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The Effects of Ethanol on the Regeneration and Reaction of Dugesia Tigrina

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Abstract

The purpose of this study is to determine the effects of ethanol (EtOH) on the regeneration and reaction time (RT) of *Dugesia tigrina*. Studies have shown that ethanol can negatively influence planaria's brain function and increase regeneration time. Forty, randomly-selected planaria were assigned to four concentrations of ethanol (0%, 0.01%, 0.1%, 1%). The planaria were exposed to their designated concentrations and then decapitated. The planaria's lengths (head and tail) were measured on Days 0, 4, 8, 12, and 16. Results showed that as the ethanol concentration increased, the length regenerated decreased. However, results were shown to only be statistically significant when ethanol concentration was at its highest (1%) signifying that low and moderate concentrations of ethanol did not affect planaria regeneration. The tail of the planaria generally regenerated slower than its corresponding head. After the initial regeneration experiment, in order to observe ethanol's effect on planaria reaction time, planaria were placed in a Y-maze with negative (light) and positive (hard-boiled egg yolk) reinforcements. The groups that were exposed to higher ethanol concentrations took a longer time to complete the maze, displaying a decrease in their reaction time and mobility. Results were significant in the 0.1% and 1% groups. Ethanol has been shown to decrease planarian regenerative abilities as well as their reaction time and mobility. Due to the similar central nervous system between humans and planaria as well as planaria stem cells sharing at least one gene with those of humans, findings can support and reveal the harmful effects of alcohol on regenerative processes, motor function, and reaction time within humans.

Keywords: Ethanol, Planaria, Regeneration, Reaction, Stem cells

1. Introduction

Recent studies have demonstrated the negative impacts of ethanol on nervous systems in various organisms. Ethanol, the independent variable, is known to disrupt neural function and impair cognitive processes. A link between alcohol consumption and diminished regenerative capabilities is caused by alcohol's damaging effects on cell proliferation and tissue repair processes, which has been evidenced in various species. Planaria, with their impressive regenerative capabilities, have been used as a model organism in regenerative biology and neurobiology research. Sharing a similar central nervous system and sharing the presence of stem cells with humans makes them particularly relevant for investigating the effects of ethanol. As planaria stem cells are known to share at least one gene with human stem cells, the implications of this research may extend beyond planaria and have significant implications for understanding the harmful effects of alcohol on regenerative processes, motor function, and reaction time in humans. The objective in this study is how different concentrations of ethanol affect the stem cell-induced regeneration of planaria as well as their mobility and reaction time. The hope is to study the extent of planaria's ability to regenerate when exposed to ethanol, how this exposure affects reaction time, and apply our findings to the neurobiology of human regeneration and reaction. The audience will understand the risks that may be imposed on their stem cells when taking

