**Science Research Proposal for Review Board: Living Specimen Experiment**

# Impact of Caffeine on Memory Retention in Acephalous Planarian Flatworms

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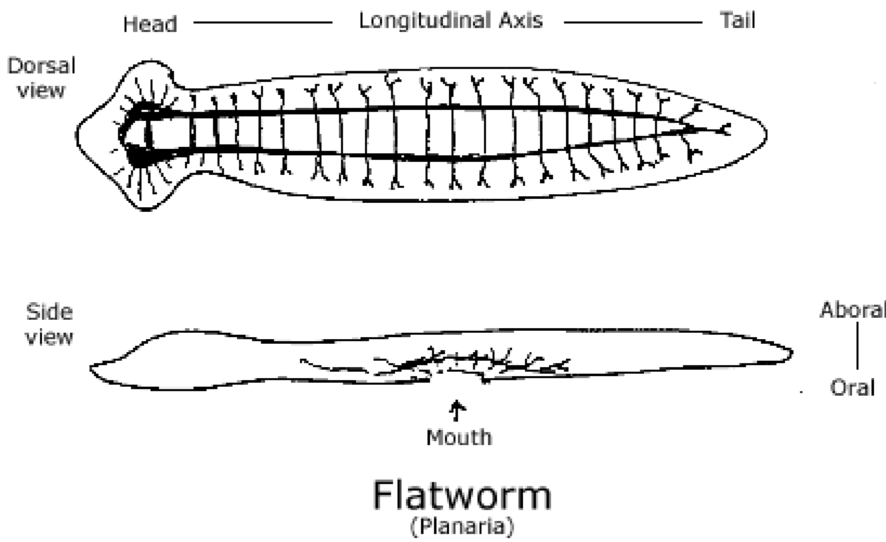
## I. Introduction

The proposed experiment would test the impact of caffeine on memory retention of acephalous planarians. Research has shown that planarian flatworms are able to repeat conditioned responses, or "learned" responses, after having their heads removed and regenerating new ones. (Levin and Shomrat, 2013).This phenomenon shows that memory in planarian flatworms may not be based solely around the central nervous system, and that memory can be retained through head regeneration. In addition, research on caffeine as an adenosine inhibitor has shown that in many organisms, caffeine can improve long term memory (Kopf, Silvia R., et al., 1999).While caffeine's impact as a stimulant on planarian behavior has been studied before, the effect of caffeine on memory retention in planarian flatworms remains unexplored.

This experiment would require planarian flatworms to be conditioned to a specific response through a conditioning paradigm. The planarian flatworms would then be divided into groups. One group would not be exposed to caffeine, to serve as a control group. The other groups would be exposed to caffeine. The heads of the planarians would then be removed. The ability of the planarians to repeat the conditioned response would be measured, and the results would be compared. My hypothesis is that long term memory is improved with the introduction of caffeine.

## II. Background

Planarian flatworms are primarily carnivorous free-living invertebrates. They can be all different sizes and colors, but have many of the same traits regardless of species. Planarian flatworms are hermaphroditic; some species are capable of sexual reproduction, some reproduce through fission, and others reproduce through both depending on environmental conditions (Newmark and Sanchez-Alvarado, 2001). Planarian flatworms have come to prominence recently as a way of studying stem cells, regeneration, behavior, and memory (Gentile, et al., 2011).




In the late 1700's, Peter Simon Pallas noted that planarians were able to generate an entirely new body from no more than a fragment of the head (Newmark and Sanchez-Alvarado, 2001). Pallas' findings were the first of their kind, and were soon confirmed by both Shaw and Draparnauld with the discovery that planarians undergo asexual reproduction through fission (Newmark and Sanchez-Alvarado, 2001). In 1898, Thomas Hunt Morgan, the "Father of Genetics", published his article "Experimental studies of the regeneration of Planaria maculata". In his article, he describes some of the various experiments he performed in testing the regenerative abilities of planarian flatworms. Morgan found that planarians have a natural sense of polarity, enabling them to identify which way is "up" during regeneration. In addition, he found that planarians could be divided in any direction and still be capable of regeneration. Not only could they be divided in multiple directions, but they could be divided into up to 279 pieces (Morgan, 1898).

