, L. M. (2015). The presence of cortical neurons in striatal-cortical co-cultures alters the effects of dopamine and BDNF on medium spiny neuron dendritic development. *Frontiers in cellular neuroscience*, 9, 269. <https://doi.org/10.3389/fncel.2015.00269>