Methylene Blue as a Facilitator to Reverse Effects of Developmental Iron Deficiency

Ashley Horejsi, Thomas Bastian, Lorene Lanier, Michael Georgieff

Introduction

Iron deficiency is a common nutrient disorder, affecting nearly 2 billion individuals globally, including 30% of women and their offspring. Iron is a nutrient with a vital role in proper mitochondrial function. This is important in developing neurons; lack of proper amounts of iron can lead to impaired memory and learning functions as well as social deficits. When supplemented early enough, iron supplements may reverse these effects. However, this time frame is very narrow and by the time iron deficiency is detectable in newborns, this window has passed.

Methylene blue is a drug that acts as an alternative electron carrier in the electron transport change. This drug improves mitochondrial function and enhances memory in some cases. Thus, this would be an ideal option to test to reverse the effects of prenatal and early postnatal iron deficiency in newborns as well as other individuals later in life.

The hypothesis for this experiment is that treatment with methylene blue alone will not yield any significant changes from iron deficient neurons and treatment with iron and methylene blue together will improve dendrite complexity to be similar to control neurons.

Methods

Chronic Iron Deficiency and Treatment of Primary Hippocampal Cultures
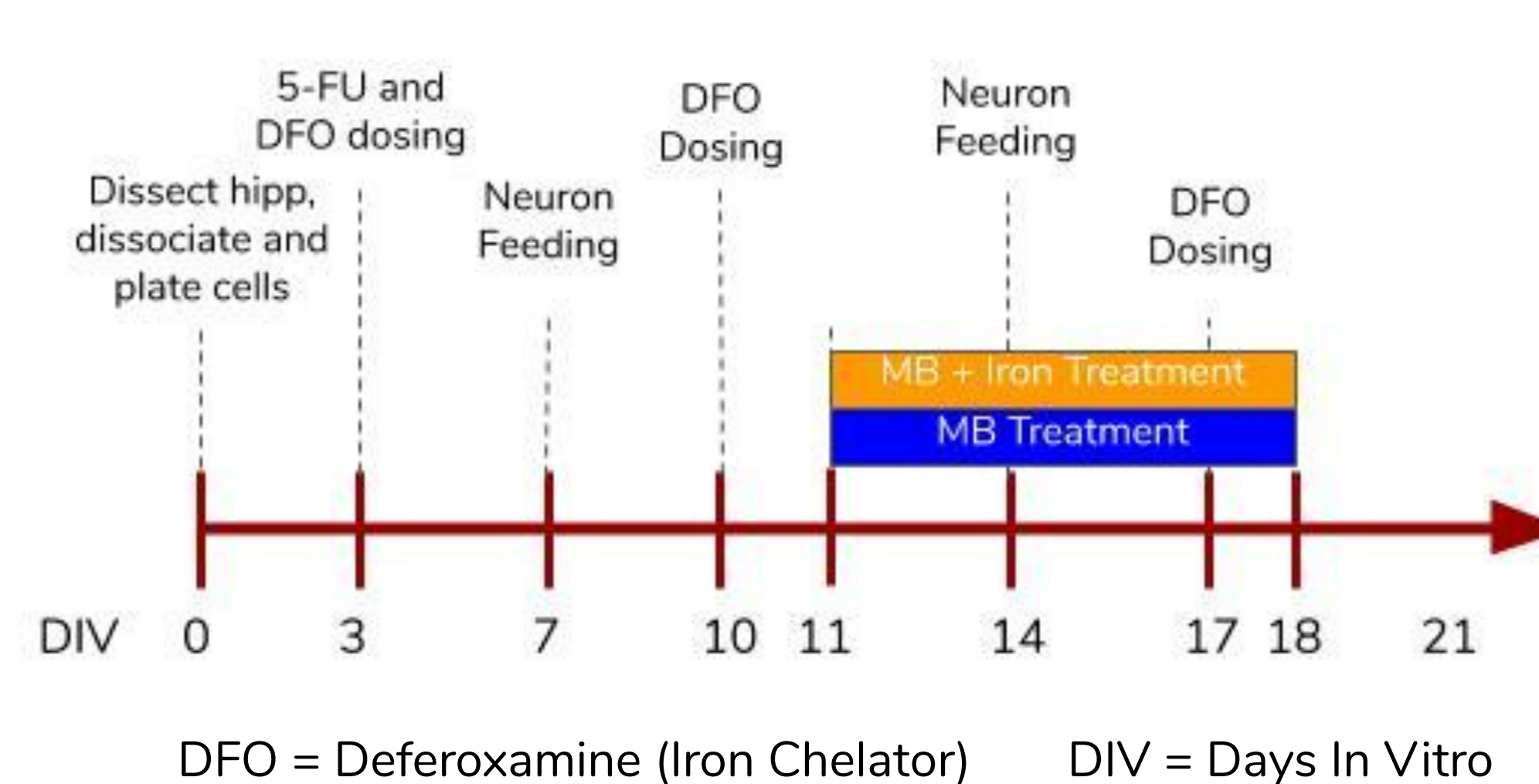
Treatment Groups:
1. Control
2. Control + MB
3. DFO
4. DFOR
5. DFO + MB
6. DFOR + MB