Planarians regenerate quickly, making them an ideal model for experiments on regeneration. Additionally, planarians can regenerate indefinitely (Tana, et al., 2012) allowing scientists to perform as many experiments as they feel necessary. This is because planarian flatworms have the ability to create endless telomerase, an enzyme that regulates the length of telomeres (Tana, et al., 2012). Telomeres trigger cell death and aging. As a telomere becomes shorter, a cell becomes less able to regenerate. This shortening causes cells to begin to show signs of aging, until it eventually dies (Tana, et al., 2012). Planarian flatworms also contain pluripotent stem cells called neoblasts throughout their body. These cells enable them to recreate any missing tissue through blastema based regeneration (Alvarado-Sanchez, 2010).

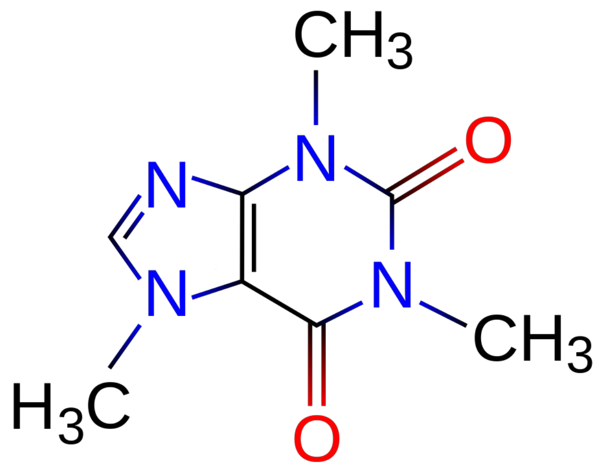
Planarian's incredible regenerative abilities enable them to regenerate fully functioning central nervous systems. In 2013, Michael Levin and Tal Shomrat, trained planarians to become familiar with a specific environment. This was done by putting planarians through a training paradigm consisting of a chamber utilizing planarians natural photosensitivity. Once properly conditioned, the researchers amputated their heads. The regenerated offspring were then tested for retention of learned behavior and their responses were measured. The planarians repeated their conditioned response, and it was deduced that each planarian remembered its conditioning, despite having developed a new central nervous system (Levin and Shomrat, 2013).

**III. Rationale**

The objective of this study is to determine if caffeine has an impact on long-term memory through something as traumatic as head regeneration. My hypothesis is that caffeine will have a positive impact on the long-term memory of planarian flatworms after head regeneration.

Research has shown that the central nervous system of planarian flatworms is different from other invertebrates. The planarian brain is actually more similar to the brain of a vertebrate organism in the way its synapses are organized and in some of the features of its neurons (Sarnat H.B. and Netsky M.G., 1985).The planarian central nervous system has been compared to that of mammals, including mice and humans. Scientists have discovered numerous homologues between human genes and planarian genes, and the presence of enzymes in planarian brians found in human brains (Sarnat H.B. and Netsky M.G., 1985).

Studies have shown a direct correlation between caffeine and long term memory. In mice, as well as a number of other organisms, caffeine has been shown to inhibit the enzyme adenosine. By inhibiting adenosine production, memories are retained for longer periods of time (Kopf, Silvia R., et al., 1999). In mice, not only has it been shown to improve long term memory by inhibiting adenosine, but it has been suggested that when caffeine is given to mice, it can prevent Alzheimers (Arendash G.W. and Cao C., 2010). While caffeine has been tested on planarian flatworms to study behavioral patterns and regeneration, its effect on long term memory has not been explored.




Recently, stem cells have been suggested as a remedy for diseases of the brain (Abdel-Salam, 2011). This study would increase the understanding of how stem cells developing into brain tissue are able to recall old memories, and perhaps could even help to preserve memories that could be lost. All this could be done without the use of the controversial model for stem cell research - human fetuses. As no research completed thus indicates that planarian flatworms feel pain, and in fact reproduce through fission or “dropping their tails” naturally, the dissection of planarian flatworms is a far more humane, more economically viable, and less controversial alternative to the use of fetuses.

