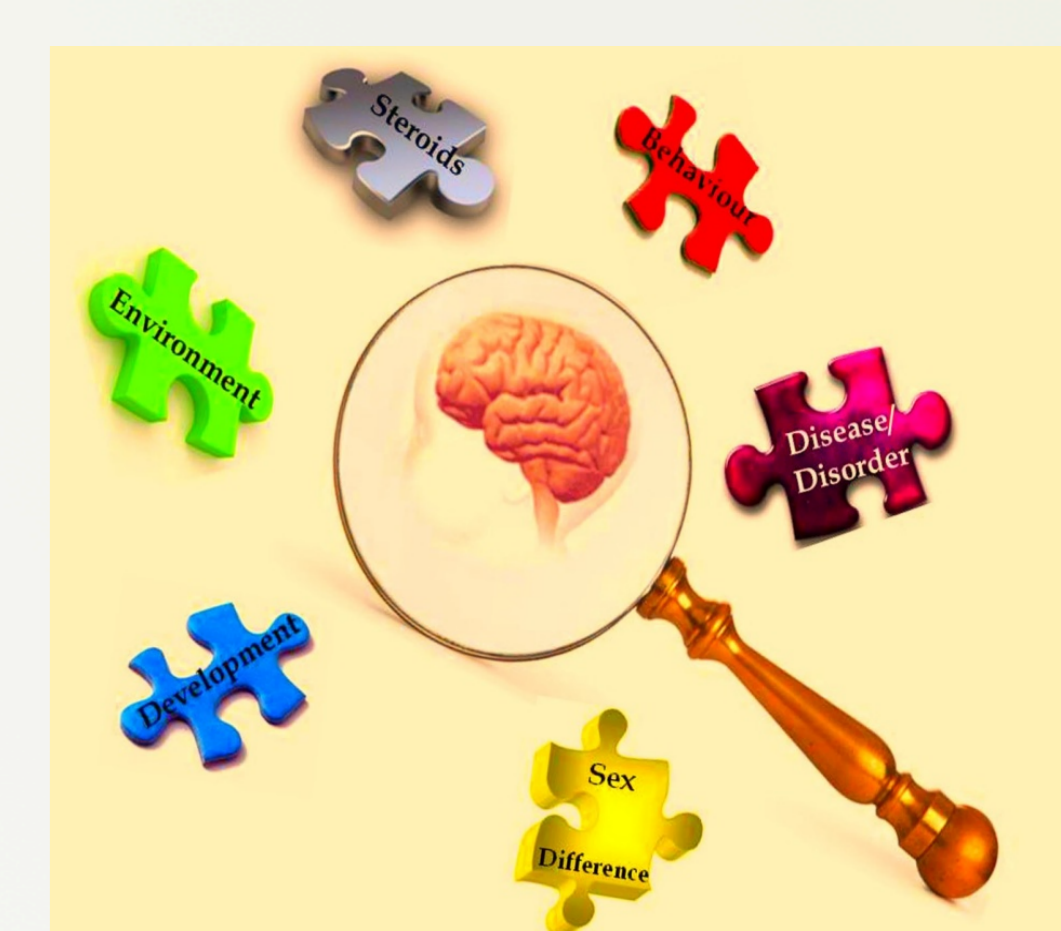


Neuroglia development in the male rat hippocampus as a consequence of early life nicotine exposure

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Introduction

Smoking during pregnancy is a serious concern due to linked fetal developmental complications that may have adverse health consequences during adulthood. Nicotine can cause long lasting deficits in hippocampal function, which can further disrupt memory and cognitive development.

Nicotine replacement therapy (NRT) products have been produced as a safer alternative to smoking during pregnancy and breastfeeding. However, there is currently limited evidence on the long-term effects of chronic low level nicotine exposure on the infant's neuronal development.

This research examines brain development following early life low dose nicotine exposure, mimicking NRT. This is done via an analysis of the relative numbers and the morphology of glial cells in the male rat hippocampus. Immunohistochemistry with a glial fibrillary acidic protein antibody and the Image J software were used to compare the number and complexity of astrocytes between nicotine and saline treated rats.