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part in alcohol consumption. The hypothesis states that ethanol will significantly decrease the stem cells' ability to regenerate the planaria while also deteriorating their reaction time and motility. The anticipated findings will demonstrate a negative correlation between ethanol concentration and successful planaria regeneration. A decline in the reaction time and mobility of planaria is expected to decrease as ethanol exposure increases as well. The results are expected to accurately reflect the hypothesis and mirror the same consequences in humans as humans and planaria both have stem cells and similar central nervous systems. Ethanol (EtOH), more commonly known as absolute alcohol or drinking alcohol, is a substance or matter that a vast population abuses. While moderate amounts of alcohol can yield pleasing effects, large ingestions of alcohol have severe detriments to the human nervous system. Consumption of alcohol slows down nerve activity and communication in the brain, specifically in the hippocampus. Alcohol can also destroy nerve cells or neurons, which are the fundamental units in the brain and nervous system. People who drink too much alcohol are often deficient in vitamin B-1, or thiamine. This vitamin is vital to providing energy to brain and nerve cells (Martin, 2003). Heavy alcohol abuse is linked to illnesses such as dementia, cancers, diabetes, liver problems, and other health conditions. Extreme exposure may also cause unconsciousness, asphyxiation, and death. The dependent variable is the function and memory of stem cells in planaria. Planaria or Tricladida are flatworms of the class Turbellaria (phylum Platyhelminthes). In this particular experiment, Brown planaria (*Dugesia tigrina*) will be used. D.tigrina is most commonly found in North America in freshwater habitats such as lakes, ponds, and streams. The worm has a flattened body and exhibits cephalization, a trend in evolution where nervous systems and sensory organs are positioned near the animal head. The body surface is covered with cilia which allow for the worm to glide around. “Sensory lobes known as auricles make the head region triangular, and eye spots called ocelli are found on the head” (Saccomanno, n.d.). The body is usually brown with white and yellow spots and the average length is 9 to 15 mm. D.tigrina are hermaphrodites, meaning they yield both female and male sex organs. Depending on the population and environment, they can reproduce both sexually and asexually. Planaria have an amazing regeneration capacity, which makes them an acceptable model for regeneration studies and molecular biology. They can regenerate new body parts and/or entire organisms in a few days to weeks. “This flatworm has been increasingly used as a model organism for educational and research purposes to better understand both tissue regeneration as a result of wear and tear and brain development as the main neural processing center in animals" (Saccomanno, n.d.). There are different characteristics that can be noted to observe planaria’s behavior: negative phototaxis (avoidance of light), C-like movements (C-shape of the planaria’s body when exposed to a stressor), etc. Genetic research for these organisms is being conducted to expand knowledge of human growth, development, and hopefully tissue regeneration. The cells responsible for regeneration are called stem cells and they are the basis of all cells. Specialized functions are generated from stem cells. In a lab, if the stem cells are under the right conditions, they divide into daughter cells. “Stem cells can be guided into becoming specific cells that can be used in people to regenerate and repair tissues that have been damaged or affected by the disease” (Mayo Clinic Staff, 2022). This is why stem cell research is so prevalent because many groups of people can benefit from regenerative medicine derived from stem cell research. Flatworms’ abilities to regenerate arise from an abundant population of adult somatic stem cells known as neoblasts which are distributed throughout the worm’s body. When a part of a flatworm is amputated, neoblasts are activated and they migrate to the wound. The neoblasts divide and their offspring form a blastema which is a mass of undifferentiated cells that will eventually develop into specific organs or body parts. Researching the effects of ethanol on planaria can provide insight into the consequences of alcohol consumption on regenerative processes in living organisms. Considering the existing knowledge, this study aims to utilize planaria's capabilities to assess the impact of increasing ethanol concentrations on the regeneration of planaria over a time interval as well as investigate ethanol effects on their reaction time. This research will reveal the implications of excessive alcohol intake on human health, specifically on humans’ regenerative biology.

2. Materials and Methods

The research subjects in this experiment consisted of 40 Brown planaria (Dugesia tigrina). The specific species of planaria was decided on because they are the most common species, they do not require high maintenance, and previous studies have used Brown planaria as well. The planaria were purchased from the Carolina Biological Supply