**IV. Method**

This study will test the impact of caffeine on planarian flatworms through head regeneration, based on the hypothesis that that long term memory can be improved through head regeneration with the introduction of caffeine. Planarian flatworms will be conditioned to maneuver through an environment. They will be guided through using the aid of light and electrical shocks. Planarian flatworms naturally recoil from light, so they will be conditioned to move towards a lighted area. This goes against their natural instinct, which would exhibit a learned behavior.

I will set up four groups of planarian flatworms: Group A will consist of the regenerated offspring of conditioned worms; Group B will consist of the regenerated offspring of conditioned worms exposed caffeine prior to regeneration; Group C will consist of the regenerated offspring of conditioned worms exposed to caffeine during regeneration; Group D will consist of the regenerated offspring of conditioned worms exposed to caffeine after regeneration. This will show at what point caffeine is most effective in memory retention.

One issue involved in studying behavioral patterns of an organism is observer bias. The observer may unintentionally influence the test organisms or even observe something incorrectly based on a conjecture regarding the experiment. To avoid observer bias, I plan to seek the help of my colleagues in measuring memory prior to and following the experiment. Data will be collected in a data table displaying how many electrical shocks were required to guide the planarian toward its target area.

**V. Procedure**

Materials:

In order to carry out the experiment, a number of different materials will be needed. Many of these are needed to procure and sustain a colony of Shmidtea *mediterranea* planarian flatworms. To provide a reliable sample, the experiment calls for at least two hundred flatworms. This will require a large plastic container, Instant Ocean salts, and beef liver to feed the planarians. The experiment will also need caffeine, which will be diffused into the water of the experimental groups. A training paradigm will be constructed out of plastic, a light source, an anode, and a cathode to condition the planarian flatworms. A dissection kit will be required to dissect the planarian flatworms in the most humane way possible.

Experimental Procedure:

1. Separate groups - Groups of planarian flatworms will be separated at random. The planarians will be divided into four groups, and will live in separate containers. Each container will be labeled with A, B, C, or D.

2. Expose Group A to caffeine - The planarians in Group A will have caffeine introduced into their water. Due to the limited research done involving planarian flatworms and caffeine, experiments will need to be done to determine the appropriate dosage of caffeine.

3. Condition - All groups of planarian flatworms will be conditioned. See Appendix B for more information on the process of conditioning.

4. Amputation - All groups of planarian flatworms will undergo head amputation.

5. Expose Group B to caffeine - The planarians in Group B will have caffeine introduced into their water.

6. Allow regeneration - The flatworms will require time to regenerate their heads before their memory can be tested. It should take approximately one week for each flatworm to regenerate a fully functioning central nervous system. See Appendix C for more information on planarian regeneration.

7. Expose Group C to caffeine - The planarians in Group C will have caffeine introduced into their

water.

8. Measure memory - In order to measure memory, I plan to ask for the aid of third party observers, thus preventing any kind of observer bias. Each observer will evaluate a group of worms, recording how many times the worm must be shocked in order for it to navigate towards the light. The data will then be compiled and analyzed.

9. Record findings - All data will be compiled in a data sheet, which has been attached to the end of the proposal.

10. Analysis - All data will be analyzed, looking for a correlation between the addition of caffeine and memory retention. If none is found, then my hypothesis may be incorrect. All steps shall be reviewed and any potential variables that may come up during the experiment will be looked into.

**VI. Significance**

The mechanism of memory is still a topic of debate among scientists. While over time, theories regarding memory retention have evolved, scientists are only beginning to truly understand how memory is stored. If approved, this study would add to the body of knowledge on memory retention. Additionally, despite using a planarian brain as opposed to that of a mammal, the study would still provide insight about stem cells and their ability to hold memories. Scientists predict that stem cells will be used more frequently in medicine in the future. Some even suggest using stem cells as a therapy for Alzheimers (Abdel-Salam, 2011). This would mean reconstructing parts of the brain. By increasing the understanding of the relationship between stem cells and memory one could potentially be able to save many memories that could be lost in such a procedure.

