Effects of Strattera (atomoxetine) on the regeneration and behavior of brown planaria (*Dugesia dorotocephala)*

Dena Lipschitz, Department of Biological Sciences, York College of Pennsylvania
Mentor: Dr. Sean Georgi

Background

- Attention Deficit Hyperactivity Disorder (ADHD) is one of the most common neurological disorders in children, affecting 171.6 million people worldwide (CDC, 2019). ADHD is caused by a decrease in the levels of norepinephrine (NE) in the brain.
- The most common treatment is stimulants, which have been shown to repress symptoms in about 70-80% of the individuals that take them (CDC, 2019). Individuals may also take nonstimulants, but they are less widely used.
- Strattera (atomoxetine), a nonstimulant, acts as a selective norepinephrine reuptake inhibitor to increase the levels of NE in the brain. Studies have been performed that show atomoxetine as a useful treatment for ADHD (Shaywitz, 2017), but further research must be done to find the effects of it on the developing brain and nervous system, and in other model organisms.
- Planarians are excellent model organisms for pharmacology studies, as they have a defined central nervous system and have nearly every neurotransmitter as found in mammals (Agata, 1998). Their regeneration abilities are very unique, as they are able to regenerate from very small segments through the use of their stem cells, or neoblasts.
- Planarian species *D. dorotocephala* are not as commonly used as other species, and atomoxetine has not been studied in planarian regeneration.

Objectives

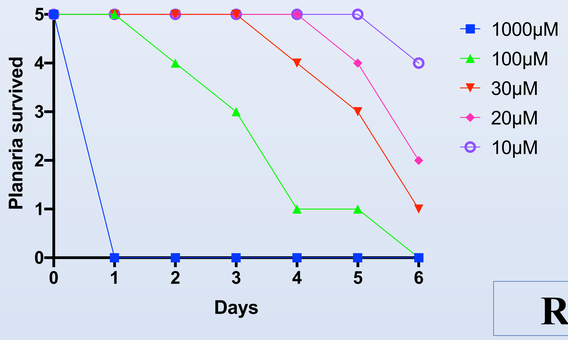
- Determine how atomoxetine affects behavior of planaria
- Observe differences of head regeneration and neoblast distribution between control and atomoxetine-treated planaria

Acknowledgements

I would like to hugely thank Dr. Sean Georgi for his extensive help and guidance through this entire experiment. I also would like to thank the York College Biology Department for allowing me the resources to perform and present my research.

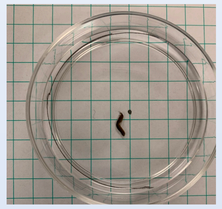
Methods

Determine viable concentration for future experiments by testing varying atomoxetine concentrations on planaria to observe survival rate over 6 days



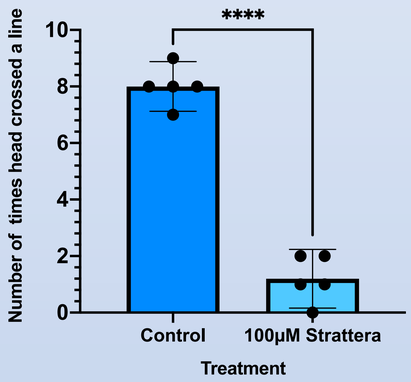
Stages of head regeneration were averaged after 5 days post amputation of control and 10µM atomoxetine-treated planaria

Performed behavior analysis comparing control and 100µM atomoxetine-treated planaria kept in solution for 15 minutes

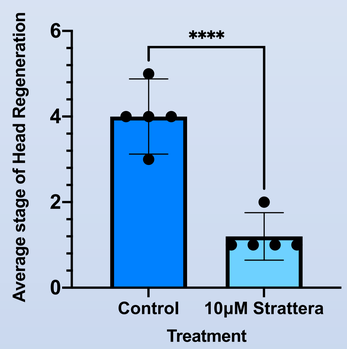


IHC for newly regenerated head fragments to observe differences in head and nerve cord regeneration by staining synapsin antigens with anti-synapsin antibodies (DSHB), and neoblast distribution by staining PH3 antigens with anti-PH3 antibodies (Cell Signaling Technologies)

