Effects of Dopamine on Regeneration Rates of *Planaria*

Purpose

The purpose of this experiment is to see how dopamine affects the regeneration rates of *planaria*.

Introduction

Planarians are small flatworms in the order Tricladida and are common to many parts of the world. The planarians possess the ability to regenerate lost body parts. Planarians are considered the simplest living animal that have a bilateral body plan and cephalization. Most things like synaptic organization, multipolar neurons, or dendritic spines are not found in primitive invertebrates but are found in planarians. The planarians also contain several different neurotransmitters including dopamine that were identified in the human brain. This makes planarians an ideal model to study the human nervous system. In this experiment brown planaria will be used which are a species of planarian.

Neurodegenerative disease occurs when neurons in the brain lose their structure, function, or die. As more and more neurons die, motor skills and intellectual acuity declines. Currently an estimated 10 million people have Parkinson's disease, a common neurodegenerative disease. The ability to regenerate nerve cells diminishes as the human brain matures. Scientists say that certain regions of the hippocampus are neuroregenerative but there is no proof to support this statement. Since there is speculation of neuroregeneration, using models with regenerative abilities might be useful in finding the cure to neurodegenerative diseases.

The central nervous system of a planarian contains a "brain" which is a bi-lobed mass of nerve tissue. This cerebral ganglia is attached to nerves that extend down from the head and contains regenerative abilities. A planarian that was cut would regenerate into two individuals. In fact even 1/279th of a planarian can regenerate back to the original size. At a wound site of the planarian, cells would proliferate and form a blastema. This blastema would differentiate into the needed tissues and regenerate the necessary body parts. This occurs because pluripotent stem cells are distributed throughout the planarian's body. These stem cells can turn into any tissue that is needed. Also an excess of dopamine is found in the planarian ganglia during regeneration suggesting that dopamine plays a role in planarian regeneration. The planarian absorbs oxygen and other chemicals through diffusion.

Phenergan, commonly known as promethazine, is a medication used to treat motion sickness. It works by blocking postsynaptic mesolimbic dopaminergic receptors in the brain. The mesolimbic pathway is one of the four dopaminergic pathways in the brain and is known as the reward pathway. This pathway connects the ventral tegmental area in the midbrain to the ventral striatum which is found in the basal ganglia in the forebrain. The lowest dosage for this medication is 12.5 mg and the highest dosage is 25 mg.

Ropinirole is an anti-Parkinson's agent. In Parkinson's dopamine producing nerve cells die in the substantia nigra, a structure found in the midbrain. Ropinirole works by imitating the D_2 and D_3 dopamine receptor subtypes. There is no precise mechanism of action for ropinirole, but it thought that it works because of the stimulation of postsynaptic dopamine D_2-type receptors in the caudate putamen in the brain. The lowest dosage of this medication is 0.25 mg and the highest dosage is 4 mg.

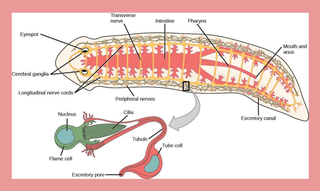

Fig. 1. A graphic from https://courses.lumenlearning.com showing the anatomy of a planaria.

Results

The *planaria* in both dopamine stimulating environments had an increased rate of regeneration, but the *planaria* in the 2 ng of ropinirole per 30 ml of spring water completed their process of regeneration in seven days, average growth of 1mm per day, while the *planaria* in the 0.5 ng per 30 ml of spring water completed their process of regeneration in eight days, average growth of 0.9 mm per day. To simulate natural conditions 20 *planaria* were kept in spring water. These *planaria* completed their process of regeneration in 10 days with an average growth of 0.7 mm per day. The dopamine inhibiting environments slowed down the regeneration rates of the *planaria* severely. The *planaria* in the 10 ng of promethazine per 30 ml of spring water regenerated to an average length of 8.7 mm, average growth of 0.2 mm per day, and the *planaria* in the 3 ng of promethazine per 30 ml of spring water regenerated to an average length of 10.2mm, average growth of 0.33 mm per day.

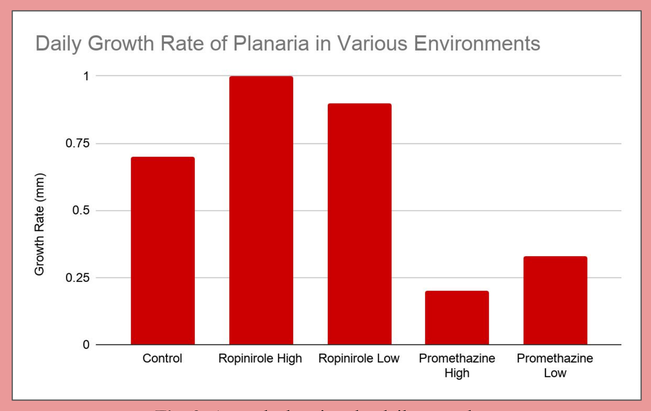

Fig. 2. A graph showing the daily growth rates of *planaria* in various environments

