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The Behavioral Effects of Oral Psychostimulant Ingestion on a Laboratory Rat Sample: An Undergraduate Research Experience

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Presented is a lab exercise designed to augment an upper-level undergraduate class covering the topics of psychopharmacology, biopsychology, physiological psychology, or introductory neuroscience. The exercise was developed as a tool to allow students to investigate behavioral correlates of oral psychomotor stimulant ingestion and observe firsthand the benefits and challenges of using animal models to study behavior. The purpose of the exercise was to observe the unconditioned, natural behaviors of laboratory rats prior to, and following, the oral administration of commonly used, over-the-counter psychomotor stimulants, and for students to experience the process of neuroscience research. Of specific interest was the comparison of behaviors demonstrated by the animals following ingestion of the nonprescription stimulants caffeine and pseudoephedrine. Students went through the steps of a research project by actively participating in all aspects of experimental design, including construction of testing apparatus, animal care, drug measurement and dosage, data collection, and analyzing behavioral data to determine animal response to psychomotor stimulant exposure. Through repetition of treatment conditions separated by a clearance phase, students observed experiment replication and learned about a research design commonly applied in animal research. Successful replication of treatment effects also served to exemplify the concepts of reliability and validity in behavioral research, while observable responses in animal models provided students with the opportunity to extrapolate important considerations for differential behavioral effects of psychostimulant consumption in humans.

Key words: animal research; anxiety; caffeine; elevated plus maze (EPM); pseudoephedrine; psychomotor stimulants; psychopharmacology

The laboratory exercise described here, which imitates the activities and experiences one would have through the course of a full experimental study, was developed to accompany an undergraduate, senior-level psychopharmacology course, offered in a psychology department. This lecture-based course previously had no laboratory associated with it and was typically taken as an elective. As demand has risen for experiences that are relevant to students interested in pursuing postgraduate training in neuroscience and professional fields (e.g., medicine, pharmacy, nursing, etc.), a pair of interested undergraduates were recruited to explore the feasibility of developing a formal laboratory component to accompany the lecture-based course.

Undergraduate students who choose to take an elective psychopharmacology course are typically doing so because of a genuine interest in the study of the brain and behavior, and the effects of drugs on both. While classroom instruction introduces all the essential concepts related to this subject, the addition of laboratory experience allows students to investigate further topics of interest, and in doing so, glean a deeper understanding of theory and the scientific process. This lab exercise can be modified to fit lab courses in any area where chemical signaling and chemical interactions within the nervous system are topics (e.g., biopsychology, physiological psychology, and introductory neuroscience).

Using an animal model to demonstrate the varying effects of psychoactive substances provides students with the opportunity to gain personal experience understanding the strengths and limitations of the most common approach to translational medical research. Of particular importance, is developing an understanding of the model itself and its necessity in fields such as neuroscience and psychopharmacology. The current activity provides students with the opportunity to gain hands-on experience working with one of the most common animal models in these fields, the laboratory rat.

Furthermore, developing an understanding of the validity of animal models, and areas where this may be lacking, is nuanced, and tends to be poorly understood by many undergraduates (Metzger, 2014). One way to clarify these issues is to give students first-hand experience interacting with and observing the daily operations of an animal research laboratory, and the behavior of live, functioning animals. Students who participated in this exercise were instructed in the basics of laboratory animal husbandry, and briefly participated in the care for the lab animals.

An added benefit of this experience, working closely with an instructor or laboratory personnel, is the opportunity to further discuss the importance of ethical considerations when working with animals. This understanding of ethics can be enhanced by having the students write and submit their own Institutional Animal Care and Use Committee (IACUC) protocol for the project. Although undergraduate students typically do not have the technical expertise or experience to fully address all of the concerns for approval

of an IACUC protocol, participants in this project provided empirical justification for the question being studied and the methodologies used. Additionally, students were instructed in an item-by-item walk through of the university's IACUC form, explaining why these questions were asked, identifying appropriate and inappropriate responses, and answering any further questions.

The addition of a laboratory experience to an undergraduate-level course on this topic serves to guide students in developing a stronger background in the experimental methods used in neuroscience and pharmacology disciplines, an understanding of the challenges associated with translational research, laboratory technique, and scientific communication, as well as strengthening their grasp on relevant theory. Additionally, this laboratory exercise provides instructors the opportunity to model appropriate laboratory methods, proper animal handling techniques, and demonstrate hands-on scientific practices. Finally, depending on which elements the instructor decides to include, this experiment reinforces the application and interpretation of more advanced forms of statistical analyses, such as a mixed-design ANOVA. Thus, through active participation in the various elements of this research-like exercise, students learn about the scientific process by experiencing each of its individual elements.

