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Two Experiments using Conditioned Taste Aversion in Rats for Use in a Learning Course

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The purpose of this manuscript was to provide faculty with two possible experiments that could easily be replicated in a learning type course. The first experiment uses classical conditioning to measure conditioned taste aversion, while the second measures the effects that sleep deprivation has on memory. The strengths of these two experiments are that, if the department already has an animal colony, they are relatively quick (4-days) and cheap to run, but provide students with research experience and possibly aid in their understanding of designing, collecting data, analyzing and interpreting that data and writing the experiment up in an APA style manuscript. Additional benefits and problems are also discussed.

Keywords: *Learning, rats, conditioned taste aversion, flow pot technique*

In 2007, the American Psychological Association (APA) created a number of undergraduate psychology major learning goals and outcomes: namely knowledge base of psychology, research methods, critical thinking skills, application, values, information and technological literacy, communication skills and personal development. One such area of psychology that covers a number of these goals and outcomes is a course in Learning. In fact, a course in learning (and/or memory) has the potential to cover all the above areas, especially if a laboratory experience is required for the course. Most conventional learning (and/or memory) courses evaluate classical and instrumental/operant conditioning, possibly including comparative cognition, which would demonstrate knowledge, critical thinking, and application, among other possible areas. Furthermore, these courses review the classical studies in these areas, but also may incorporate other articles to further the students understanding. These additional readings may be associated with a required laboratory experience as well. Therefore, the authors goals are to provide some laboratory ideas and share experiences of such endeavors, some borrowed from previous mentors or personal lab experience, which could be referred to as "best practices," but also cover a number of the APA goals and outcomes for psychology majors.

The authors will focus on two quick experiments (1-2 weeks) that are simplistic in nature, but also allow for student input in the design of the experiment, which is critical for student engagement of the experience. The two experiments use rats and vary around Conditioned Taste Aversion (CTA), also known as the Garcia Effect, after John Garcia first studied and wrote about the phenomenon (Garcia, Kimeldorf & Koelling, 1955). Conditioned Taste Aversion, which can be analogous to food poisoning in some humans, relies on classical conditioning to first pair a neutral stimulus (i.e. food or drink) with an unconditioned stimulus (US, i.e. bacteria) to produce an unconditioned response (UR i.e. illness). Later, when exposed to the food (or drink) the animal tends to avoid it as it becomes associated with the previous illness that followed the consumption of the food (or drink). If this happens, the food (or drink) now becomes the conditioned stimulus (CS) and the illness (or in this case avoidance of the stimulus that is associated with illness) becomes the conditioned response (CR). Obviously, this is an important survival mechanism for animals.

In rats, this process typically involves a sweetened solution (CS) followed immediately by an injection of Lithium Chloride (LiCl: US), which induces illness in rats. One important point is that the rats' stomach structure prevents the ability to vomit (Hofstetter, Suckow & Hickman, 2006), but instead will lay down and sleep off the illness. This also eases the conscience of students (and IACUC: Institutional Animal Care and Use Committee, which reviews animal experimental protocols). The benefit of this procedure is it is a one-trial learning event and

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can be tested 24 hours later for retention with a choice period of both the sweetened solution and an alternative solution (i.e. water). The second experiment investigated how amnesetic procedures may affect memory by introducing a sleep deprivation procedure using the "Flower Pot Technique" (Mendelson, Guthrie, Frederick, & Wyatt, 1974) before beginning CTA. An alternative amnestic agent would be hypothermia (Hinderliter, Musci, Pollack, Misanin, & Anderson, (2004), but few students choose this method, for various reasons. Hypothermia also changes the research methodology as it follows the learning event, which serves as a useful discussion topic in the design of the experiment and aids in assessing critical thinking.

The goals of these experiments are to have students aid in the design of an experiment, do a literature review, critically evaluate research, perform a study, collect data, analyze that data using statistical procedures and interpret and write up the results in a manuscript suitable for publication. These objectives not only aid in the students understanding of the material, but also cover a number of the previously described American Psychological Association's (APA) undergraduate psychology major learning goals and outcomes: knowledge base of psychology, research methods, critical thinking skills, application, values, information and technological literacy, communication skills and personal development.

Experiment 1

Methods

Subjects. The present study used Long-Evans rats (Harlan) that were approximately 10 months old. The experiment began with a total of 15 rats, eight females and seven males. Three rats, two females and one male were dropped from the experiment due to insufficient data. This left 12 total rats included in the experiment with six females and six males.