DFO = Deferoxamine (Iron Chelator)   DIV = Days In Vitro

Analyses
Dendrite complexity (branching and length) was determined through dendrite tracing and sholl analysis.

Statistics
Statistical significance was determined using a one-way and two-way ANOVA tests. Asteriks represent a significant difference between that treatment group and the control. The same letter above bars on the graphs represent no significant difference between the groups.

Conclusions

The DFO treatment group results were in line with results from previous experiments. In none of the groups was the treatment group of MB treated DFO and MB plus iron treated DFO neurons statistically similar to the control group while being statistically significant from the DFO and DFOR groups. This means the MB treatment did not work as expected, and in some cases made the groups worse. This is likely due to the dosage amount of MB used. In testing done since the data collection from this experiment, it has been shown that there is only a very narrow range in which MB treatment will be helpful in aiding mitochondrial function and that in some cases too much MB can be harmful. This experiment would need to be repeated with a smaller MB dosage amount in order to come to a more accurate conclusion about the viability of MB as a treatment option for reversing the effects of prenatal and early postnatal iron deficiency.

Results

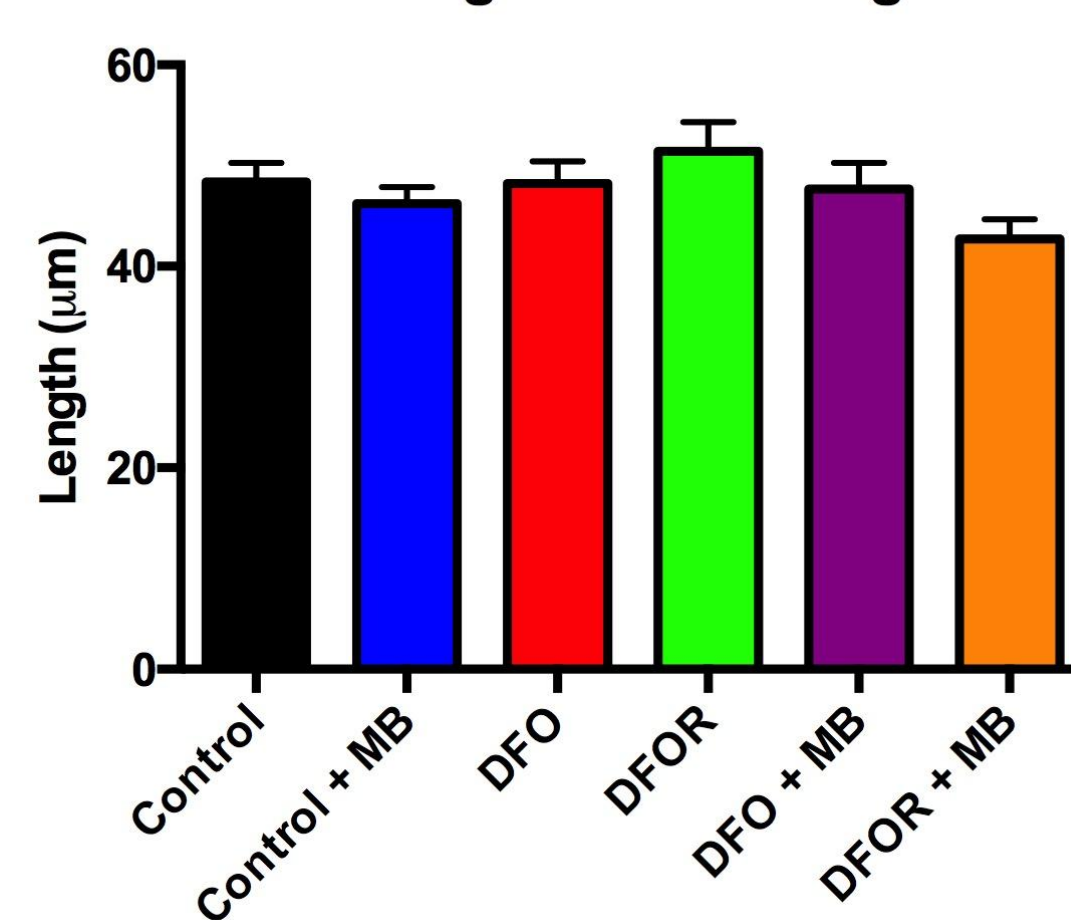Figure 1: Average dendritic branch lengths within each treatment group (µm).