Learning Objectives

Collectively, this laboratory exercise develops and enhances the following principles for participants:

1. Understanding the importance of animal models in research, including benefits and limitations.
2. Expansion of concepts related to good experimental design and protocol, including reliability and validity.
3. Understanding of the principles of drug dosage and experience calculating appropriate dosages based on body weight.
4. Experience with real-time identification, observation, and recording of behaviors exhibited by rodents in an elevated plus maze (EPM).
5. Enhanced understanding of how specific brain regions and neurotransmitters are influenced by caffeine and pseudoephedrine, and their subsequent behavioral outcomes.
6. Application of statistical knowledge to understanding and interpreting results from an advanced experimental design containing multiple between- and within-subjects conditions.
7. Effective summarization and reporting of experimental results.

Feasibility

This laboratory exercise is accessible to any institution that contains a rodent housing facility. Although the presented results include counterbalancing to account for sex differences, the observed outcomes are robust and the project does not require this condition. Likewise, this project does not require an identical number of subjects to what is presented here, and this number can be increased, or decreased. Instructors are free to omit or alter several elements of the design while retaining the overall outcome of the study. The only specialized equipment required for this exercise is an Elevated Plus Maze (EPM). An EPM can be readily obtained from a variety of different scientific equipment vendors. Alternatively, for those where cost is an inhibiting factor, or instructors who wish to provide students with experience in apparatus design and construction, an EPM can be easily constructed from materials obtained at a local home improvement store.

The current exercise was conducted over the course of a 16-week semester, in the fall term of 2017. Data collection took place for four weeks, beginning at week 10 and running through the end of week 13. In the weeks leading up to data collection, students conducted a literature review to explore the rationale for the project, assisted in the development of an IACUC protocol, constructed the necessary equipment, and learned the skills needed to care for the test subjects and effectively collect data in the project.

This lab activity was conducted as an independent study, with one student enrolled in a stand-alone, 1-hour laboratory course, and a second electing to complete the study on a volunteer basis. Both students were enrolled in the accompanying three-hour, senior-level psychopharmacology lecture. The lab met once per week for all weeks where data was not being collected, and the lecture met three times per week. During data collection, students were assigned half of the subjects used in the study, and met daily to record observations. This approach does require some flexibility on the behalf of the instructor, who needs to be available to stand in for data collection in the event that a student is unavailable. In a lab section whose enrollment is more than two students, data collection responsibilities can be divided differently to accommodate scheduling.

Effects of Psychomotor Stimulants

Psychomotor stimulants are a broad class of psychotropic medications whose general shared characteristic is to stimulate the central nervous system, resulting in increased spontaneous motor activity at doses too low to produce convulsions (Schuster, 1981). Included in this class of drugs are nicotine, caffeine, and a variety of different amphetamines. One intriguing observation about these substances is that some produce an enhanced level of attention and hyperactivity, whereas others produce an excess level of hypervigilance leading to anxiety-related behaviors. This experiment demonstrates the differential effects of two common, over-the-counter psychomotor stimulants: caffeine and pseudoephedrine.

Because it is found in more than 60 known species of plants, and is an included ingredient in numerous dietary sources such as coffee, tea, soda, chocolate, and energy drinks, caffeine is by far the most commonly consumed psychoactive substance (Acquas et al., 2012). Similarly, pseudoephedrine is one of the most common over-the-counter medications, found most often in allergy-relieving preparations, medications intended to relieve sinus congestion associated with common viruses, and also some nonprescription pain medications. Both chemicals, despite their legal availability as non-controlled substances, are potent psychomotor stimulants, and are frequently used for

the purpose of reducing fatigue, increasing attention, and improving physical performance (Ruksee et al., 2008; Acquas et al., 2012; Meeusen et al., 2013; Pritchard-Peschek et al., 2014;).

Although the mechanisms and effects of specific psychomotor stimulants are complex and varied, one that is well established is their collective tendency to elevate the extracellular quantity of monoamine neurotransmitters present in the central nervous system. Monoamines, in particular dopamine, have been determined to play critical roles in the regulation of motor activity, motivated behavior, effort, and reward. These effects are all mediated by the large quantity of dopaminergic neurons found throughout the striatum. Dopamine signaling in the nucleus accumbens, located in the ventral striatum, plays a key role in the processing of reward value of stimuli, reinforcement, and enhancing appetitive and fear responses (Berridge and Kringelbach, 2013). Additionally, increases in dopamine in the dorsal striatum has been found to promote motor activity via the direct pathway, which includes the caudate nucleus, putamen, and globus palladus (Alexander and Crutcher, 1990).