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Company. They arrived in four glass containers filled with spring water. The planaria were kept in the basement in the dark at room temperature. The planaria were cultured in the same glass containers until the experiment began, which was a week after they were received. To culture the planaria, the spring water was changed every couple of days using 'Poland Spring' spring water purchased from the local grocery store. The worms were also fed with hard-boiled egg yolk by placing it in the water before the water was changed. After 45 minutes to an hour, the egg yolk had to be retrieved and the fouled water was cleaned out and replaced with fresh spring water. As a safety measure, the scientists wore goggles, face masks, and gloves. To dilute the different concentrations of ethanol, the stock solution which is 1% of ethanol, was created. Using a graduated pipette, 5 mL of ethanol was measured from a flask. The ethanol was placed into a 500 mL graduated cylinder. Using a normal pipette, the graduated cylinder was filled up with distilled water up to the neck of the cylinder. Then, the cylinder was inverted several times before continuing to fill it up to the line of the cylinder, until the bottom of the meniscus was touching the line. A similar approach was used to create the 0.1% ethanol solution and the 0.01% solution. 5 mL of the stock solution was measured and mixed with distilled water in a 50 mL graduated cylinder. Finally, 5 mL of stock solution was mixed with distilled water in a 500 mL graduated cylinder. All solutions were poured into separate flasks for storage purposes and a stopper was put on all three flasks to prevent evaporation. To create a Y-shaped maze for the planaria, a piece of green plastic was used as the base. The green color allowed contrast between the brown color of the planaria and the plastic so they could be seen easily. For the walls of the maze, cardboard was used. Two "long" pieces with a width of 2 inches, a length of 4 inches, and a height of 3 inches were cut. The 6 smaller pieces had measurements with a width of 2 inches, length of 2 inches, and height of 3 inches. Then, hot glue was used to glue the cardboard pieces together on the base in the desired Y-shape. On Day 0 of the experiment, 16 glass Petri dishes were disinfected by spraying them down with alcohol. 8 Petri dishes were labeled as the "heads" group and labeled the remaining as the "tails" group. Two "head" Petri dishes were designated to each concentration of ethanol (0%, 0.01%, 0.1%, 1%). Similarly, two Petri dishes would be labeled as the "tail" groups for each concentration. For example, the Petri dishes in the control group (0% ethanol solution) were labeled as "0% head group 1," "0% ethanol head group 2," "0% tail group 1," and "0% head tail group 2." After labeling, the Petri dishes were placed into the freezer for around 10-15 mins. Using a plastic dropper, 5 planaria were put into each cooled Petri dish labeled as the "head" group. The Petri dishes labeled as the "tail" group were left empty. The cooling slowed down the planaria's movement, making it easier to accurately measure each planaria. Using the same dropper, around 8 drops of spring water were dropped into each Petri dish, allowing the planaria to stretch to their full length. To measure the planaria's initial length, a centimeter ruler was used. Each planaria was placed into a different Petri dish after measuring to ensure that no planaria were measured twice. After all the planaria in a group were measured and recorded, they were placed back into their designated Petri dish to soak in their designated ethanol groups for 45 minutes. From here, the decapitation process began. Using a disinfected scalpel, each planaria was decapitated horizontally across the middle of their abdomens. The "heads" and "tails" were then placed into their designated groups and Petri dish. Each Petri dish was then filled to the top with spring water. On Days 3, 6, 9, 12, and 15, the spring water was switched out. The planaria were fed with hard-boiled egg yolk on Days 6 and 12. When the regenerating planaria "split," the smallest, immobile part was removed to ensure the data would not be skewed. The measurements of the planaria – heads and tails – were recorded on Days 0, 4, 8, 12, and 16. On Day 16, the second part of the experiment was initiated. A small piece of hard-boiled egg yolk was placed in the left leg of the Y-maze as a positive reinforcement. A light was shone on the right leg of the Y-maze as well as the starting line as a negative reinforcement, due to planaria's negative phototaxis. One person supervised the maze by placing the planaria into the maze at the "starting line" using a dropper, directing the other person on when to start the timer and when each planaria reached the egg yolk, and taking each planaria out of the maze once they reached the egg yolk. The other would record the time (in seconds) it took for each planaria to reach the positive reinforcement. All 16 Petri dishes were tested through the maze using the same procedure and the times were recorded for each planaria in their designated groups. Statistical analyses included two sample t-tests comparing the control group (0% EtOH) to an ethanol solution (0.01%, 0.10%, or 1% EtOH) for mean differences, in both regeneration and reaction time.Statistical analyses encompassed two-sample t-tests comparing the control group (0% EtOH) to an ethanol solution (0.01%, 0.10%, or 1% EtOH) to assess mean differences in both regeneration and reaction time. Two-sample t-tests are a type of hypothesis test used to compare the means of two groups to determine if there is a significant difference between

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them. In this experiment, t-tests were employed to compare the "control group" (0% ethanol) with each of the ethanol solutions (0.01%, 0.10%, and 1% EtOH) separately. These tests assess whether there were statistically significant differences in both the regeneration and reaction time between the control group and each ethanol solution. The p-value, or probability value, is a statistical measure that indicates the strength of evidence against the null hypothesis. In the context of this experiment, the null hypothesis would be that there is no significant difference in the measured parameters (regeneration and reaction time) between the control group and each ethanol solution.
The alternative hypothesis would suggest that there is a significant difference. The p-value quantifies the probability of obtaining the observed results, or more extreme results, assuming that the null hypothesis is true. In other words, a small p-value suggests strong evidence against the null hypothesis, while a larger p-value indicates weaker evidence. In this experiment, if the p-value is less than the chosen significance level (alpha, set as 0.1 for regeneration and 0.05 for reaction), researchers would conclude that there is enough evidence to reject the null hypothesis in favor of the alternative hypothesis. Alternately, if the p-value is greater than alpha, it suggests that there is not enough evidence to reject the null hypothesis.

3. Results

Table 1. Linear regression line slopes of the average planaria length in respect to days.