Methods

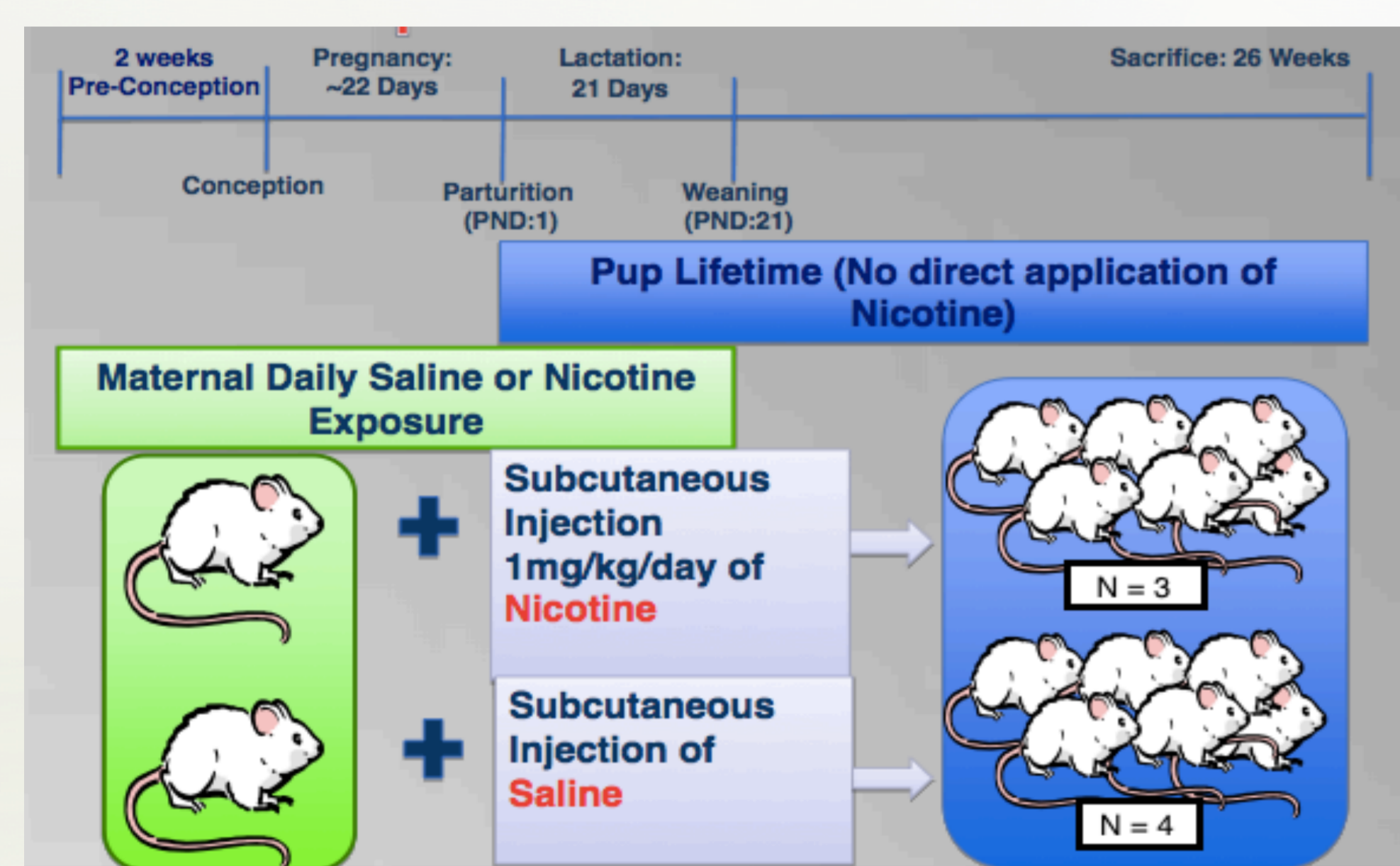


Figure 1. Representation of how dams were randomly assigned to receive saline or nicotine bitartrate daily for 2 weeks prior to mating until weaning (PND:21).¹ Male offspring were sacrificed and their brains sectioned at 30 μ m and used for analysis.