However, psychomotor stimulants have also been linked to activation of the same brain regions that are hyperactive in individuals who experience symptoms of anxiety, specifically, activation of the medial prefrontal cortex, ventrolateral prefrontal cortex, cingulate cortex, and the amygdala. Additionally, neurotransmitter responses to stimulant ingestion mimic neurotransmitter activity associated with anxiety. This is especially the case for pseudoephedrine, which increases the release of serotonin and dopamine, while also inhibiting the reuptake of these neurotransmitters. Increased levels of serotonin in conjunction with elevated levels of dopamine are found to be strongly associated with anxiety reactions (Martin et al., 2009; Acquas et al., 2012; Roy-Byrne, 2015). Caffeine, to a lesser extent, produces similar effects through a separate mechanism, by acting as a competitive inhibitor of adenosine. This amplifies the stimulant effects of dopamine, and also works to increase the sensitivity and activation of serotonin receptors, however, caffeine does not stimulate the release of excess neurotransmitters. Therefore, high doses of pseudoephedrine will stimulate anxiogenic behaviors, whereas high doses of caffeine will only stimulate increases in exploratory behaviors, which would otherwise be suppressed.

The observed outcome of the study is that the differential activity between the two psychomotor stimulants produces differing effects in an animal model: The pseudoephedrine triggers new, or exacerbates preexisting, anxiety-like responses, resulting in a large amount of time spent in an enclosed, ‘safe’ area. Caffeine, on the other hand, produces an exaggerated state of arousal that counteracts any anxiety associated with enhanced dopaminergic activity, producing the tendency to explore open areas that, under normal circumstance, would be avoided. Laboratory rats are the ideal subject for this lab experience for at least two reasons. First, this species’ response to various drugs share similar responses to humans (Iannaccone and Jacob, 2009). Second, this species demonstrates natural behaviors that are anxiety-type at baseline, thus substances that enhance these behaviors produce robust effects (Walf and Frye, 2007).

MATERIALS AND METHODS

Animal Subjects

The University of Nebraska – Kearney IACUC, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, approved the use of all animals, and procedures followed. Subjects were a sample of 16 adult Longs-Evans laboratory rats (*Rattus norvegicus*), eight males and eight females. These animals had no previous use in behavioral experiments, nor were they exposed to any psychomotor stimulant agents. Animals were housed individually in standard, clear, polycarbonate laboratory cages and were provided with free access to food during all phases of the study. Additionally, animals had free access to water during baseline and clearance phases of the study. During test phases, the active substances were dissolved in each animal’s daily water supply, which was limited to a premeasured, average daily intake. To optimize the animal’s level of activity during the daytime hours when data collection took place, animals were maintained on a reversed day-night cycle.

For purposes of introducing students to the daily operations of an animal research facility, and training them on correct husbandry procedure, students were trained in the care of rats and participated, briefly, in the care of test subjects. This included training in correct technique for handling animals to minimize stress, daily feeding and watering procedures, and instruction on changing bedding and cage washing. Students were responsible for care and health monitoring of their assigned test subjects during the data collection portion of the experiment.

Experimental Design

The study used a mixed-design experiment, with one within-subjects variable and two between-subjects variables, to examine the effects of caffeine and pseudoephedrine on anxiety-type behaviors displayed by test animals during each of four experimental conditions: baseline, initial stimulant ingestion, drug clearance, and a second stimulant ingestion. Both drug ingestion conditions of the experiment contained the same drug for each animal, with drug doses held constant, based on the individual weight of each animal on dosing days, throughout the duration of ingestion testing. The within-subjects variable was the presence of psychostimulant drug in the animal’s daily water supply. The between-subjects variables were the type of stimulant (either caffeine or pseudoephedrine) administered to each group of animals, and the sex of the animal. Anxiety-type behavior for each animal was defined as the tendency to avoid open areas, in favor of enclosed spaces. This variable was quantified and measured as the duration the animal would spend in an enclosed, ‘safe’ environment, versus the time they would spend in an open, ‘dangerous’ environment.

The experimental approach used in this study was developed by the students, with strong guidance by the instructor of the lab. During the first two scheduled lab meetings, students reviewed common methodologies used

in pharmacology research, and identified which would be the most appropriate to answer the current research question. Next, additional variables that may be relevant to the outcome of the study were determined. Finally, students considered what type of statistical analysis would be most appropriate for analyzing the results of the study, drawing heavily on previous coursework and other laboratory experiences.