Ethanol group	Regression Line Slope (mm/day)
0%	0.1758
0.01%	0.1195
0.10%	0.0456
1%	0.1406

Table 2. Average number of seconds taken to complete maze by planaria separated by ethanol groups displayed in a chart.

Ethanol Group	Time (in seconds)
0% solution	97
0.01% solution	109.75
0.1% solution	136
1% solution	169

Table 3. P values for t-tests comparing regeneration means; set at an alpha of 0.1

2 Sample t-test for Mean Differences (Regeneration)	
Ethanol groups	P value
Control vs 0.01%	0.9542
Control vs 0.1%	0.2702
Control vs 1%	0.0964

Table 4. P values for t-tests comparing reaction means; set at an alpha of 0.05

2 Sample t-test for Mean Differences (Reaction)	
Ethanol groups	P value
Control vs 0.01%	0.7219
Control vs 0.1%	0.0045
Control vs 1%	0

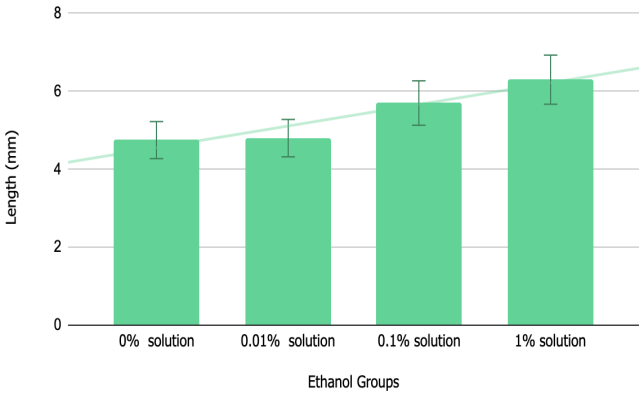

Figure 1. Mean Difference of Planaria Initial Length (mm) and Regenerated Length (mm), Amount Required for Planaria to Reach Initial Length.

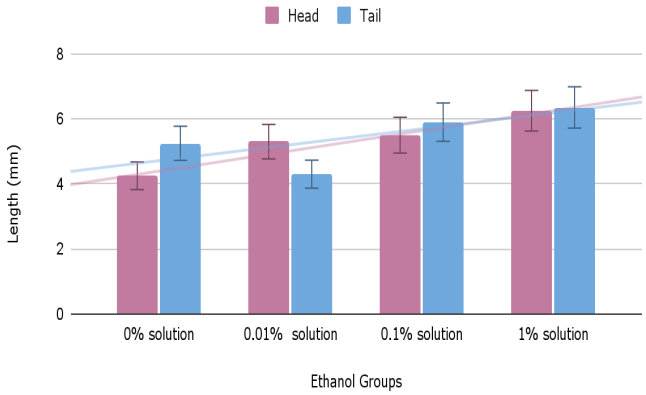

Figure 2. The Length (mm) Required to Reach Initial Length from Day 0 Blocked by Head and Tail
Error bars represent 1 standard deviation from the mean

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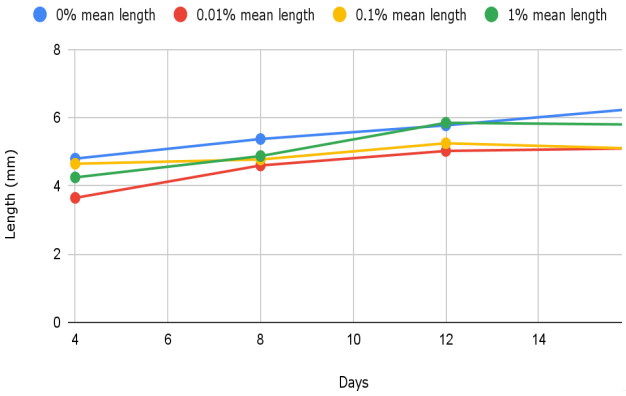

Figure 3. Average Planaria Length (mm) in Respect to Days

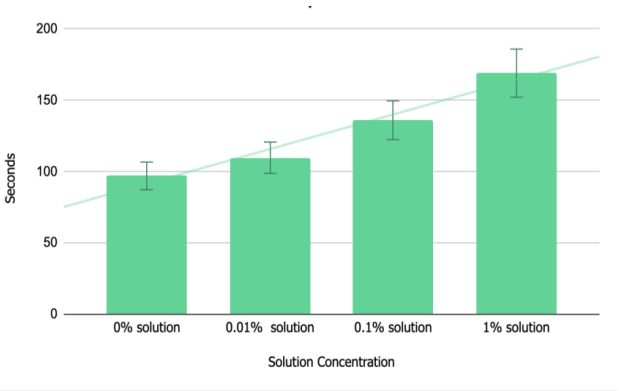

Figure 4: Average number of seconds taken by planaria to complete the maze separated by ethanol concentrations.