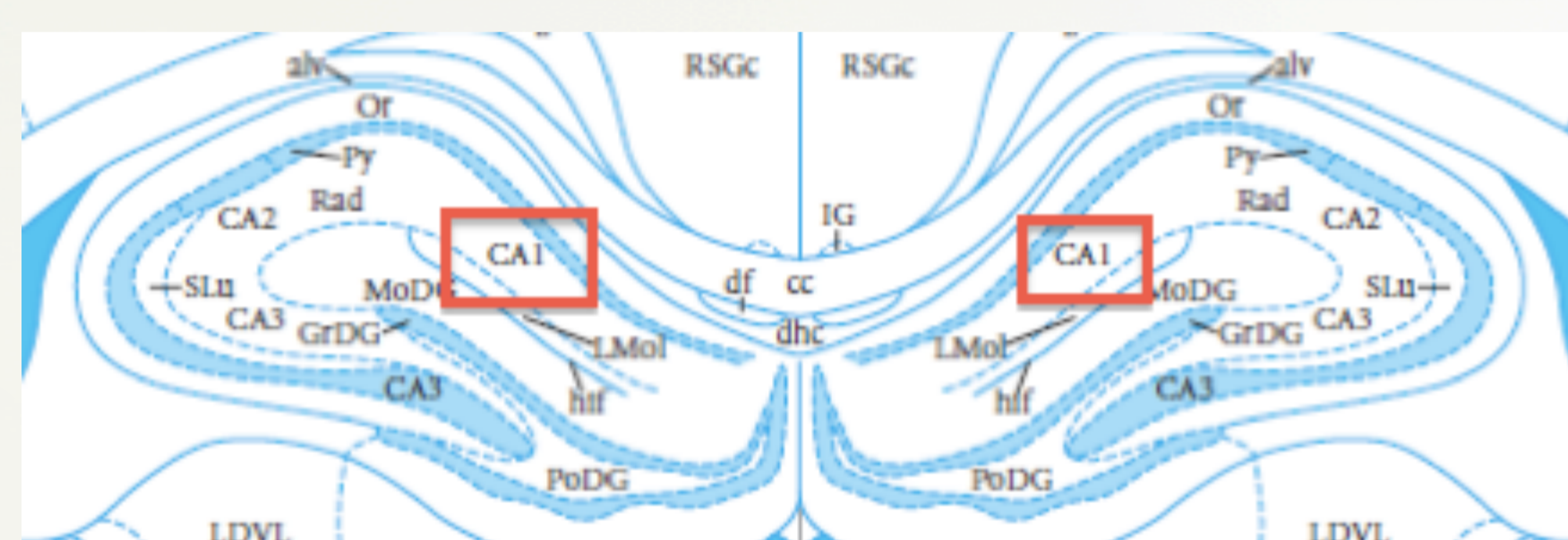


Figure 2. Left and right hippocampal region of the male rat and the CA1 area that was photographed (in red). Regions were determined using Paxinos and Watson (2007) rat brain atlas.