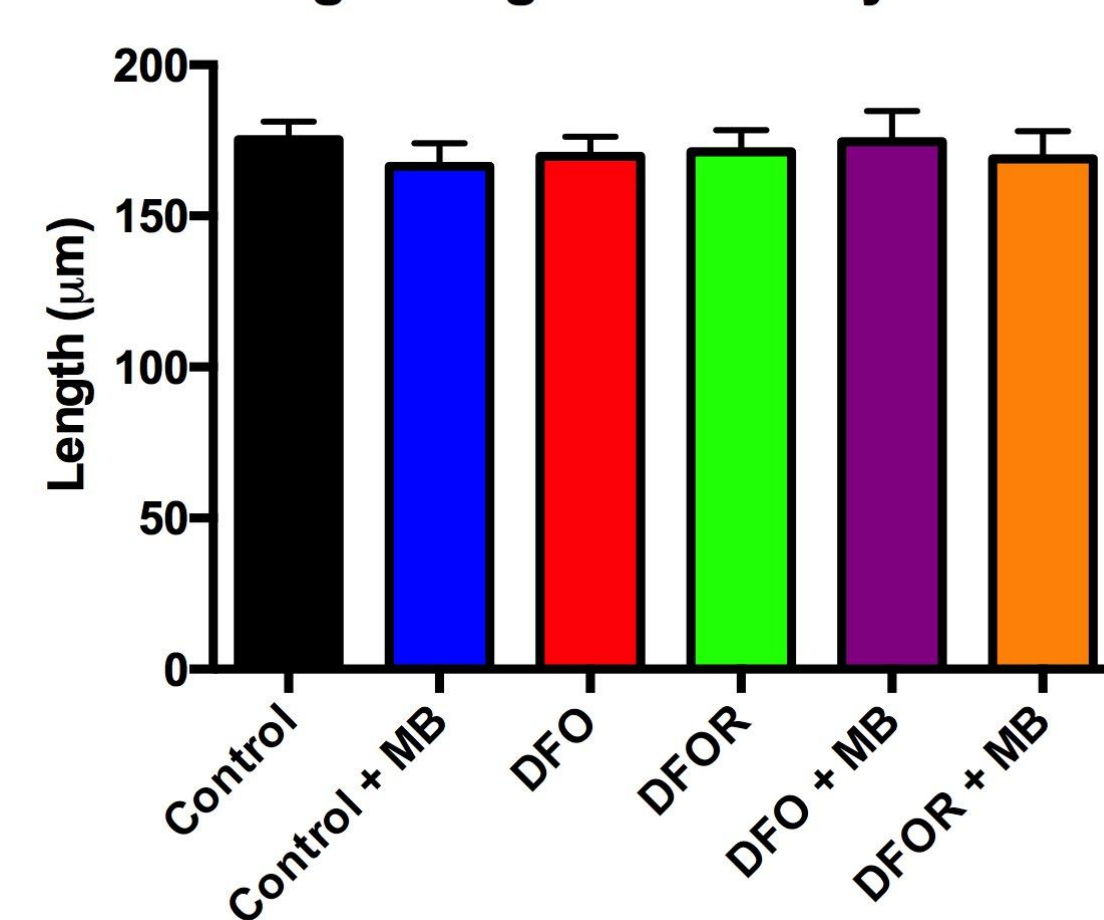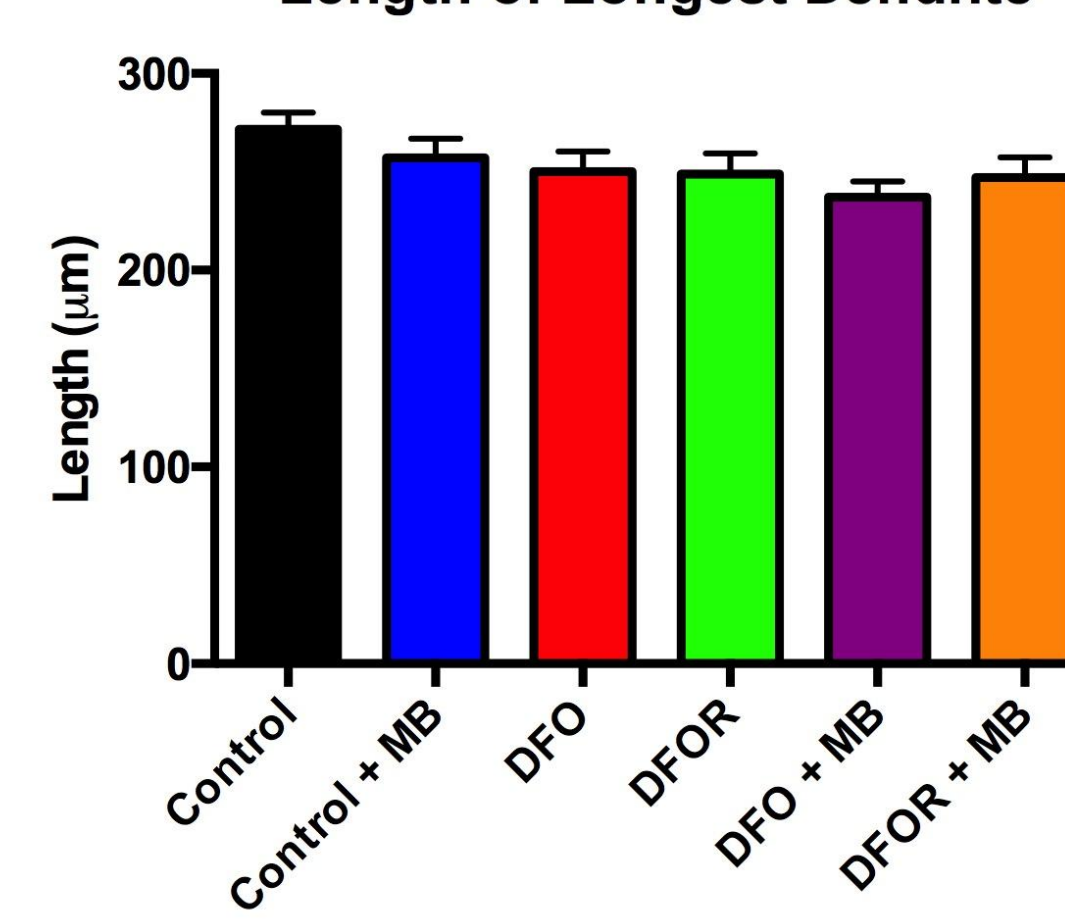Figure 2: Average branch length of primary dendrite for each neuron within each treatment group (µm).