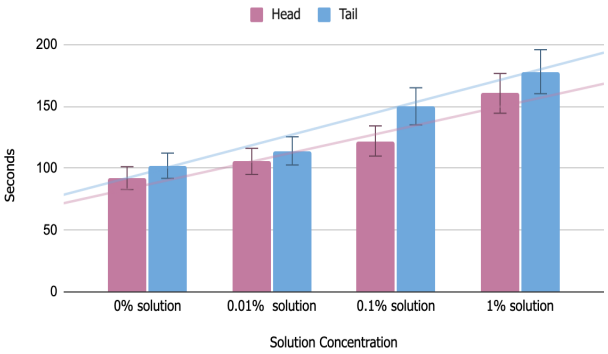

Figure 5. Average number of seconds taken by planaria to complete the maze, separated by ethanol concentrations and blocked by head and tail

4. Discussion

Statistical analysis (refer to Table 3) comparing the data between the control group (0% EtOH) and the 0.01% EtOH group attempting to support an effect of ethanol on regeneration has shown to be statistically insignificant. The minimal amount of ethanol within the solution has not caused a significant difference in the regrowth of the planaria. Because humans and planarian stem cells share at least one gene that may cause regeneration, they are widely used to research human regeneration (Planaria: A window on regeneration, n.d.). Also, since EtOH is used in alcohol products meant for human consumption, these results may apply to alcohol's effects on human regeneration; a nominal amount of alcohol – which contains ethanol – consumption may not affect the stem cell-induced regeneration of human cells. This conclusion is consistent with the results comparing data from the control group and 0.1% EtOH solution. However, the p-value is lower and closer to alpha than the p-value in the 0.01% EtOH group. Further, Figures 1 and 2 show a clear distinction between the amount left to regenerate in the 0.01% EtOH group and the amount left to regenerate in the 0.1% EtOH group. The 0.1% EtOH group has a much higher difference between its initial and regenerated length meaning it has not regenerated as fully as the 0.01% EtOH group. While both groups have been shown to be statistically insignificant, the group with a higher ethanol concentration may more likely affect regeneration due to the large difference between the p values for the 0.1 group and the 0.01 group.. Therefore, while moderate alcohol consumption (represented by the 0.01 group) may not significantly affect human cell regeneration, it is still much more likely to have harmful effects on regenerative properties in comparison to a low alcohol intake. Unlike the previous results, a statistical analysis comparing the data between the control group and the 1% EtOH group clearly supports the effect of ethanol on regeneration (refer to Table 3). The highest ethanol concentration has caused the planaria to grow back significantly less than the control group as seen in Figure 1. Since the 1% EtOH solution caused planaria to regenerate significantly less than the control, human consumption of higher percentage ethanol in the form of alcoholic beverages may harm regenerative properties such as wound and tissue healing. To put it simply, an excess in drinking may cause the diminishing of tissue regeneration. This conclusion is consistent with a previous study that found delayed wound closure in alcohol-exposed subjects (Curtis et al., 2014). Further, as seen through the liver's profound regenerative properties, stem cells are located in the liver. Many studies have supported