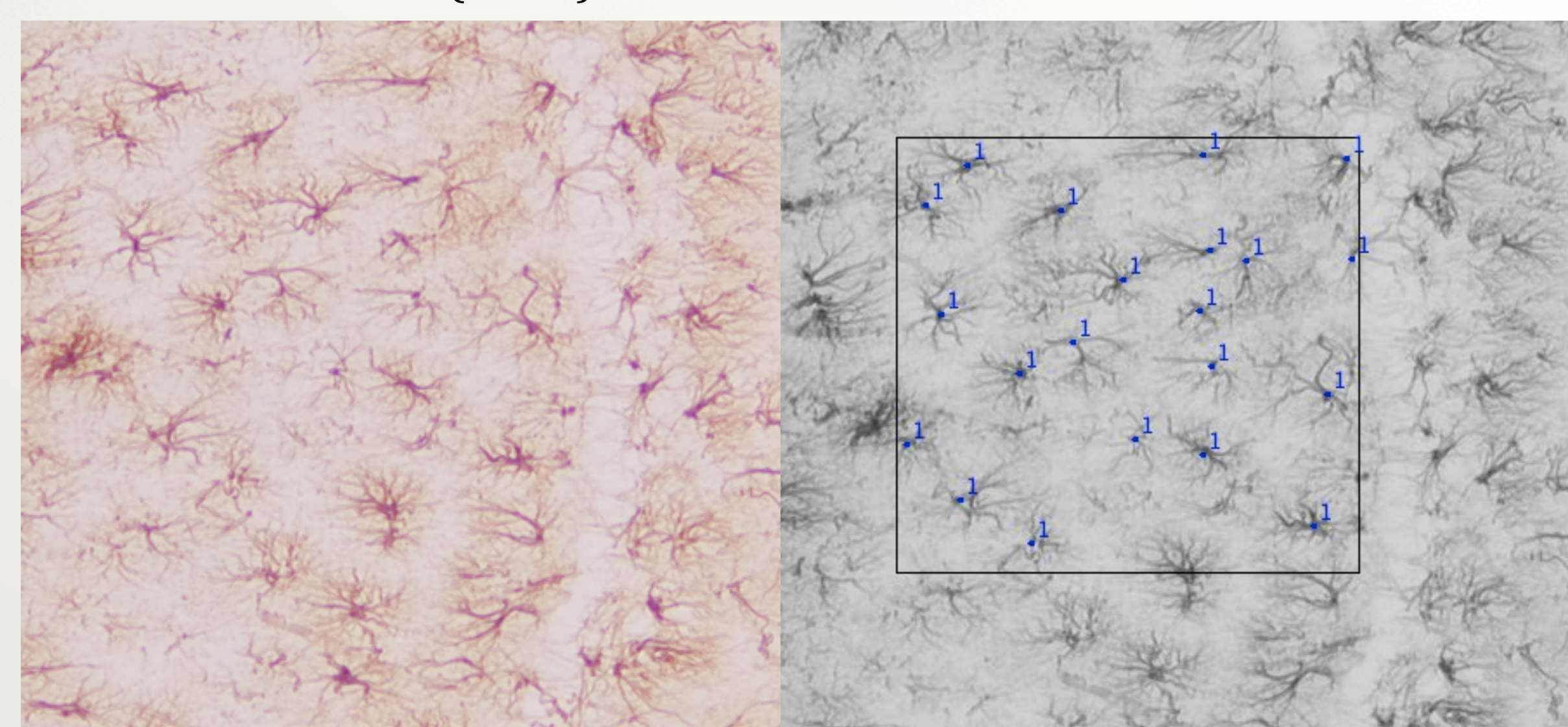


Figure 3. Using a 10X magnification, cells were counted using the Image-J computer software and a 3x3 inch box was placed in the most cell dense area of the stratum radiatum area. Positive cells required a visible cell body that was completely present in the box. Counts were done by two experimentally blind investigators in order to verify reliability of the counts.

