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Exposing Undergraduates To Animal Research in Psychology

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Abstract:

To expose students to the details of research involving animals for modeling and understanding psychological disorders, the course began by investigating published original research and concluded with an original experiment. Specifically, during the first 8 weeks of the semester students read and discussed pivotal papers covering important considerations of animal model research before conducting a pre-designed experiment and finally an original experiment. The original experiment exposed common goldfish to differing levels of alcohol (0, 0.04, 1%) and recorded their latency to feed and overall movement patterns, as measured by the number of sections entered in the test tank. Results illustrate that with increases in alcohol concentration latency to feed decreased while the number of sections entered increased. Details of the course and experimental set up are discussed as well as the potential for implementation in similar environments.

Keywords: psychological disorders, animal models, alcohol, goldfish, course design

Introduction

To overcome the challenge of engaging students at a small liberal arts institution in the high impact practice original research (Murray, 2018), a semester long course was designed and culminated in the completion of an experiment involving goldfish. Specifically, an upper level Psychology course, *Animal Models of Psychopharmacology*, was taught at a small liberal arts institution in southern Iowa. Through collaboration with two other areas on campus, a space and some funding for equipment were possible during the spring 2017 semester. Overarching goals of this course were exposure and analysis of animal research of psychological disorders, introduction to animal modeling with pre-designed studies and designing, conducting and analyzing an original experiment using an animal model to explore a psychological phenomenon. Course specifics pertaining to the first two goals, details of the experiment design, conduction, results and ideas for ways to expand this in similar settings are explored in the present work.

To achieve familiarity with animal research literature, students' explored literature focused on the role of animal models in understanding and treating psychological disorders. In particular students were first exposed to the work of Bird and Parlee (2000), highlighting the ethical and scientific implications of using animal models in research. By reading van der Staay (2006), students developed knowledge of the different types of animal models that exist and which is best suited for addressing particular research questions. In addition, van der Staay et al. (2009) was reviewed to highlight the importance of continuously evaluating a particular animal model's relevance, replicability/reliability, predictive, construct and external validity/generalizability. Next, the limitations of animal models of schizophrenia, depression and bipolar disorder were explored through Nestler & Hyman's (2010) review.

To begin to summarize the basic framework for understanding animal model development and use, the work of Miczek and de Wit (2008) was reviewed prior to the class as a whole creating a checklist (Appendix A) to guide reading of future research. Over the next week students read and applied the checklist to discussions on traditional models of anxiety and depression (Kalueff et al., 2007) and the novel chick anxiety-depression model (Sufka et al., 2006; Sufka et al., 2009).

At this point students were asked to find, explore, analyze and provide a class presentation on an animal model of a neuropsychiatric disorder not previously discussed (Appendix B). Students delivered group presentations on these models and used the aforementioned checklist to evaluate the validity of the models. Primarily students used a PowerPoint group presentation to discuss and evaluate particular animal models (ex; cat model of schizophrenia).

To gain familiarity with animals in research, initially students completed pre-designed experiments measuring the influence of nicotine on action potentials in crickets. In this simple experiment crickets were injected with nicotine, anesthetized, a leg removed and action potentials in the leg recorded with a spiker box (Backyard Brains, 2017). This experiment allowed students to begin to explore the basics of animal husbandry and experimentation in a format where there were expected results with which to compare. Students were then asked to find original research articles and propose simple experiments that could be conducted with either crickets or common goldfish (as these were readily obtainable and easy to maintain). After students made some proposals there was class-wide discussion of feasibility (i.e.; cost, time line, ethics) of proposed projects. The class as a whole elected to conduct an experiment in goldfish that assessed alcohol's immediate effects on movement and feeding behavior. Given the small size of the university no animal research committee was present to approve the project, but the primary investigator had previous training related to research with animals from another institution. Future researchers should attempt to at least contact a neighboring institution if there is not a committee at their institution to approve research involving animals.