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the fact that liver regeneration is harmfully affected by binge drinking, and this study only further supports that conclusion by gathering evidence that exhibits ethanol exposure inhibiting stem cell regenerative capabilities. However, although planaria stem cells and regeneration were studied in this experiment, they are only used as a general model that can aid researchers in gathering evidence applicable to human regeneration, so any conclusion applied to human regenerative properties should be taken loosely. Similarly to this experiment, which displays slower stem cell induced regeneration in planaria when exposed to high amounts of ethanol, studies have found slower neural stem cell growth in humans when exposed to high amounts of alcohol. (Content: Alcohol Inhibits Cell Growth, n.d.). Neural stem cells or NSCs are self-renewing cells that can generate neurons; impeding NSC regeneration lessens the amount of neurons within the brain. This neuron deprivation in the hippocampus leads to memory and learning issues which means binging alcohol can severely damage brain functions. NCSs can also repair peripheral nerve injury and if neural stem cell growth continues to slow down due to ethanol/alcohol exposure, processes involved in regeneration and such injuries may be impaired (Wang et al., 2017). Since binge drinking can cause improper NCS amounts, the consequential peripheral nerve injury can then cause weakness, numbness and pain, commonly found in the hands and feet. Thus, the decrease in planarian regeneration when exposed to high ethanol concentrations supports various findings of decreased stem cell growth due to high alcohol consumption. As stated, Figures 1 and 2 display that as the concentration of ethanol increases, the amount left needed to regenerate also increases, exhibiting slower regeneration for planarian in higher ethanol concentrations. Interestingly, 3 of the 4 solution groups displayed a higher difference in initial and final length in the tail groups than the heads. This difference may mean that planaria heads regenerate faster than their tails, signifying most of their stem cells and regeneration inducing factors lie within the upper half of their bodies. Additionally, while the amount left to regenerate between the different concentrations displayed a consistent positive trend (as seen in Figure 1), Table 1 and Figure 3 display a fluctuation. For the 0%, 0.01%, and 0.1% groups, Table 1 shows a continual decrease in regression line slopes as the concentrations increased, meaning that as the concentration increased, the rate of regeneration decreased. However, the 1% concentration group deviated from this trend as the steepness of its slope (refer to figure 3) and linear regression line slope values were even higher than that of the 0.01% groups. These results may either support ethanol-increasing regeneration for a short period of time or inconsistency in data due to human error when measuring the planaria during the regeneration period. While the planaria were initially cooled for around 10-15 minutes, inconsistency in cooling took place during the 16-day trial, causing errors in measuring their lengths. To combat this error, the groups could have been cooled prior to every measurement rather than only on the initial day to ensure optimal results in planaria measurement. Although the 1% EtOH group regression slope displays an inconsistency with the hypothesis, Figures 1, 2, and the t-tests continue to exhibit the fact that the 1% EtOH groups regenerated much less than any other group. Figure 3 exhibits a continual increase in planarian length until Day 12, where all groups besides the control slightly decrease in their length. This drop may signify that ethanol can reduce the planaria's ability to continue regenerating as the control group did not experience this "drop" in length.

However, this conclusion lacks much support and should be further investigated in order to confirm. Much of this study's findings support the beginning of the hypothesis that ethanol impairs planaria regeneration, although the experiment more specifically points out that only high amounts of ethanol concentration seem to have an effect. These findings correlate with a similar study where the ethanol groups had an initial delay in regeneration; however, this study found that development in ethanol groups assume normality after the initial delay (Collins, 2007).

Statistical analysis (refer to Table 4) comparing data from the control group and the 0.01% EtOH group that attempted to show an effect of ethanol on planarian reaction and movement was statistically insignificant. A low percentage of EtOH may have no effect on planarian reaction; however, an analysis comparing the control group with both 0.1% EtOH and 1% EtOH groups displayed very strong evidence supporting the hypothesis. Therefore, when planaria were exposed to moderate and high concentrations of ethanol – subjective to the planarian – their reaction time and movement significantly decreased as evidenced by the greater amount of seconds taken to complete the maze. Figure 4 and Table 2 offer a graphical and numerical, respectively, display of higher ethanol concentration increasing the amount of time taken for planaria to finish the maze. A study's results suggested that alcohol impairs cognitive performance, specifically premotor reaction time (Hernandez, 2007). Premotor reaction time refers to the crossover phase when motor neurons in the brain activate muscle contractions. The slow reaction time of planaria in higher

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EtOH concentrations, as evidenced by the time it took for the planaria to complete the maze and move away from the light, may offer support as to why humans experience impaired premotor reaction time when alcohol is consumed. Figure 5 exhibits an increase in maze completion time as ethanol concentration increases, but also points out that the tail groups took a longer time to complete the maze, signifying that movement and reaction of planaria are slower within the regenerated tails in comparison to their head counterparts. Nerve cells – responsible for reaction time – are present in a planaria's brain. If EtOH generally hindered the regeneration of the planaria tails more than heads (refer to Figure 2), it can be assumed that the tails have fewer nerve cells compared to the head counterpart. Fewer nerve cells lead to slower reaction time, as evidenced by the tail groups' slower completion time for the maze. During the experiment, some planaria were observed to crawl towards the light before reaching the egg, demonstrating a positive phototaxis - an organism's reaction towards light - as compared to their usual light aversion. Studies have shown that negative phototaxis is temporarily suppressed after the decapitation of planarians, which suggests that the head regeneration process is when the photoreceptor system regeneration occurs (Inoue et al., 2004). However, due to confounding variables within these experiments, it is unclear whether the negative phototaxis in planaria was due to ethanol or regeneration. Future studies could determine the cause of negative phototaxis and due to similar neurobiology of planaria and humans, extending research upon regeneration and ethanol-altering planarian phototaxis may aid in research about alcohol affecting human light aversion.