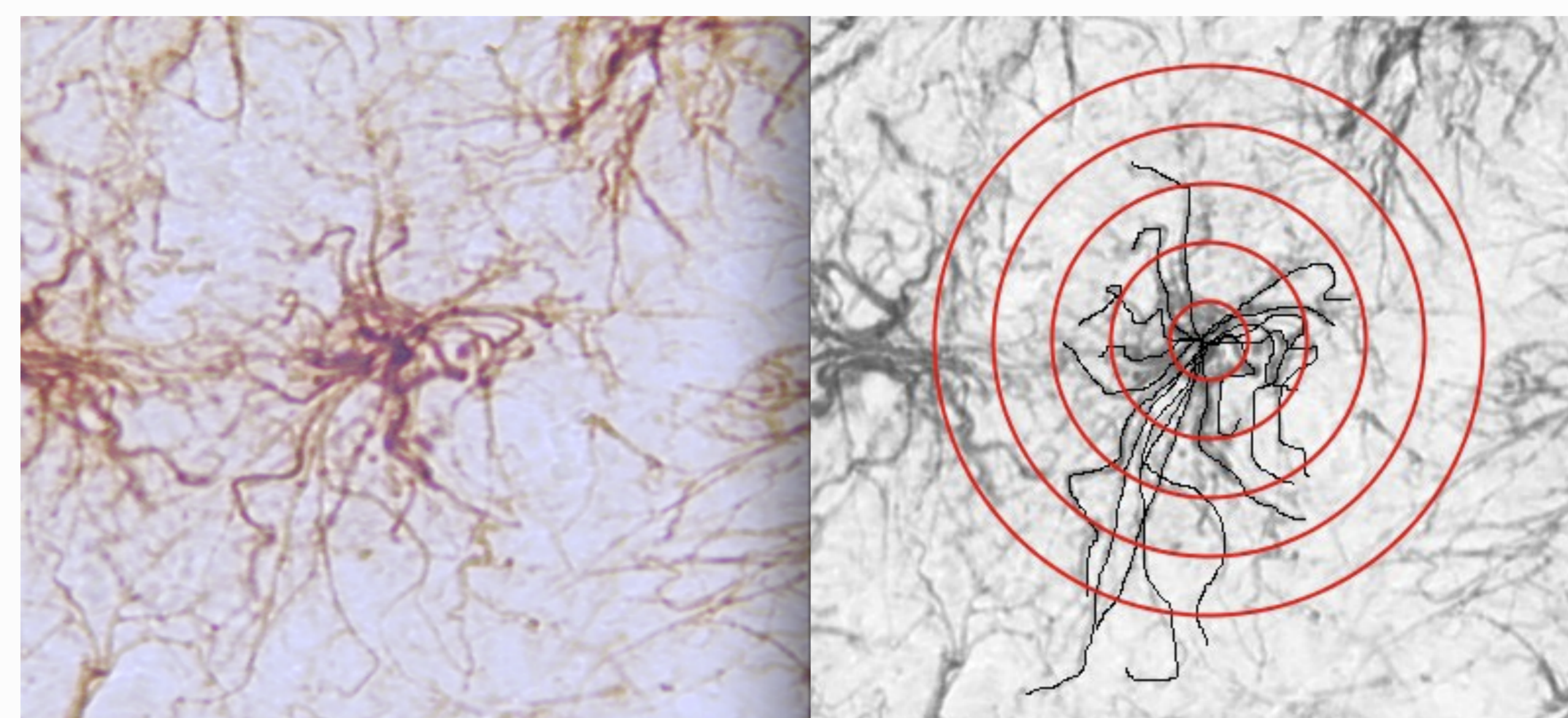