Behavioral Testing Apparatus

All animal behavior was evaluated with an EPM, which is a widely used and accepted model for measurement of anxiety related behaviors in rodents, and has been validated for use in screening of anxiolytic and anxiogenic pharmacological effects (Walf and Frye, 2007; Lalonde and Strazielle, 2010). For use with rats, this apparatus consists of a cross-shaped maze elevated 50 cm from the floor with two open arms, and two arms with enclosed sides and an open roof. Each arm measures 50 cm long and 10 cm wide, with a 10 cm x 10 cm center platform located where the arms cross. Under normal conditions, rodents avoid the open arms of the maze because of a natural aversion to heights and a preference for enclosed spaces. Thus, this serves as a simple, unconditioned test based on the natural behavioral tendencies of rodents, in conjunction with their spontaneous exploratory behavior in novel environments. After initial placement on the center platform, animals have access to move freely between the four arms of the EPM.

Purchased commercially, the acquisition of a simple EPM, designed for rat use, as described previously, would cost a university department approximately $2,200.00 (based on pricing information from Maze Engineers, Braintree Scientific Inc., Stoelting, and Kinder Scientific). To enhance experiment feasibility, students designed and built a maze that met experimental standards, for a budget of $140.00 (for a slightly different approach to constructing an inexpensive EPM, see Fox et al., 2018). This build price reflects the purchase of finish-grade oak planking and oak panels, oak trim, turned pine pedestal legs, quality polyurethane waterproof finish, and miscellaneous hardware.

Students participated in all parts of the design and construction of the EPM. This included obtaining schematics for a typical EPM, determining the correct dimensions for a rat-appropriate apparatus, and identifying suitable building materials. Actual EPM construction took place during lab time, and took approximately two lab meetings to complete.

Testing Environments

The testing environment consisted of an open room with standard fluorescent overhead lighting, and minimal furnishing, only a table and a chair for the experimenter, to control for environmental distractions. Standard laboratory attire and safety equipment were provided for all personnel involved in the experiment. The EPM was centered in the testing area, with a tripod mounted video camera (Samsung, model HMX-F90) positioned at the end of one open arm of the maze. The recorded data was used in a demonstration of inter-rater reliability for the student participants. Recordings of two, five-minute testing sessions were coded by the instructor and compared with student data to demonstrate this principle. Additional recordings were randomly selected to check for accuracy in data recording technique. The limited number of students in the development of this exercise did not permit a full evaluation of inter-rater reliability, due to time constraints. However, given a larger group of students, this would provide a valuable lesson in developing effective operational definitions and data collection techniques. A lab notebook, a stopwatch with a silence feature, and a timer were used to record arm entries, duration in each arm, and to time each session. To minimize any anxiety related to handling, animals were transported from the vivarium in their home cage to the table in the test area. Animals were only removed from their home enclosures immediately prior to testing, and once testing was completed, placed back in their home enclosures and returned to the vivarium. Following the return of each rat to the vivarium, and prior to bringing the next rat to the testing area, the EPM was cleaned and disinfected with cleaning cloths containing a 1:10 bleach dilution, allowing 1 minute for drying, to prevent carryover of any olfactory cues, which may influence the behavior of subsequent test subjects.

Pharmaceutical Preparation and Dosage

Animals treated with caffeine were dosed with over-the-counter caffeine tablets (generic brand, 200 mg tablets), purchased at a local drugstore. To prepare the caffeine/water solution, tablets were crushed into a powder to speed dissolution in water. Animals were provided a daily dose of 110 mg/kg, with individual doses determined based on each animal's daily weight immediately prior to dosing. The treatment dosage of caffeine was chosen as the appropriate oral dosage required to obtain psychomotor, and presumably mental, stimulation without prompting the unpredictable and potentially aggressive behaviors that can be elicited from rodents at higher dosages (Braun, 2011).

Subjects in the pseudoephedrine condition were dosed with over-the-counter pseudoephedrine tablets (generic brand, 90 mg tablets), obtained from the same local drugstore. The pseudoephedrine/water solution was prepared the same way as mentioned previously, but at a daily dose of 137 mg/kg, again determined based on individual animal weight. The dosage of pseudoephedrine was determined by choosing a convenient-to-administer amount, which had a value falling between the amount required to elicit preference in a population of rats previously conditioned with amphetamine (40 mg/kg), and half of the established lethal dose of 320 mg/kg (Pilla et al., 2013; Tongjaroenbuangam et al., 1998).

Daily fluid intake of the animals was monitored for one week prior to testing, averaging 40 mL per day, per animal, and subsequent drug doses were dissolved in this quantity of water. Upon administration of the appropriate dosage, animals self-administered the test medications over the course of the next 24 hours and were tested immediately afterward. The dosing procedure was repeated for five consecutive days, beginning on Sunday and running through Thursday, resulting in five consecutive days of data collection from Monday through Friday. This self-

administration method for both caffeine and pseudoephedrine has been shown to be an effective means of dosing rodents in previous studies (Pilla et al., 2013; Tongjaroenbuangam et al., 1998).