The design for the original research study was based off the research of Ryback (1969) who suggested goldfish could be useful in studying alcohol's impact on memory. Being aquatic, goldfish can easily be exposed to various concentrations of alcohol and possess livers capable of alcohol metabolism. Following exposure to alcohol, goldfish demonstrate ataxic swimming, altered feeding patterns and memory impairments (Rayback, 1969). Additionally, Goodwin et al. (1971) used goldfish to study escape reflexes after exposure to 0.8% ethanol solution or water for 0.5 to 48 hours. After half an hour of exposure to 0.8% ethanol goldfish reactivity increased, demonstrating the highest reactivity after 1 hour of exposure, then showing a decline in reactivity, and tolerance, defined as non-response to an aversive light, after 30 hours. Interestingly, a second experiment showed that this tolerance can be eliminated following 24 hours spent in a tank of water (Goodwin et al., 1971). Based upon this finding the present experiment involved tests sessions separated by 48 hours to ensure alcohol from the previous test session was no longer an impact on behavior. Further, using zebrafish, Gerlai et al. (2000) contrasted the behavior of treatment naïve fish exposed to 0, 0.25, 0.5 or 1.0% alcohol concentration in a holding tank prior to measures of locomotor activity in a novel tank. Overall movement patterns were measured by dividing the experimental tank into four sections and counting entries into each section. Specifically, measures of swim patterns were taken 60 sec following exposure to the tank and again for 60 sec after 10 min of tank exposure. Results showed that when fish were exposed to low concentrations of alcohol (0.25% and 0.5%), movement activity increased, but at a higher concentration (1%), movement decreased relative to exposure to 0% (Gerlai et al., 2000). The present study was designed using a similar division of the tank to record movement behaviors.

To further expand existing literature (Rayback, 1969; Goodwin et al., 1971; Gerlai et al., 2000) and provide students with an original research opportunity, the present study investigated the movement and feeding behavior of goldfish following exposure to various concentrations of alcohol. Congruent with the work of Gerlai et al. (2000), it was expected that movement behaviors would to be slowed or impaired following alcohol exposure. Specifically, as alcohol concentrations increased, the sections entered was predicted to decrease and the amount of time to eat to increase.

Materials

Nine common goldfish obtained from PetSmart in Des Moines, IA were kept in a 'home' tank (filled with tap water and store-bought water treatment) with filtration, faux plants, fish rocks, and natural lighting. Fish were fed once daily in the early evening, except on test days when they were only fed during

the experiment. A test tank was kept completely bare and divided into six equal sections. Each section was denoted with a dry-erase marker on the outside glass of the tank. For inebriation, a plastic, cylindrical container—plain—was used, and the applicable amount of alcohol to achieve desired concentrations was added. The fish were fed with regular Tetra fish flakes. Time was kept via stopwatches on a cell phone and one experimenter announced times for fish to eat.

Methods

Each testing session occurred with 48 hours in between them, as the same nine fish were used in each session. The three test sessions were conducted within the same week. On the first test day, the nine fish were transported via net to the plastic absorption tank, with no alcohol added (for a 0% concentration of alcohol in plastic absorption tank water). The fish were kept in this container for twenty minutes. Based on the work of Ryback (1969), after 20 minutes in the plastic container, fish would show differential levels of blood alcohol levels dependent upon the amount of alcohol present in the plastic container. After the twenty minute period, fish were transported to the test tank via net and given three minutes to acclimate to transportation before timing began. Food was then dispersed in the center of the tank and the timer was started. Each student researcher was charged with tracking one fish—each fish was claimed before the food was dispersed—and recorded how long it took their fish to start eating in seconds. Student researchers also recorded each time their fish entered a different section to track overall movement.

After five minutes in the test tank, the fish were transported back to their home tank. The second and third test sessions happened in the same manner. However, these sessions involved adding alcohol to the plastic container water. On the second test day, the container's water had a 0.4% alcohol concentration and on the third, a 1% alcohol concentration.