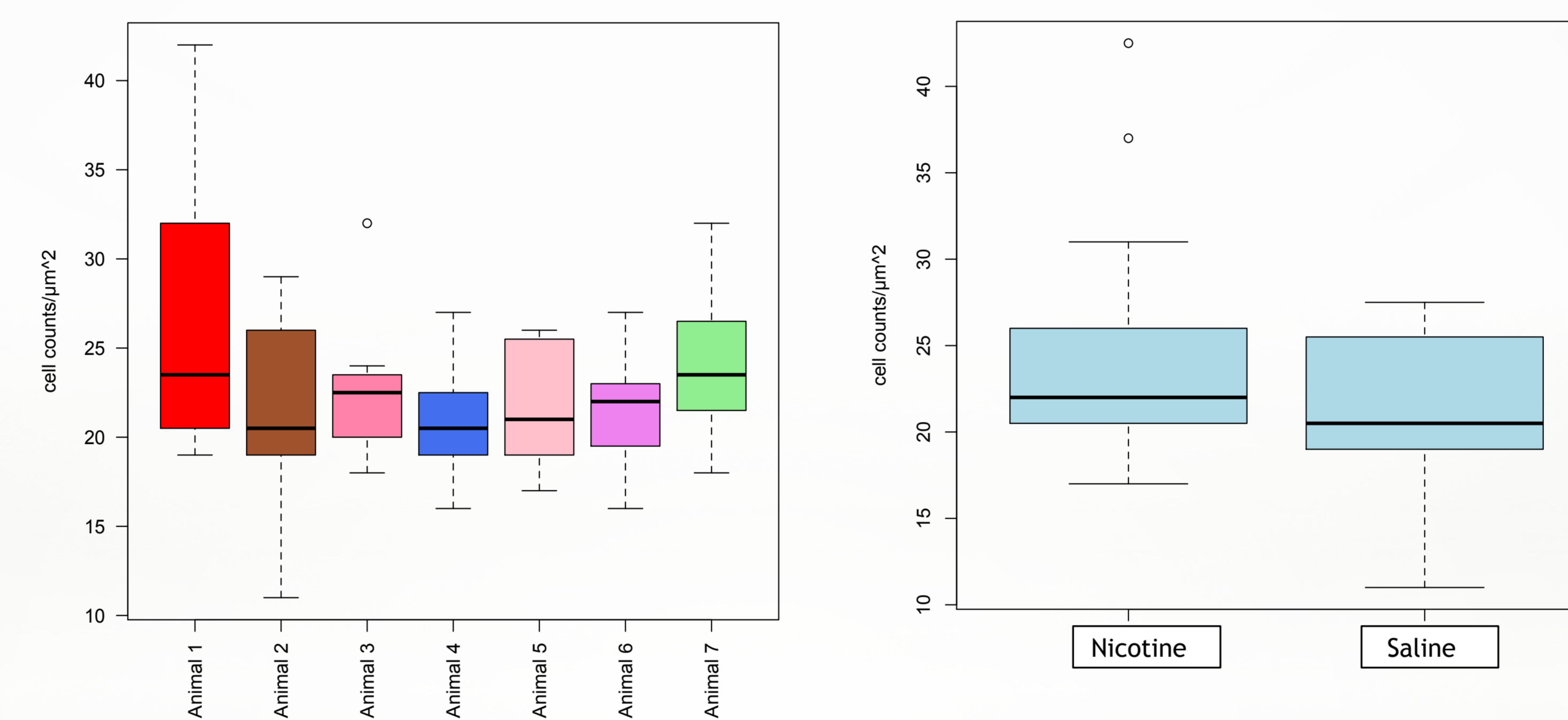