Figure 3: Average branch length of the longest dendrite for each nuron within each treatment group (µm).

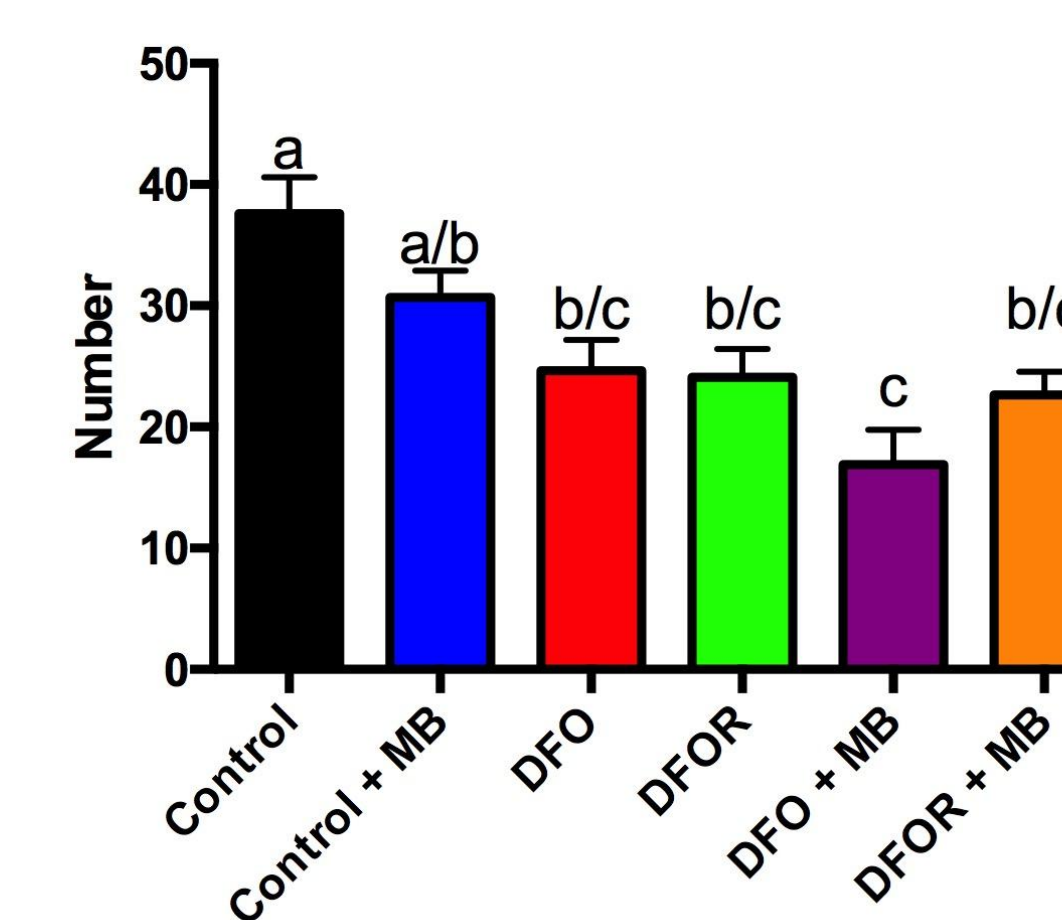Figure 4: Average number of dendritic branches for each neuron within each treatment group. Same