Results

The impact of the different alcohol concentrations on latency to feed are summarized in Figure 1. As alcohol concentration increased the latency to feed decreased. Consistent with these observations, a repeated measures ANOVA revealed an overall significant difference between groups, Wilks's $\lambda = .022$, $F(2) = 45.08$, $p = 0.005$. Surprisingly, pairwise comparison between 0% and 1% concentration only approaches significance, $p = 0.09$. No other comparisons were found to be significant.

As alcohol concentration increased, the number of sections entered increased as summarized in Figure 2. Consistent with these observations, a repeated measures ANOVA revealed an overall significant difference between the groups, Wilks's $\lambda = .128$, $F(2) = 20.46$, $p = 0.002$. Pairwise comparisons illustrate a significant difference in sections entered between the 0% and 0.4% concentration, $p < 0.005$. No other comparisons were found to be significant.

Discussion

This course was designed with three goals: familiarize students with animal research by asking them to read and analyze previous research; further explore animals in research through conducting simple pre-designed experiments; and employ an animal model in a novel experiment to investigate a psychological disorder. The first two goals were achieved through completion of course work. Specifically, after reading numerous articles on animal models of psychological disorders students created a checklist to guide evaluation of other animal models, demonstrating their understanding of the nuances of this field. Using this checklist students evaluated a novel animal model and presented it to the class. All students demonstrated their understanding by earning a score of 70% or higher on this assignment. Demonstration of experimental replication was shown by housing and maintenance of a dozen crickets who showed similar action potential patterns as that in Backyard Brains (2017). Analysis of the results from the original experiment involving goldfish are discussed in relation to previous research, limitations and future directions.

Results of the experiment illustrate the impact of alcohol on feeding and movement patterns. Contrary to the hypothesis and results of Gerlai et al. (2000), the amount of time that it took fish to start eating

decreased as their blood alcohol levels increased, but the time decrease only approached significance between the 0% and 1% concentrations. Perhaps time to begin eating decreased as a result of repeated testing and alcohol's anxiolytic effects. Goldfish may have experienced high levels of stress during the first test session, and so were experiencing a fight-or-flight response that prevented them from focusing on food. Previous research, in other species, has illustrated the negative impact of novelty on feeding behavior and this procedure has previously been used to investigate anxiety related behaviors (Samuels & Hen, 2011). On the second and third period, time to eat was expected to increase due to alcohol's depressing effects on physical activity (Addicott et al., 2007), we observed the opposite. Due to repeat testing, the fish may have acclimated to the test procedures and no longer experienced a stress response, leading to decreases in latency to feed.

Overall, the goldfish entered more sections following alcohol exposure. However, pairwise comparison only showed a significant difference in sections entered between the 0% and 0.4% concentration, where increased alcohol concentration increased total sections entered. This is particularly interesting, as Gerlai found that a lower alcohol concentration led to increased activity, but a higher one (also 1% in their work) led to decreased activity as compared to the control group. Again, present results could be due to acclimation combined with decreased stress response to a novel situation. The impact of repeated testing may have led goldfish to display increased exploratory behavior later in the week because fish had acclimated to the netting and movement procedure. Their acclimation to this procedure is similar to the results of Christianson et al. (2008) who demonstrated increased social exploration in rats previously exposed to an escapable stressor compared to those exposed to an inescapable stressor.

To minimize repeat effects, testing was separated by a day, as Goodwin et al. (1971) showed the impact of alcohol to no longer influence results following a 24 hour delay. However, there may have been some sort of carryover effect we were unable to record. Fish may have been experiencing high levels of stress during the first session, due to novelty (Samels and Hen, 2011) and so moved far less and didn't rush for the food. They also may have learned by the third session that food would be administered if they simply waited.

Materials and subjects were limited by resource constraints. The use of several subject groups may have led to different results. Furthermore, the use of multiple tanks that were similar to the home tank, rather than tanks with different environments, may have resulted in different movement pattern and stress response. Lastly, it's possible that longer alcohol absorption periods, acclimation periods, and testing/data collection periods may have led to different findings. Future research might investigate how lengths of time for each section of a test session affects behavior and alcohol's effects. Further, comparison of data collected from designs with repeated subjects and separate subject groups could determine what carryover effects might exist when subjects are repeated.