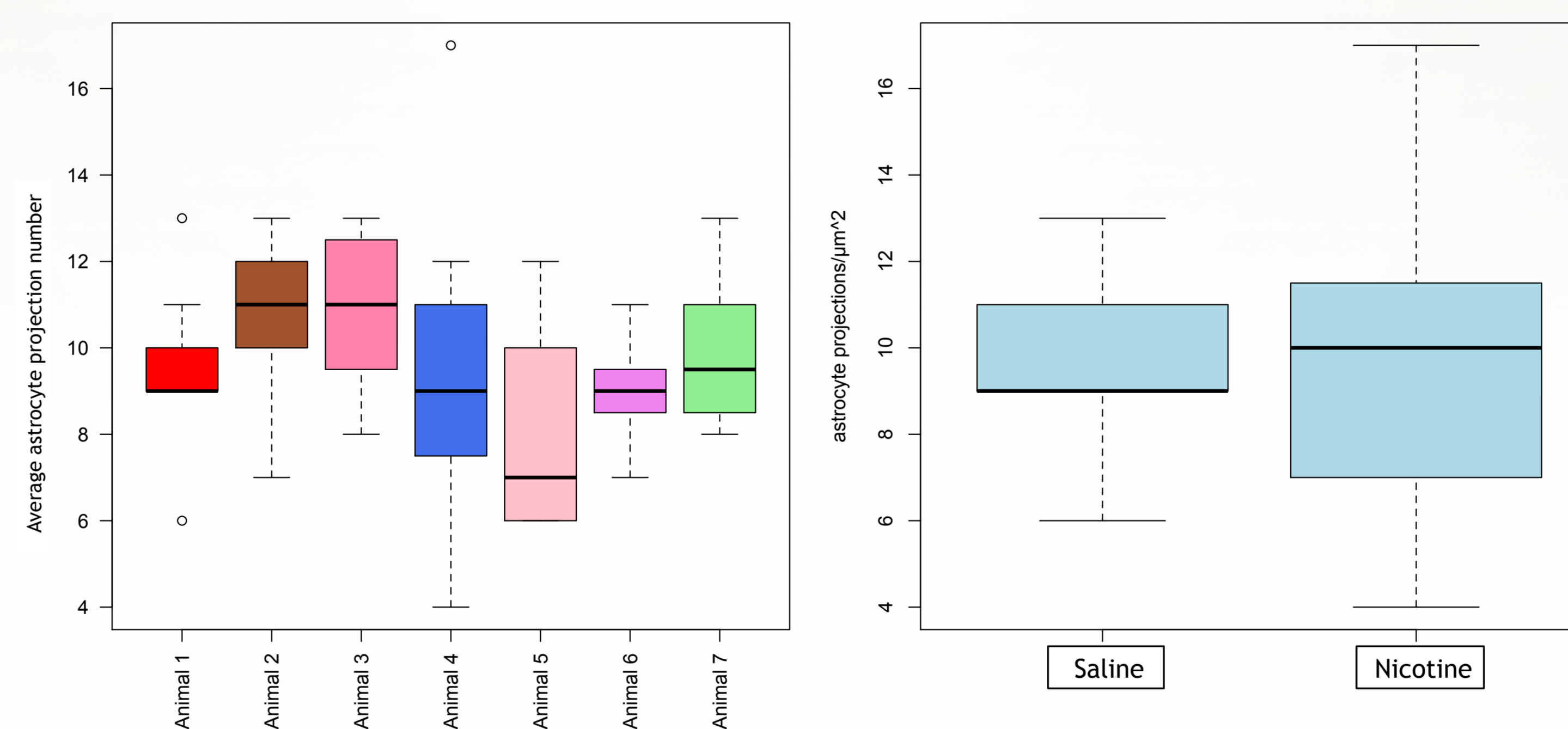
Figure 4. Representation of the complexity analysis. Image-J was used to trace and count each astrocyte projection from the center of the cell body and 5 concentric circles with consistent inner and outer radii were used to measure the length of each projection at the 40X magnification.