letter above bar represents statistically similar.
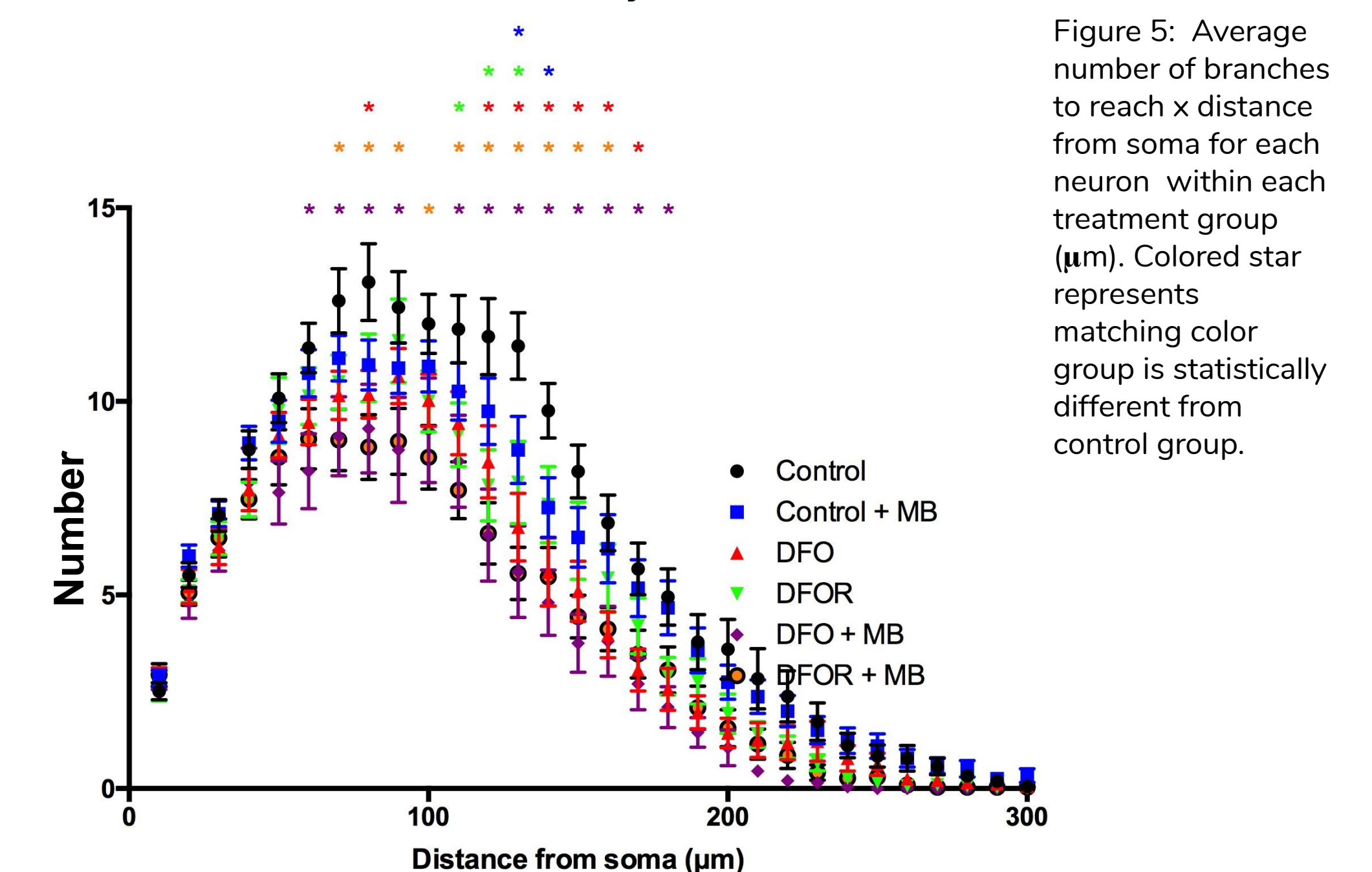
Figure 5: Average number of branches to reach x distance from soma for each neuron within each treatment group (µm). Colored star represents matching