This study exposed undergraduate students to the core principles of animal research in a relatively inexpensive model. This experiment provided the opportunity to study a reagent with which many people have lay understanding in an animal model system. By beginning the course with a literature review of frameworks for understanding and evaluating animal models students were equipped with a base from which to design an experiment using goldfish. In addition to the final class presentation, 4 students also presented at Tri-State Undergraduate Psychology conference on November 4, 2017. Future research could use a similar set-up to investigate a variety of other reagents on behavior. For example, fish could be exposed to caffeine and behavior assessed. To understand inter-rate reliability, measures could be taken by numerous students and inter-rater reliability calculated. This was not accounted for in the current study and may have had an impact on results.

Conclusion

The applicability of the present research to the larger population is limited, but as a teaching demonstration it was a success. Students were involved in all stages of the research process including design, experimental manipulations, data analysis, and reporting the results in papers and for some a presentation. Since this experiment requires minimal financial and physical resources, it can offer research experience to undergraduates at institutions where research facilities are limited.

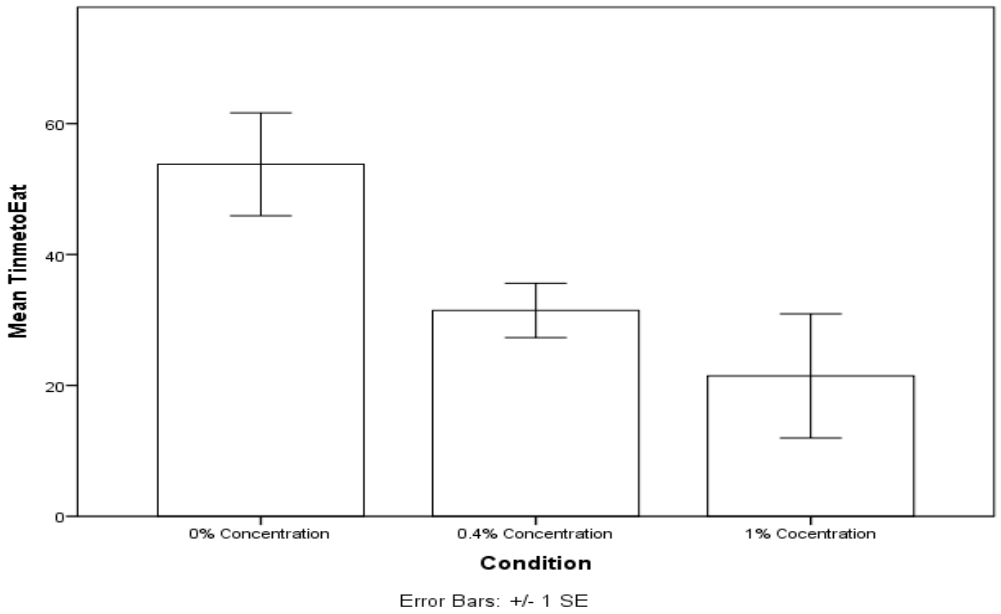

Figure 1: Mean time to eat as a function of alcohol concentration condition. SE-standard error of the mean.