Results

The intra-rater reliability was estimated at 0.92; consistency of scoring based on a Pearson correlation coefficient was $r=0.959$. A non parametric analysis was carried out and the results are as follows:



Figures 5&6 . Representation of the median and interquartile range of each animal tested and the overall median and respective interquartile range for each treatment group. The relative number of cell counts were recorded using the method outlined in Figure 3. There is no significant group difference among both treatments, Mann-Whitney U: $p>0.05$



Figures 7&9. Representation of the median and interquartile range of each animal tested and the overall median and respective interquartile range for each treatment group. The relative number of projections were recorded using the method outlined in Figure 4.

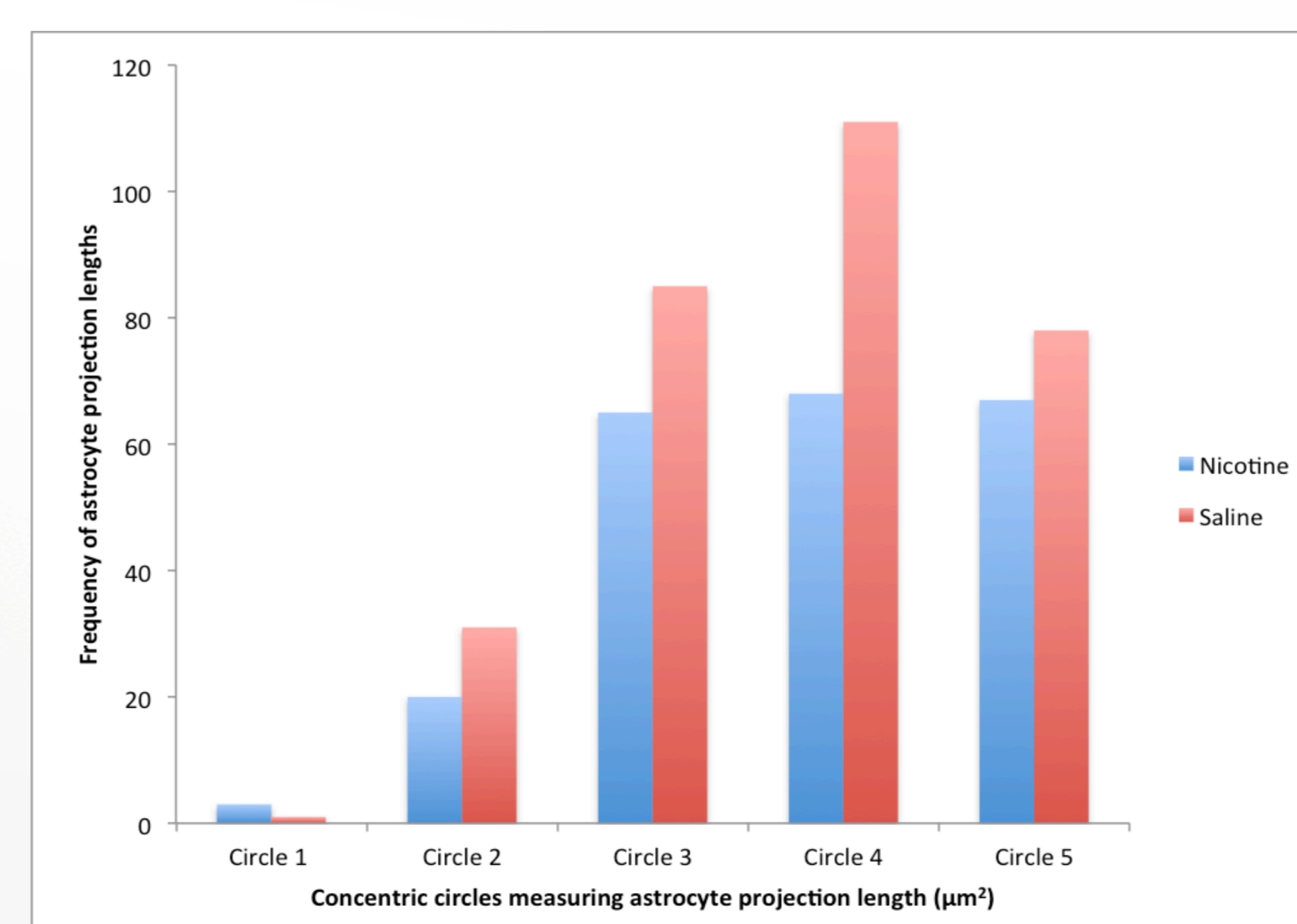


Figure 9. Representation of the frequency distribution (frequency of astrocyte projection length versus concentric circles measuring the length μ m²) to compare the complexity of astrocyte projections using method outlined in Fig. 4 between the two treatment groups.

Conclusion

The initial hypotheses were that maternally ingested nicotine would cross the mother's placenta, blood-brain barrier and be transferred in the milk to the developing offspring. This would cause an increase in the number of astrocytes in the CA1 region of the hippocampus.

However, there was not a statistically significant effect of nicotine on cell number in the hippocampal layers and area studied. This could be because the concentration of nicotine used in this study was below the threshold of affecting the astrocyte number and complexity.

In addition, because of the limitations, the results of this study do not ensure the safety of NRT or for a mother to expose her offspring to any level of nicotine.

Limitations and Future Directions

Our sample size for the treatments was very small ($n=4$ and $n=3$, nicotine and saline, respectively) and this is problematic for finding a true statistical difference between the nicotine and saline treated groups – power is very low. In addition, a concrete pattern was not observed in the projection counts, perhaps because of the random selection of only a single astrocyte and hence producing a small “n” value to work with.

Further research must account for a larger sample size to improve the internal validity of the study and introduce an equal number of rats in each treatment group.

While nicotine, even in NRT has the potential to affect the developing fetus; a comparison could be done with an NRT exposed group where the mother is chronically stressed during her pregnancy. This could mimic the mother undergoing withdrawal symptoms from chronic smoking. Physiological changes induced from chronic stress in utero may be worse than low-level nicotine exposures on the brain.

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References & Contact

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