5. Conclusion

The purpose of this experiment was to reveal ethanol's effects on the stem cell regenerative abilities present in planaria and their reaction time and movement. The experiment produced results that were somewhat expected. Higher concentrations of EtOH decreased planaria's regeneration and reaction/motility; however, regeneration results were only statistically significant for the 1% groups and reaction results were only significant for the 0.1% and 1% groups. Only high concentrations of ethanol notably affected regenerative abilities while only moderate and high concentrations of ethanol significantly affected planaria reaction and movement. While the general trend of this experiment exhibited ethanol decreasing planarian regenerative abilities as well as their reaction time and movement, the rate of regeneration for the 1% EtOH groups was the second-highest compared to the other ethanol groups, deviating from the hypothesis. Researching the effect of ethanol on stem cells and the neurobiology of planaria can help scientists understand the adverse effects of alcohol on stem cells and thus, the neurobiology and regenerative properties of humans.

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References

Chong, et al., (2017, December). *Roles of neural stem cells in the repair of peripheral nerve injury*. Neural regeneration research. Retrieved December 5, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5784362/

Content: Alcohol Inhibits Cell Growth. The Alcohol Pharmacology Education Partnership. (n.d.). Retrieved December 5, 2022, from https://sites.duke.edu/apep/module-3-alcohol-cell-suicide-and-the-adolescent- brain/content-alcohol-inhibits-cell-growth/

Curtis, et al., (2014). Episodic binge ethanol exposure impairs murine macrophage infiltration and delays wound closure by promoting defects in early innate immune responses. *Alcoholism: Clinical and Experimental Research*, *38*(5), 1347–1355. https://doi.org/10.1111/acer.12369

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ISSN 2688-3651 [online]

Features. Microscope Imaging Station. Planaria: A window on regeneration. (n.d.). Retrieved December 5, 2022, from http://annex.exploratorium.edu/imaging_station/research/planaria/story_planaria1.php

Hernandez, O. H., Ke-Aznar, V. I., & Vogel-Sprott, M. (n.d.). *Alcohol impairs the cognitive component of reaction time to an omitted stimulus: A replication and an extension*. Journal of studies on alcohol and drugs. Retrieved December 5, 2022, from https://pubmed.ncbi.nlm.nih.gov/17286346/

Mayo Foundation for Medical Education and Research. (2022, March 19). *Answers to your questions about Stem Cell Research*. Mayo Clinic. Retrieved December 5, 2022, from https://www.mayoclinic.org/tests- procedures/bone-marrow-transplant/in-depth/stem-cells/art-20048117

"Planaria: A Window on Regeneration." *Exploratorium*, https://www.exploratorium.edu/imaging_station

Planarians. Brain and Cognitive Sciences. (n.d.). Retrieved December 5, 2022, from https://bcs.mit.edu/planarians#:~:text=Planarians%20have%20cephalic%20ganglia%20(the,myriad%20types%2C%20including%20complete%20decapitation.

Saccomanno, R. (n.d.). *Dugesia tigrina*. Animal Diversity Web. Retrieved December 5, 2022, from https://animaldiversity.org/accounts/Dugesia_tigrina/

Takeshi, et al., (n.d.). *Morphological and functional recovery of the Planarian photosensing system during head regeneration*. Zoological science. Retrieved December 5, 2022, from https://pubmed.ncbi.nlm.nih.gov/15056922/

The effect of caffeine and ethanol on flatworm regeneration. (n.d.). Retrieved December 6, 2022, from https://dc.etsu.edu/cgi/viewcontent.cgi?article=3389&context=etd

U.S. Department of Health and Human Services. (n.d.). *The role of thiamine deficiency in alcoholic brain disease*. National Institute on Alcohol Abuse and Alcoholism. Retrieved December 5, 2022, from https://pubs.n