Although it may not be plausible or desirable in all instances to implement training on the measurement of chemical compounds, students in the current exercise were instructed on this skill. Students were initially instructed in how to use the cumulative weight of the animals within each condition to identify the total quantity of each drug needed to dose the animals for the upcoming 24-hour period. Next, they were taught how to calculate the exact amount of drug needed per individual animal to give a proportionally correct dose based on the individual animal's weight. Identifying information was marked on a sticker on each water bottle to prevent errors in dosing. All students were required to demonstrate correct calculations and proper drug weighing technique prior to participating in mixing drug solutions for the actual experiment. Dosing was alternated between the students weekly to maintain a consistent dose time, with the lab instructor dosing animals on Sundays. The lab instructor reviewed all drug dosage calculations prior to students measuring individual proportions, to ensure these were correct.

Behavioral Procedure

Testing was conducted over the course of four consecutive weeks, midway through the term to avoid breaks and holidays, with each week consisting of five consecutive days of testing in the EPM. All testing took place between Monday and Friday, during the hours of 8 AM to 5 PM. With the exception of two Sundays, where the lab instructor came in to begin a 24-hour dosing period, animals received free access to clean water and were not tested in the EPM on weekends.

The current protocol consisted of four separate conditions, with one condition being tested each week. These conditions consisted of the following: a pre-manipulation baseline to determine typical rat behavior in the EPM; an initial drug dosing condition; a clearance phase to allow for animals to recover from the first drug exposure; a second drug dosing condition. Depending on time availability and desired outcomes, this element of the experiment offers instructors a large degree of flexibility in its implementation.

Animals were tested once per day, for a total of five minutes (300 s), in the EPM. Daily testing involved independently placing an individual rat in the center of the maze facing an open arm to begin a testing session. The number of arm entries and total time spent in each arm during the session were recorded. Time spent in each arm was recorded only as the time that an animal entered or exited an arm. Total time spent in each arm was then calculated after the daily data recording was completed. Incidental observations of anxiety-type behaviors (grooming, scratching, leaping, twitching, and rearing), as well as physiological signs of well-being (urination and defecation) were also noted, but not recorded systematically enough for inclusion in the data analyses.

The first condition tested was simply to establish a baseline level of anxiety-type behavior for each animal in the EPM. Animals were placed in the test apparatus and their natural behavior in the novel environment was recorded. Anxiety was operationally defined as the amount of time an animal spent in the closed arms of the maze, with greater amounts of time spent in closed arms equating to a greater level of baseline anxiety. Upon completion of this condition, animals were separated into two test groups, with four animals of each sex in both groups for a total of eight animals per group. To optimize validity, animals were separated using a matched-sample design, so baseline anxiety levels were approximately equivalent between groups. Students were instructed in the rationale for a matched-sample design, its necessity in research scenarios like those described here, and how to effectively implement this approach.

Both the second and fourth conditions were identical, where each group was dosed with its respective drug. These differed only in the week they were administered, the drug each group was administered did not change between the two times. Therefore, one group of animals was treated with caffeine, and the other with pseudoephedrine. Drugs were prepared as previously described, and animals self-administered the drug preparation that was continuously available to them over the course of 24 hours. This treatment was repeated daily for five consecutive 24-hour periods. Observations of animal behavior in the EPM were measured and recorded beginning 24 hours after the first dose was administered. Therefore, animals received their first drug dose 24 hours prior to testing, beginning on Sunday, and received their last dose beginning on Thursday. After testing was complete on Friday, animals were provided clean drinking water.

Between the two drug treatment conditions, a drug clearance condition was provided. This condition consisted of continued daily testing in the EPM, in the absence of the test substances. During this time, all animals had unrestricted access to clean water. Behaviors in the test apparatus were assessed to determine if they returned to baseline level. At this point, students were instructed on the importance of an ABA design, why baseline and clearance phases were necessary, and discussed the implications and importance of whether a groups' behavior returned to original baseline levels.

Student Assessment

Two students participated in the development of this laboratory activity. Students were formatively assessed based on their weekly participation in the development of the study's design, construction of test apparatus, demonstration of proper animal care and handling technique, correct calculation and measurement of chemical compounds for daily dosing, and accurately recording data. Because the successful outcome of this exercise depended on effectively mastering all of these techniques, these were evaluated on a pass/fail criterion.