color group is statistically different from control group.
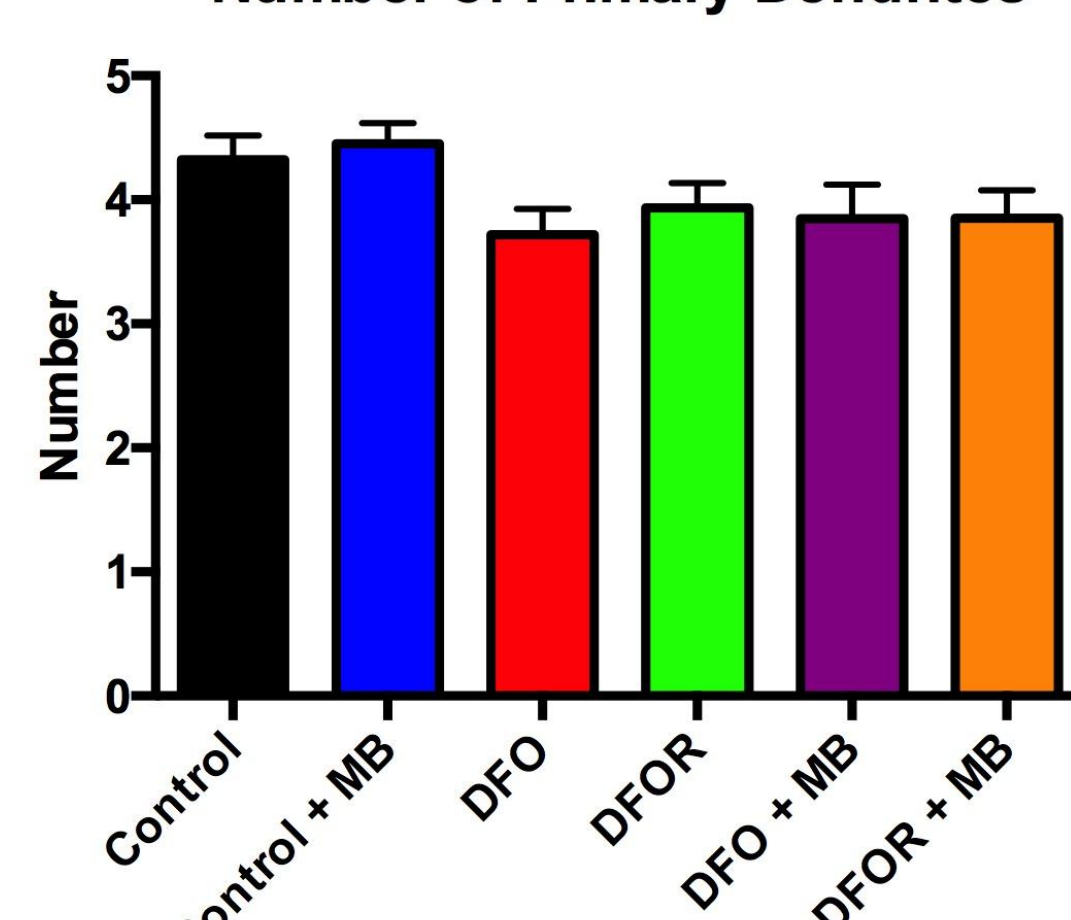
Figure 6: Average number of primary dendrites for each neuron within each treatment group.
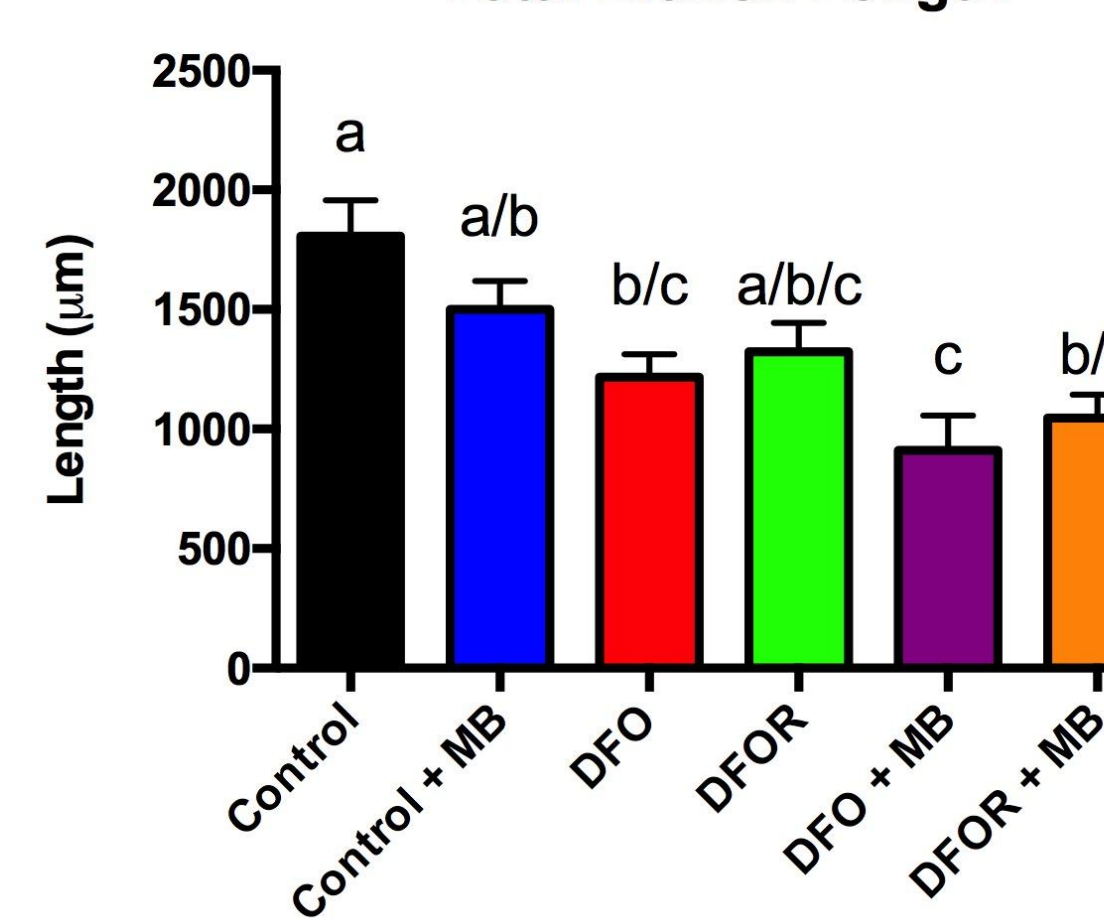
