Category: Animal Science

Research Plan

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Can Green Tea Alleviate the Effects of Stress on Learning and Memory in the Great Pond Snail (*Lymnaea stagnalis)*?

Rationale:

As of 2017, it has been reported that 46.6 million Americans live with a mental disorder. The most common is generalized anxiety disorder (GAD) with over 40 million Americans diagnosed (The National Institute of Mental Health 2019). In 2015, the American College Health Association concluded that 85.6% of students had felt overwhelmed in the past year (Carlson 2016). When students are stressed and anxious there is a negative effect on their learning and memory (Carlson 2016). As a result, academic performance may be negatively affected. Due to an increase in the number of individuals diagnosed with memory associated diseases and the correlation between stress and memory, researchers are investigating holistic ways to improve memory. In 2017, Centeno found that there are micronutrients called flavonoids in green tea (such as epicatechin and epigallocatechin) that improve cognitive brain function, memory, and even learning. Green tea, and the flavonoids present in it, have been previously tested and shown to improve the memory of the Great Pond Snail (Lukowiak 2003). The Great Pond Snail is a freshwater snail commonly used in tests on learning and memory because the snail has easily observable behaviors linked to memory and large neurons (Dalesman 2013). They also respond to stressful events in a similar way to mammals, making them an excellent model species to study the effects of stress on memory (Science News 2013). The purpose of this research is to determine if green tea is a natural and effective way to alleviate the effects of stress related to learning and memory.

Hypothesis(es), Research Question(s):

1. Will stressed snails lose their ability to learn, form, and retain memories?
2. If stress does affect the snails' learning and memory retention, can green tea help alleviate the effects of stress?

If the stressed snails lose their ability to learn and form memories, then green tea will be able to alleviate the effects of the stress.

Procedures

A. Care and Maintenance

- The Great Pond Snails are kept in calcium-rich Poland Spring water that should be kept 18 to 22 degrees Celsius
- Snails are fed fresh romaine lettuce three times per week
- Water is changed once a week

B. Learning (Conditioned Taste Aversion)

- Food deprive snails for twenty-four hours before procedure
    - **Pre-Test**
        - Place snail in petri dish with pond water
        - Place 5 mL of 0.01 M sucrose solution near the mouth of the snail using a syringe
        - Observe and record the number of bites the snail takes in one minute
    - **CTA**
        - Wait 10 minutes
        - Expose the snail to 5 mL of 0.01 M sucrose solution, wait 15 seconds
        - Expose snail to 5 mL of 0.01 M of KCl solution

- **Post-Tests**
  - 10 min, 1 h, and 24 h after conditioning, repeat the pre-test method and record number of bites in one minute for each time interval

- Repeat procedures for a total of 40 snails

C. Stressing Snails

- Two weeks later, use the same snails and complete the pre-test as directed above
- Stress snails by crowding 20 snails in 100 mL of pond water for one hour. According to Lukowiak (2014), effects of stress last for several hours
- Retrain (CTA) the snails in pond water and complete post-tests

D. Stress and Green Tea

- Two weeks later, using the same snails, complete the pre-test
- Stress snails using the stressing procedure as directed above
- Retrain (CTA) the snails in a 1:4 dilution of green tea solution and complete the post-tests

Data Analysis

The number of bites the snails take during pre-tests, training, and post-tests, will be recorded in a data table. The data tables will be used to create pie charts that represent how many of the snails remembered their training. Bar graphs will also be created to represent the number of snail bites. Since the snails are essentially being compared to themselves, an Tukey Test will be used as a statistical test to determine if the differences in the groups are statistically significant and where the differences lie.

D. Bibliography

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NO ADDENDUM EXISTS