The final evaluation of students consisted of a scientific write up of the study that included a literature review justifying the research, methodology, results of statistical analyses, and a discussion of the implications of their

findings. The preparation of this research paper served as the final submission for the laboratory course, and the final laboratory grade, where applicable, were largely determined by its contents. The paper was assessed using a rubric developed by the faculty in the psychology department, which is used to evaluate all scientific write-ups of undergraduate laboratory research at this institution. An outline of the categories used in this evaluation are presented in the Appendix.

RESULTS

Experimental outcomes

A graphical summary of the average weekly total time (number of total seconds out of a possible 1500 s) spent in an open arm of the EPM, separated by drug exposure group, are presented in Figure 1. (Note: animals were assigned to drug conditions *after* the baseline data were collected.) As can be seen, at baseline animals spent between 350 and 400 seconds exploring the open arm, with the two groups differing by only 27 s (11.4s per day). After introducing the two drugs, rats in the pseudoephedrine group remained similar to baseline, increasing time spent in the open arm by a total of 8.5 s (1.7s per day). The animals in the caffeine group, on the other hand, increased the time spent exploring the open arm by 446 s (89.2 s per day). During the clearance phase, both groups failed to return to baseline. While the caffeine group's open arm time predictably decreased toward the original baseline, the decrease below the original baseline in the pseudoephedrine is unusual. The most likely explanation for this change is place conditioning, During the first drug exposure, typical rodent avoidance of open areas may have been exasperated by the pseudoephedrine. It is plausible that this resulted in an enhanced preference for the confines of the closed arm during the clearance week. The second drug exposure resulted in a similar outcome to the first, with the pseudoephedrine group increasing time spent in the open arm slightly, but never varying appreciably from their original baseline. Descriptive statistics for data from each category are presented in Table 1.

Although a simple graphical analysis demonstrated a large behavioral difference between the animals exposed to the two test substances, statistical analyses were included to validate this observation and reinforce the application of statistical training from students' prior coursework. A 3x2x2 mixed-design ANOVA was conducted using IBM SPSS 25 analytical software to examine the within-subjects effect of treatment condition (drug exposure 1 vs. clearance vs. drug exposure 2), and the between-subjects effects of sex (male vs female) and drug type (pseudoephedrine vs caffeine).

	Pseudoephedrine	Caffeine
Baseline	361±327 s	389±141 s
Drug Time 1	370±186 s	835±194 s
Clearance	276±138 s	500±126 s
Drug Time 2	393±187 s	778±149 s

Table 1. Presented are the mean and standard deviations (M±SD) for the amount of time, in seconds, spent in an open arm of the EPM over a total of five, five-minute sessions in each condition.

Baseline data served as a covariate to control for typical rat behavior in the EPM, unrelated to the presence of psychoactive substances.

The results of this analysis revealed no statistically significant within-subjects main effect for treatment condition, $F(2, 22) = 2.82$, $p = 0.082$, $\eta_p^2 = 0.204$, though this did approach significance and show a robust effect size. While this fails to reach the criteria for a statistically supported main effect, it suggests that a larger sample size may have resulted in significance. More importantly, a statistically significant interaction between treatment condition and drug type was found, $F(2, 22) = 4.50$, $p = 0.023$, $\eta_p^2 = 0.290$.

A test of pairwise comparisons was conducted to determine the source of the statistically significant interaction between treatment condition and drug type. These revealed a statistically significant difference between the amount of time spent in an open arm between the two drug conditions at the first drug exposure number $F(1, 11) = 41.43$, $p < 0.001$, $\eta_p^2 = 0.790$. These also revealed a statistically significant difference between the amount of time spent in an open arm between the two drug conditions during the clearance phase, $F(1, 11) = 13.09$, $p = 0.004$, $\eta_p^2 = 0.543$. Finally, the comparisons revealed a statistically significant difference between the amount of time spent in an open arm between the two drug conditions at the second drug exposure number, $F(1, 11) = 26.14$, $p < 0.001$, $\eta_p^2 = 0.704$. As can be seen (Figure 1), rats treated with caffeine spent more time in the open arm of the EPM than those treated with pseudoephedrine.