Figure 7: Average branch length for all dendrites on a single neuron within each treatment group. Same letter above bar represents statistically similar.
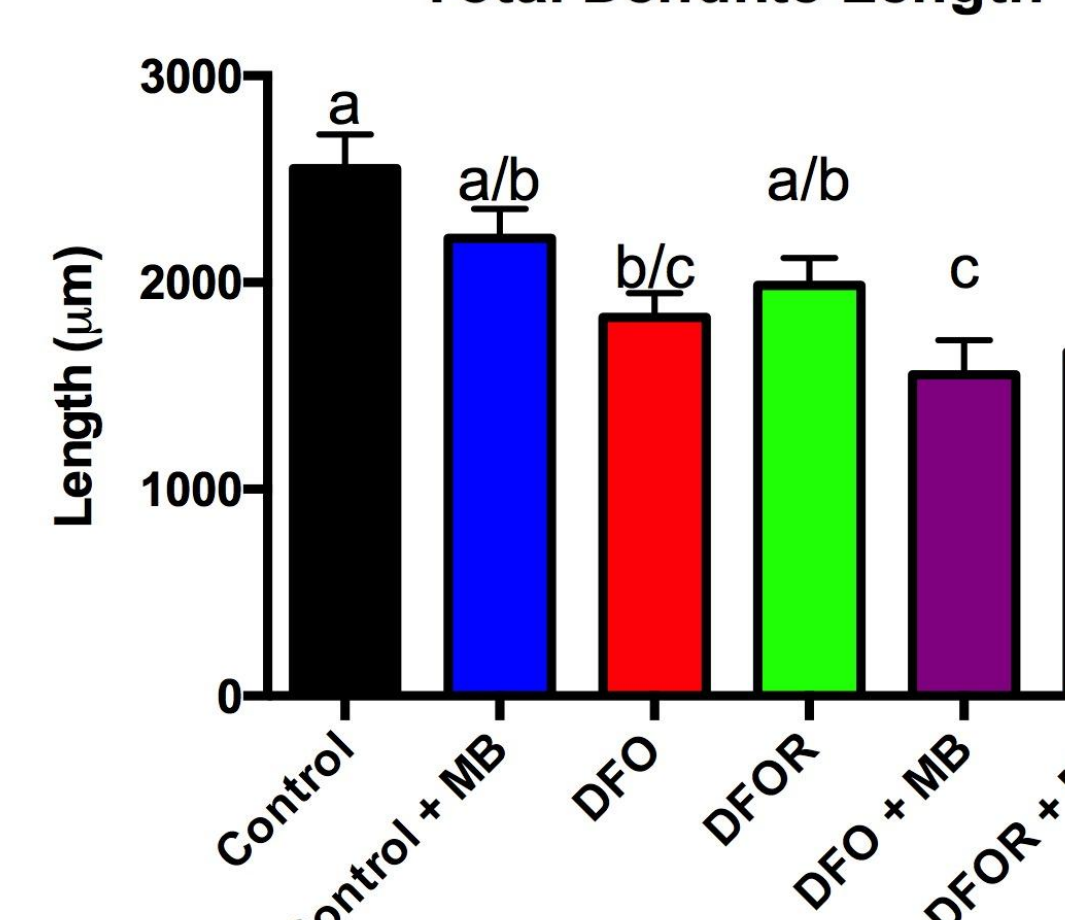
Figure 8: Average length for all dendrites per single neuron