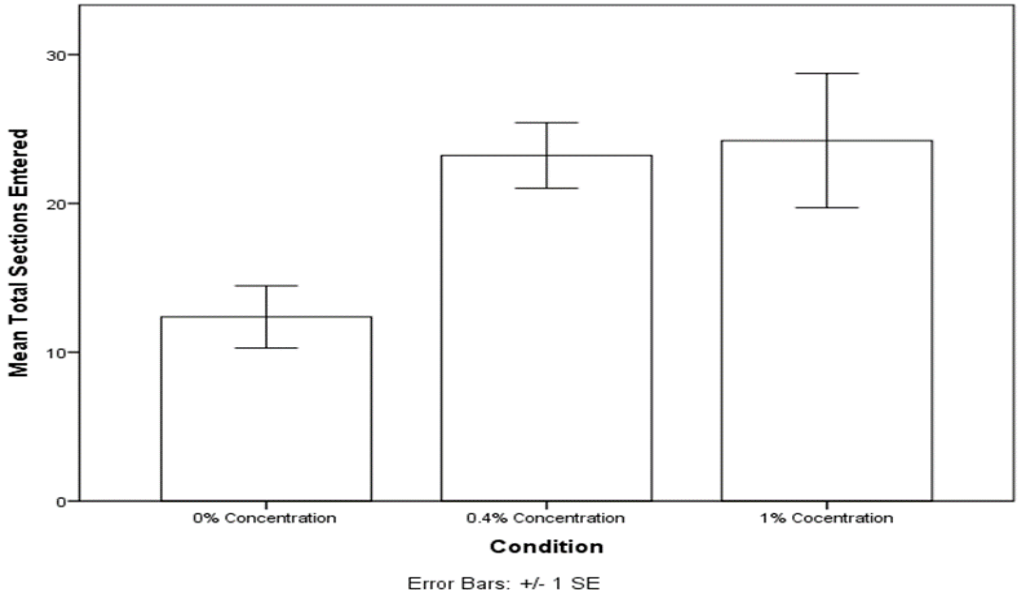

Figure 2: Mean total sections entered as a function of alcohol concentration condition. SE-standard error of the mean.

Appendix A

Questions for Evaluating Animal Models

What is the purpose of the study?

Relevance of the model

Which method(s) is used?
Screen? Simulation? Assay?

Start with endophenotypic mapping; identify the connections that made

How is the clinical condition translated to the animal?

Evaluation of types of validity for the model/screen/simulation
Face, Predictive, Construct,
Importance depends on type and goals of the study

Relationship to clinical disorder

3 R's of animal research- Reduce, Refine, Replacement (with less sentient species)

Why choose the animal for the experiment?

Are there ethical concerns or conflicts of interest? (Relate the replacement and refining of the model)

Reproducibility of the results

Generalizability of the results

Appendix B

Animal Model Group Presentation

In general, this group presentation will involve identification of an animal model for a specific psychological disorder and its evaluation. You will need to find at least 2 original research articles that utilize the chosen model. Though I am not going to time you, I suspect that successfully addressing all of the questions and engaging the class in discussion will take around 30-45 min.

Specifically, your presentation should introduce the psychological disorder under investigation including symptoms, current treatments and potential explanations. Next, you will present the animal model that is exploring the psychological disease including information about the species used, the methods and findings from the model that do or do not follow the human clinical picture. The presentation will then continue with an analysis of the types of validity that the model addresses and why this is important (see Questions for Evaluating Animal Models). The conclusion will highlight where research could go or why it should not continue using the model.

Those who are not presenting will be expected to be participating in discussion so when you design your presentation plan on class discussion.

Comments on Animal Model Group Presentation
Each presentation will allow the class to understand and picture the model. Therefore it is likely that you will use some form of visual format (ex; Powerpoint, Prezi, etc.).

Presentation by _____
Comments by _____

	Presentation clearly answers the question	Question not clearly and/or completely answered	Question not addressed
INTRODUCTION (10 pts) Clearly identifies the psychological disorder under investigation and why it matters			
What is the model being used? Why use that particular animal?			
METHOD (10 pts)			

Are the methods of the simulation, screening, model clearly described?			
Conceptually, what is/are the independent and dependent variable(s)?			
Operational definitions of constructs (how are variables manipulated and/or measured)?			
What are the levels of the independent variables, or how are the predictor variables measured? How is/are the dependent variable(s) measured?			
RESULTS (*5 pts)* Fit with hypothesis? Clearly explained?			
DISCUSSION (*10 pts)* *Relationship to other animal model findings* Relationship with clinical findings			
Threats to Validity? (10 pts) Consider questions such as endophenotypic mapping, face, construct, predictive validity. Generalizability, reproducibility			
Format (5 pts)			
References – includes every study cited in presentation - and only those studies?			

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