The proposed study would also help to either support or refute the idea that caffeine could potentially improve long term memory in humans. While the impacts of caffeine have been tested on simple mammals such as mice, it has yet to be tested on the memories of planarian flatworms. This is significant because, as previously stated, planarian flatworm brains have many similarities with human brains.

**VII. Conclusion**

If the proposed experiment is approved, this will help to increase the understanding, not only of planarian flatworms, but of the mechanisms of the mind. If this experiment were to find, as hypothesized, a positive correlation between caffeine and memory retention, then new lines of inquiry regarding caffeine's impact on the brain could be explored. If supported, this would suggest that caffeine may have applications such as being used as a way of treating memory loss. It also would explore the impact of caffeine on stem cells, which would open up opportunities of experimentation regarding the impacts of stimulants on stem cells and regeneration.

## VIII. Appendices

Appendix A:

*Planaria Care*

To create a large colony of planarian flatworms, they must be kept under ideal conditions. S. *mediterranea* planarian flatworms require clean salt water kept around 70 degrees. Water should be changed twice a week, after feeding. A pea-sized portion of beef liver is usually sufficient for about 50 planarians. After allowing the planarians to feed for an hour or two, check the water to see if they've been fed properly - if food is left behind, they've had enough; if no food is left behind, they've haven't eaten enough. The flatworms should be fed infrequently to prevent them from being overfed and becoming sluggish (McConnell, 1965).

Appendix B:

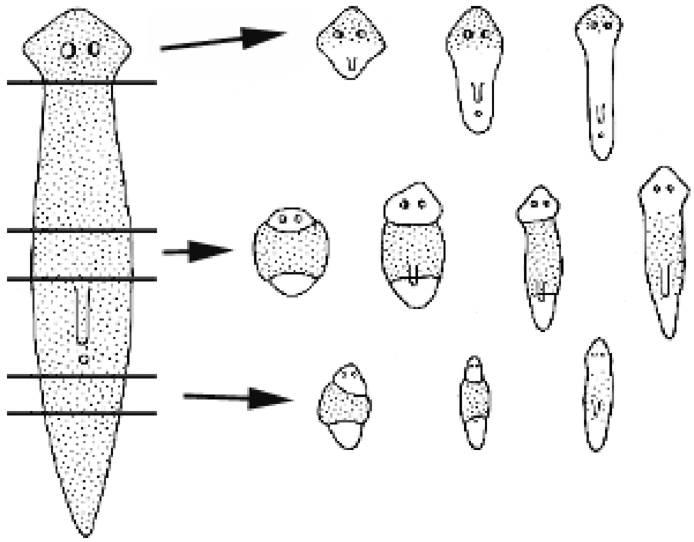
*Conditioning*

To condition the planarian flatworms, I will construct a training apparatus. To do this, I will take a smooth plastic container and create a Y-shaped maze-like structure. At one end of the maze will be a cathode and at the other end will be an anode. On one side of the Y-shaped maze will be a bright light and a food reward. The planarians will be trained by being given a light shock when they navigate away from the light (which they will naturally do, as planarians are extremely sensitive to light and will typically avoid it). When a planarian begin to move towards the light (the target area) without any shock stimulation, then the planarian has successfully been conditioned. (McConnell, 1965).

Appendix C:

*Regeneration*

Planarian flatworms contain pluripotent stem cells called neoblasts throughout their body, which enable them to recreate any missing tissue. Another factor in the indefinite regeneration of planarian flatworms is telomerase. As a telomere becomes shorter, a cell becomes less able to regenerate. The cell will begin to show signs of aging, until it eventually dies. This does not occur in planarians, making them able to regenerate indefinitely as well as biologically immortal (Tana, et al., 2012). Additionally, planarian flatworms can reproduce both sexually and asexually, depending on the species. Planarians reproduce asexually through fission by removing their tails. From their tails, a new planarian flatworm develops. New planarian flatworms can grow from fragments as small as 1/279th (Morgan, 1898).



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