within each treatment group (µm). Same letter above bar represents statistically similar.
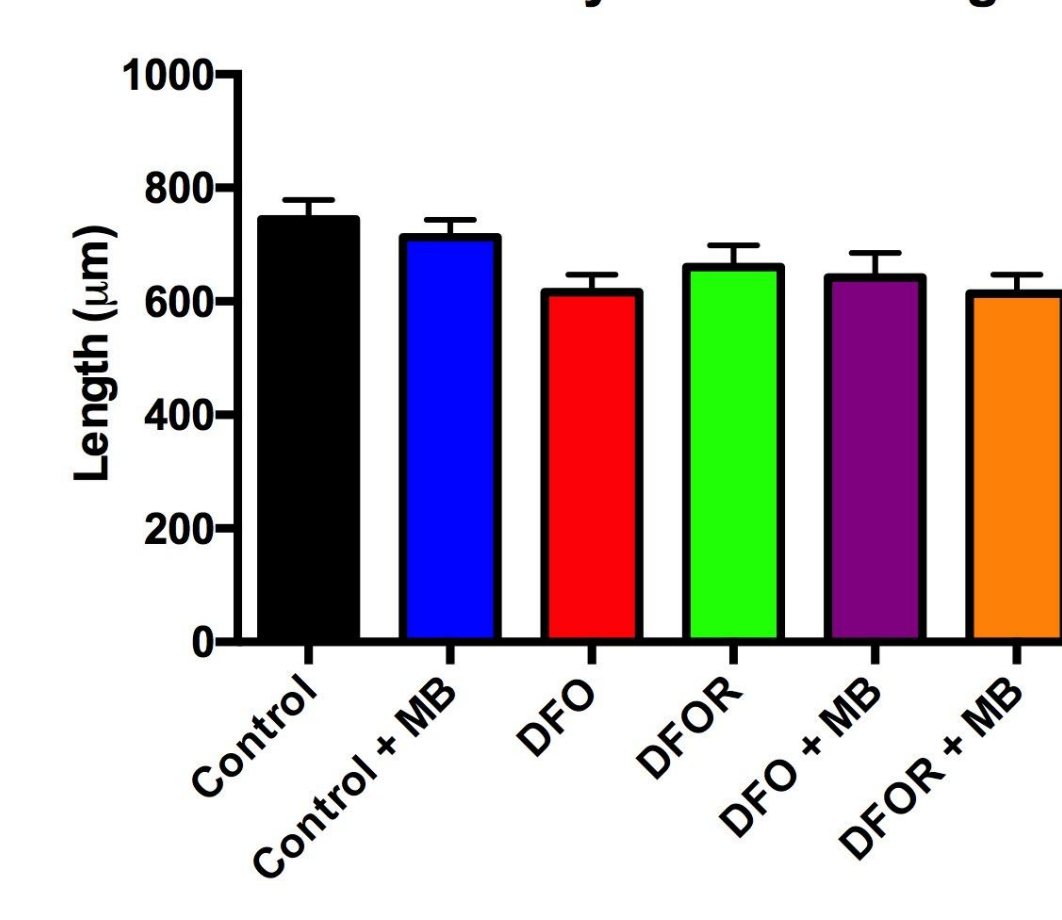
Figure 9: Average length of primary dendrite for each neurons within each treatment group (µm).

Acknowledgements