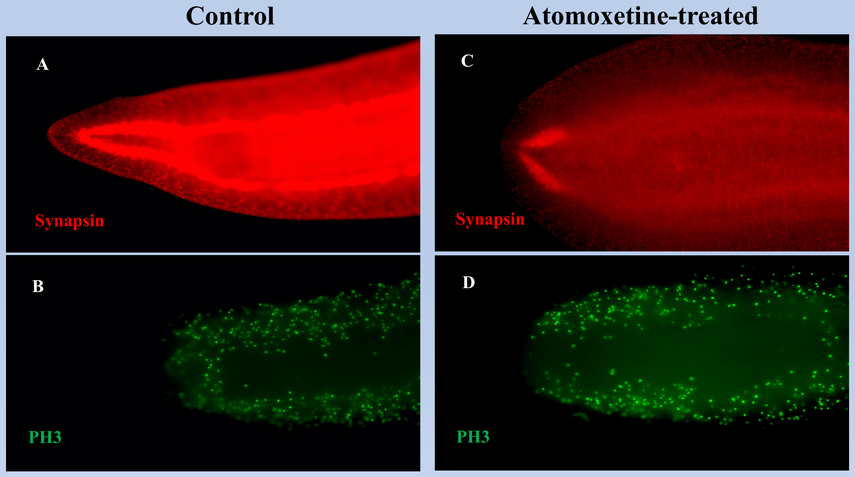
Results



Mean number of times planarian (*D. dorotocephala*) head crossed a gridline observed during a 30 second time interval used for behavioral analysis. The control planarian (n=5) were kept in spring water and the treated planarian (n=5) were kept in a 100µM Strattera solution before being placed in a dish over a 6mm^2 grid. **** notes a statistical difference, proven by a two-sample t-test performed using GraphPad Prism ver 9.0 (α=0.05, P-value<0.0001).



Average stage of head regeneration between control and Strattera-treated planaria (*D. dorotocephala)* at five days post amputation (dpa). Regeneration stages were measured from stage 1 (no new blastema growth) to stage 5 (complete head regeneration). Dissected tail fragments were treated with 10µM Strattera solution (n=5) and controls were kept in spring water (n=5). *** notes a statistical difference proven by a two-sample t-test performed using GraphPad Prism ver 9.0 (α=0.05, P=0.0002).



Immunostaining of control (A) and 10µM atomoxetine-treated (C) planaria with anti-synapsin to observe head and nerve cord regeneration. Immunostaining of control (B) and 10µM atomoxetine-treated (D) planaria with anti-PH3 to observe neoblasts in the regenerating head and CNS. Immunostaining was performed 5 days post amputation.

Discussion

- A 10µM atomoxetine concentration was found to be best for regeneration experiments and 100µM for the behavioral analysis.
- Behavior was diminished by atomoxetine, as the average number of times the planarians crossed the gridline was significantly decreased in the atomoxetine-treated planarians compared to the controls.
- Our study showed that regeneration time increased in atomoxetine-treated planarians, as the average stage of head regeneration was much lower than controls, and most planarians did not reach full regeneration in the span of the five days, but may have if the study was extended.
- The IHC showed a smaller regenerated head and less distinct nerve cords, as well as a higher concentration of neoblasts in the head region, possibly due to the head fragment still regenerating.

Future Studies

- Determine exact molecular target of atomoxetine in brown planaria, as planaria do not have exact NE neurotransmitter, but have a similarly functional neurotransmitter named octopamine (OA).
- Measure long-term effects of drug as well as sensitivity to the drug

Citations

Center for Disease Control and Prevention. 2019. Attention-Deficit/Hyperactivity Disorder. *U.S. Department of Health and Human Services.*

Shaywitz, S., Shaywitz, B., Wietecha, L., Wigal, S., McBurnett, K., Williams, D., Kronenberger, W. G., & Hooper, S. R. (2017). Effect of Atomoxetine Treatment on Reading and Phonological Skills in Children with Dyslexia or Attention-Deficit/Hyperactivity Disorder and Comorbid Dyslexia in a Randomized, Placebo-Controlled Trial. *Journal of child and adolescent psychopharmacology*, *27*(1), 19–28. https://doi.org/10.1089/cap.2015.0189

Agata, K., Soejima, Y., Kato, K., Kobayashi, C., Umesono, Y., Watanabe, K. (1998). Structure of the Planarian Central Nervous System (CNS) Revealed by Neuronal Cell Markers. *Zoological Science*, 15(3), 433-440.