Statistically significant interaction effects were observed for both between-subjects main effects. Analyses revealed a statistically significant between-subjects main effect for drug type, $F(1, 11) = 51.12$, $p < 0.001$, $\eta_p^2 = .823$), confirming the previous conclusion. Animals who were exposed to caffeine spent more time in the open arm of the maze across all three treatment conditions. Additionally, a statistically significant interaction was observed between sex and drug type, $F(1, 11) = 6.06$, $p = .032$, $\eta_p^2 = .355$. Figure 2 shows that the source of this interaction is from female rats

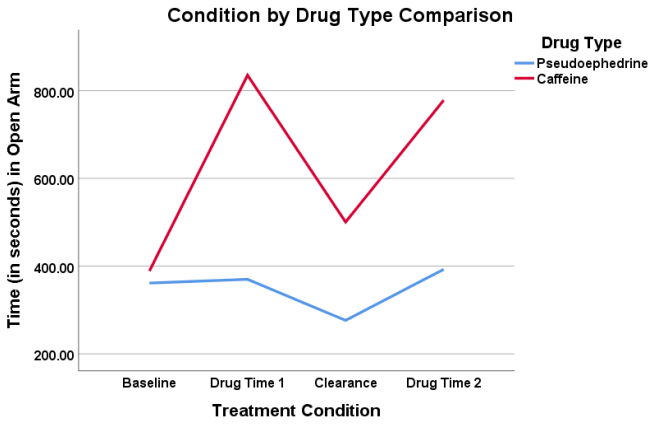

Figure 1. Presented are a comparison between the mean times spent in the open arm of the EPM between the two drug types. The source of the statistically significant interaction is easily observed, as is the failure of both groups to return to baseline during the clearance phase.

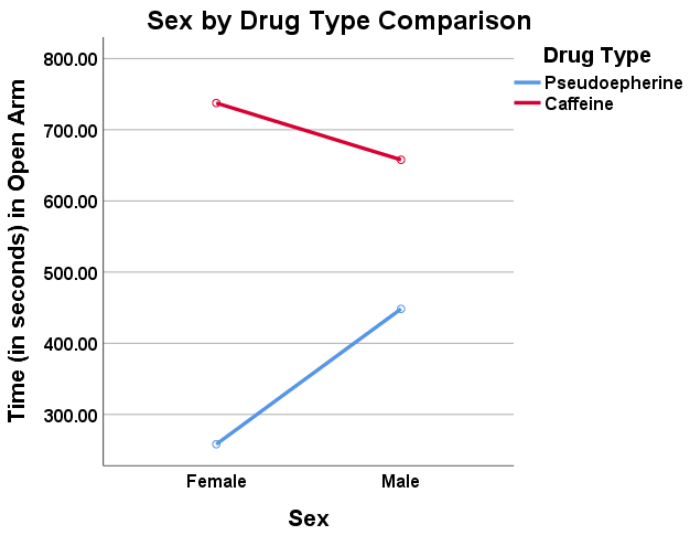

Figure 2. Presented are the mean times spent in the open arm of the EPM, comparing the effects of drug type and sex. Females spent approximately 100 s more in the open arm of the maze than males when given caffeine, but nearly 200 s fewer when given pseudoephedrine.

spending more time in an open arm than their male counterparts when exposed to caffeine, while spending less time when exposed to pseudoephedrine.

An additional one-way ANOVA was conducted to determine if differences observed in the group administered pseudoephedrine differed significantly. Of particular interest was whether the decrease in time spent in the open arm of the EPM was significantly lower than the original baseline. The results of the analysis found no statistically significant differences among the four conditions in the pseudoephedrine group, $F(3, 28) = 0.424$, $p = 0.737$.

Student Outcomes

As was previously mentioned, students were formatively assessed weekly on their mastery of numerous elements of the research project, which were essential to its completion. Included amongst these were participation in the development of the study's design, construction of equipment, correctly caring for and handling animals, determination of necessary quantity and measurement of chemical compounds, and accurately recording data. After instruction on each of these techniques, both students were able to successfully complete each necessary task within one week without assistance or prompting.

Students were also formatively assessed on their ability to write a scientific paper to summarize the work that they completed throughout the laboratory experience. In the department where this was conducted, students in all upper-level labs are required to write a paper adhering to current American Psychological Association (APA) guidelines. The department uses a standardized rubric for evaluating all APA-style papers, which uses a 5-point, Likert scale to assess 15 different elements of the student's writing. On this scale, scores of 1 are considered 'inadequate' and these increase to a rating of 'superior' for a score of 5. No element of either student's paper was scored lower than a 4. Furthermore, when compared to a sample of their peers from the same term by a faculty assessment committee independent from the faculty involved in the development of the current exercise, both papers were viewed as 'good to superior'.

Because the number of students participating in the current activity was so small, it was concluded that little value could be gained from a formal evaluation of the perceptions of student experience. This stemmed partially from concerns that students would be easily identifiable based on their responses and may feel unable to respond honestly as such. Anecdotally, both students reported that they felt the lab was a good educational experience and aided in their understanding of research designs and methodologies discussed throughout the lecture portion of the course. Students indicated that it was very helpful to their understanding of animal research to have the opportunity to be involved in the development of an IACUC protocol, and get actual experience working with live animals. When asked if they would recommend this lab experience to future students interested in the topic of psychopharmacology, both students stated that they would strongly encourage it.