The independent variable was the type of injection solution, LiCl (experimental group) vs. sodium chloride (NaCl for the control/placebo group). The rats were randomly selected for each group, after being first matched for the amount of water consumed on days one & two. There were seven rats in the LiCl group and five rats in the NaCl group.

The rats lived in Plexiglas home-cages with corn-cob bedding. Food was constantly available to the rats. Water, however, was deprived except for the exposures as part of the experimental procedures. The room was maintained on a 15:9 hour light:dark cycle and a constant temperature of approximately 22 degrees Celsius.

Procedure. The subjects were handled and weighed daily for one week before the beginning of the experiment. Twenty-four hours before the experiment began, the rat's water bottles were removed. On days one and two of the experiment, the rats were deprived of water except for a 15-minute exposure to water in graduated drinking tubes (Ancare). On both days the amount of water the rats consumed was recorded. Rats were then matched and randomly assigned to experimental and control groups on day three based on the amount of water consumption from days one and two. Conditioning began on day three, in which the animals were given 15 minutes to drink 20 milliliters of 10% apple juice/water solution, the conditioned stimulus, followed immediately by an I.P. injection of 0.15M lithium chloride (LiCl, the unconditioned stimulus) for the experimental group and 0.9% sodium chloride (NaCl) for the control group. Both groups were injected at a dose of 2% of the rat's body weight. The LiCl and NaCl were purchased from Carolina Biological Supply.

On day four, the retention test took place: both groups were given 20 milliliters of regular tap water and 10% apple juice simultaneously, and allowed 15 minutes of drinking or until one test tube was completely consumed. The amount drank from both tubes was recorded for future data analyses. The data were analyzed with an independent t-test, in order to identify if there was a significant difference between the LiCl and NaCl groups for the two dependent variables: the difference of the consumption of the apple juice (Day 3-Day 4) and the preference on day 4 between the apple juice and water (AJ-water).

Experiment 2

Methods

Subjects. The subjects were 11 three-month old male Long-Evans rats (Harlan). The independent variable was the amount of sleep deprivation, either 24 hours or none. Five rats were assigned to the experimental sleep-deprived group, and six were assigned to the control non-sleep-deprived group. Rats were randomly assigned to the experimental and control groups after matching rats based on water consumption from days one and two of the experiment (see below). Rats were housed in Plexiglas cages with corncob bedding. Room

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temperature was 22°C with a 15:9 hour light/dark cycle. Rat food was available at all times, water was rationed and closely controlled, as described below.

Materials. Sleep deprivation tanks were used to create an environment inhospitable to REM sleep. These tanks were 68 liter plastic storage tubs with 25.4 cm terracotta flower pots placed upside down which left the 12.7 cm bottom as a platform for the rats to sit. The success of this procedure assumes that when rats lose muscle tone upon entering REM sleep, they will slip off the small base of the flower-pot and into the water, thus waking them up and preventing that critical stage of sleep (Mendelson, et al., 1974). The water was mixed with 1 oz. dish soap and placed in the bottom of the tub, approximately 2.5 cm deep. All materials were purchased from Walmart. The dish soap water solution was used to prevent the water-deprived rats from drinking it. Room temperature was maintained at 22°C, and light/dark cycles were 15:9 hours.

Procedure. In order to determine if sleep deprivation can cause impaired memory to the point that taste aversions are not retained, the 12 rats were exposed to a four-day experiment. Twenty-four hours before the experiment began, the rat's water bottles were removed.

On day one, approximately 24 hours after the water bottles were removed, all of the rats were allowed a 15-minute exposure to 20 milliliters (mls) of tap water. After the 15-minute period, the water bottles were removed and the amount consumed was recorded.

On day two, all of the rats were allowed another 15-minute exposure to 20 mls of tap water. After the water bottles were removed and the consumption amounts recorded, the rats were divided into either the experimental (sleep deprived) group or the control (non-sleep deprived) group. To determine which rats would be placed in the particular groups, the water consumption rates of each rat was analyzed and rats with similar consumption rates were placed in opposite groups. The experimental rats were then placed in the sleep deprivation tanks overnight.

On day three, both groups of rats were given a15-minute exposure to 20 mls of 10% Apple Juice (AJ), the conditioned stimulus. Before exposure to the AJ, the sleep deprived rats were allowed 15 to 20 minutes to adjust and groom themselves following the sleep deprivation period. This is based on experience that has found that when the rats are returned to their homecage, following a night in the sleep deprivation chamber, most will begin to groom themselves before doing other tasks. Immediately after the rats' 15-minute exposure to AJ, all of the rats were given intraperitonal (IP) injections of .15 M of lithium chloride (LiCl) (Carolina Biological Supply; at 2% of body weight). All rats were returned to their normal cages, without water, and AJ consumption was recorded.