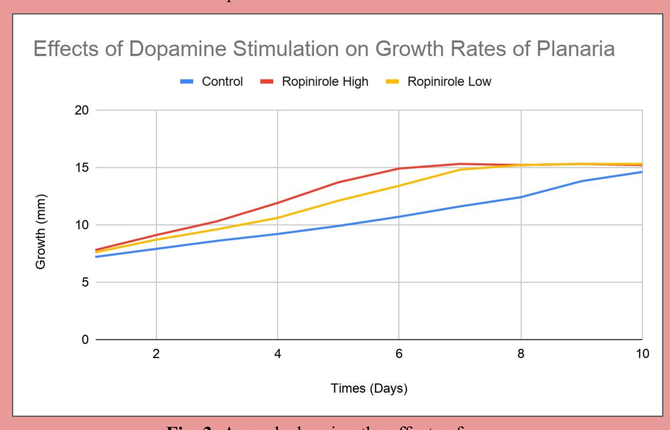

Fig. 3. A graph showing the effects of dopamine stimulation on growth rates of *planaria*.

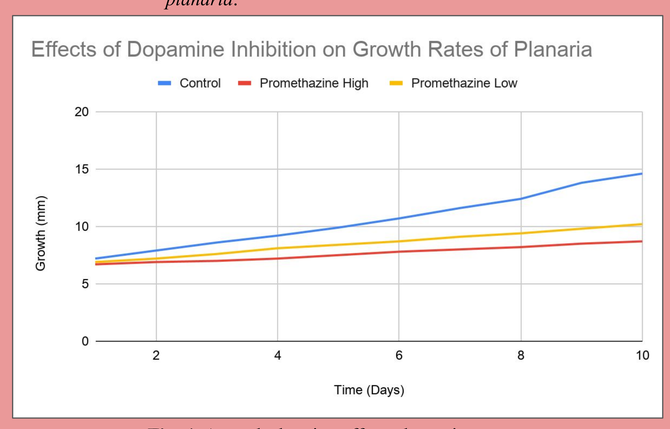

Fig. 4. A graph showing effects dopamine inhibition on growth rates of *planaria.*

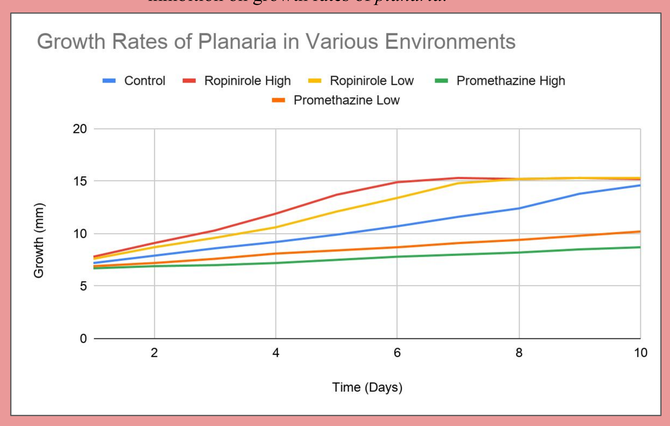

Fig. 5. A graph showing the growth rates of *planaria* in various environment.

Hypothesis

If the *planaria* are exposed to dopamine stimulants, then the *planaria* will have an increased rate of regeneration. If the *planaria* are exposed to dopamine inhibitors, *planaria* will have a reduced rate of regeneration.

Methodology

1. In 5 clean Petri dishes pour 20 ml of spring water.
2. Label the 5 dishes ropinirole high, ropinirole low, promethazine high, promethazine low, and control.
3. Crush 1 mg of promethazine and mix it into a container with 1L of spring water.
4. Thoroughly mix the solution.
5. Then take out 1ml of the mixture and mix it into another 1L of spring water. This will give you 1ng of promethazine per ml of spring water.
6. Repeat steps 3-5 using ropinirole.
7. Pour 10 ml of the promethazine solution into the promethazine high Petri dish and 3 ml into the promethazine low solution.
8. Pour 2 ml of the ropinirole solution into the ropinirole high Petri dish and 0.5 ml into the ropinirole low Petri dish.
9. Add spring water to the Petri dishes so they all have 30ml in them.
10. Place a *planaria* under a dissecting scope and as the *planaria* elongates, slice it in half.
11. Measure the length of both halves of the *planaria* and then record.
12. Repeat steps 10 and 11 nine more times.
13. Find the average length for these 20 *planaria* pieces and then move them into the control Petri dish.
14. Repeat steps 10-13 for the remainder of the 4Petri dishes.
15. Measure the average length of all the *planaria* in each Petri dish every day for 10 days.

Conclusion

The hypothesis was supported as the *planaria* in the dopamine stimulating environment had an increased rate of regeneration when compared to the *planaria* kept in spring water. The *planaria* kept in the dopamine inhibiting environment had a decreased rate of regeneration when compared to the *planaria* kept in the spring water. *Planaria* in the dopamine stimulating environment had an average daily growth rate of 0.21 mm more than average and the *planaria* in dopamine inhibiting environment had an average daily growth rate of 0.475 mm less than average.

Fig. 6. (above) *Planaria* feeding.

Fig. 7. (left) *Planaria* in control Petri dish.

Photos were taken and figures were made by experimenter except figure 1