DISCUSSION

The results of the statistical analyses confirm the intended effect of two separate psychomotor stimulants. Caffeine had an effect that overrode the rodent-typical tendency to avoid open areas, resulting in the animals being much more willing to take risks and explore the otherwise aversive spaces. Pseudoephedrine, on the other hand, induced anxiety-type behaviors leading animals to continue to prefer the confines of the closed arms of the maze. The robust nature of the observed behavioral differences provide an excellent opportunity for students to develop a clearer understanding of the impacts of different stimulants on behavior. Furthermore, these observations can be expanded to include discussions of the source of differential effects of other psychomotor stimulants, such as nicotine, cocaine, and amphetamines.

Based solely on time spent in the open arm of the maze, it would appear that both pseudoephedrine exposures produced little effect. However, the incidental observations, especially grooming and rearing, occurred at much higher rates during the drug exposure conditions. Additionally, rats repeatedly shuttled between the two open arms during testing. Therefore, while the animals still spent most of their time in the enclosed arms, they were considerably more active than during baseline. A test apparatus that employs IR sensors throughout the EPM, similar to that reported by Fox et al., (2018), would be more suitable to record evidence of changes in locomotor activity. Addressing this potential issue also provides an opportunity for students to consider the limitations of what can be measured with an EPM.

The observed sex difference in drug effect is consistent with the common observation that psychotropic drugs, especially psychomotor stimulants, tend to affect males and females differently, with females tending to show greater responses (cf. Lynch et al., 2002). The inclusion of both sexes in the demonstration allows students to observe first-hand how differences in physical composition and varying

sex-hormone levels are related to overall drug effects. An advanced version of this activity could include students carefully observing daily differences in female behavior caused by the interaction of a test substance and fluctuating sex-hormone levels through the course of the estrous cycle.

Although it was not anticipated in the development of this demonstration, rats in the pseudoephedrine condition showed greater than baseline-level anxiety-type behavior during the one-week drug clearance condition. Several plausible explanations could account for this observation. Given the relatively high dosage of pseudoephedrine administered, it is possible that the animal's behavior was the result of withdrawal symptoms. Alternatively, the heightened level of anxiety experienced by the animals may have resulted in place conditioning to the EPM itself, evoking anxiety-related behaviors even in the absence of the test substance. Another variation of this project could investigate different dosages of pseudoephedrine to determine whether there is a critical dosage at which this prolonged anxiety reaction occurs.

Having students consider the implications of the observed outcomes provides an opportunity for them to gain insight into the challenges and benefits associated with translational research. A discussion of whether the anxiety-related behaviors observed in the rats are relevant to humans is an excellent starting point. To enhance the understanding of the neuroanatomical basis of anxiety, further comparisons can be made highlighting similarities and differences in the anxiety circuit between the two species. Further considerations could include discussion of the appropriateness of certain over-the-counter drugs containing pseudoephedrine for persons who either experience, or are at high risk to experience, anxiety disorders.

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APPENDIX: Student Research Paper Grading Rubric

All students who complete an upper-division laboratory are supposed to complete a formal, APA-style research report detailing the work completed throughout the term. The following rubric is used to evaluate each paper on 15 elements, distributed over 7 categories.

Each element is evaluated on a scale of 1 to 5, where 1 is 'inadequate' and 5 is 'superior'.

Background

1. Literature review is present and ties into relevant theory.
2. The purpose/significance of the project is clearly stated and relevant to previous reports in the literature.
3. The hypothesis of the study is clearly stated and relevant to previous reports in the literature.

Methodology

4. Appropriate methodology for testing the proposed hypothesis are selected and explained.
5. Research design is clearly outlined and directly relates to the hypothesis.
6. All necessary information about research participants are clearly explained.

7. All relevant details about the procedure and required materials are listed.

Results

8. Appropriate statistical analyses are selected and correctly reported for the research outcome.
9. Results are clearly reported, description is consistent with the statistical analyses conducted, and related back to the hypotheses.

Discussion

10. Clearly and directly ties results to purpose and hypothesis and expands description to provide explanation for findings.
11. Discussion effectively relates results back to theory.
12. The relevance of the findings and implications for future research are related back to the literature. Any present limitations are noted and discussed.

APA Format

13. Adheres to all APA-style guidelines, with no egregious errors or omissions.

Readability

14. The paper lacks errors in phrasing and syntax and maintains a professional tone throughout.

References

15. Selected references are peer-reviewed, of substantive quality, and relevant to the topic of study.

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