On day four, the retention tests were administered. All rats were given 15-minute exposures to 20 mls of tap water and 20 mls of AJ. Both fluids were offered at exactly the same time and left in the cage for the same amount of time. After the 15-minute exposure period, consumption amounts for each fluid were measured and recorded.

The consumption measurements were then analyzed using the inferential independent t-test statistic. The t-test was first used to determine if there was a significant difference between AJ consumption on day three compared to day four. Next, a t-test was used to determine if the AJ consumption amounts were significantly different from the tap water consumption amounts on day four.

Results and Discussion

The independent variable for experiment one was the type of injection solution, LiCl vs NaCl, whereas the independent variable for experiment two was the presence or absence of sleep deprivation. There were two dependent variables, which were the same for each experiment. The first was the difference of apple juice before the injection (day three) minus the amount of apple juice consumed after the injection (day four). Depending on class input, this could also be expressed as a ratio of day three – day four/total amount consumed of apple juice. The students should realize that this is an important measure, because if the rats experienced/remembered the CTA, there should be a reduction of AJ consumption on day four.

The second dependent variable was called a preference score, which measured the preference of the AJ vs water drank on day four, the retention test for the conditioned taste aversion. This was expressed as a difference of AJ minus the amount of water, but again could be expressed as a ratio of AJ – water/total amount consumed on day four. The students should understand that if the rat experienced/remembered the CTA, they should prefer the water and avoid the illness producing AJ. For experiment two, if the sleep deprivation resulted in amnesia for the CTA, then the sleep deprived rats should prefer the AJ, while the non-sleep deprived rats should prefer the water and avoid the AJ. Independent t-tests were used for the analyses using

Microsoft Excel, which was readily available on almost all campuses.

Two dependent variables were used to demonstrate the importance of the need of multiple measures to identify the presence or absence of learning. Of even more value is that because the entire class participates in the experiment, with two students assigned per rat, there is much more error and rarely do both dependent variables reach significance. In fact, only two out of twelve times these experiments have been run by classroom students have both dependent variables worked to support each hypothesis and only once did the results not support both hypotheses. This is an important lesson for two reasons: 1) it shows why it is important for tight controls in experiments and how error (usually experimenter error) affects results and 2) the students have to provide their own final conclusion in terms of support or no support of the hypothesis and why. The students are always curious and quick to ask "did the experiment work," to which a typical response includes, "what do you think? You tell me in your paper." Obvious solutions to the lack of support are 1) the number of student researchers increase the error in the experiment and 2) low animal sample size. But it is important to allow the students to apply what they have learned from a statistics and research methodology course and be given the opportunity to critically evaluate and interpret their own results and produce scholarly evidence for the absence of support for the hypotheses.

One limitation of this experiment is the rare occasion of a severe fear of the rats. Early in the class it might be important to bring a rat into the classroom and introduce the rat, explaining they are a docile strain of rat and are more afraid of humans then humans are of the rat. It also helps to explain how a number of students have some fear at the beginning, but by the end of the experiment are wanting to take the rat home with them. As previously mentioned, students work in pairs with each rat, so if they don't want to touch the rat, they need to find someone who is not afraid. However, it should be noted that the students really do not have to have any contact with the rats, instead they are just placing and removing bottles. Injections are performed by the faculty member, except on the rare occasion that a student is motivated or has previous experience with injections. Only then are students allowed to perform injections under close supervision of the faculty. An additional limitation that has not arisen in my experience, but certainly could, is an individual that firmly believes in animal rights and is against animal experimentation. In our program, we have options for students to complete the lab requirements in other courses that use human participants, however objections to the use of animals can provide an opportunity to explain the benefits of animal experimentation (although discussions most likely will not result in a change in the individual's attitude). The Foundation for Biomedical Research has resources and literature to aid such purposes.

In conclusion, these laboratory experiences are invaluable to undergraduate students, who may lack other research experience for different reasons. It is important to make this a requirement for all psychology majors as it provides real world experiences, makes the students more competitive for graduate school and jobs, and requires the students to apply what they have learned from previous courses. From experience (and confirmed from others that require laboratory experiences), this is one opportunity in their undergraduate training that the students find rewarding and never forget. It also creates an exciting experience where they also learn something at the same time.

Author Note

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