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An Undergraduate Laboratory Exercise Examining the Psychomotor Stimulant Effects of Caffeine in Laboratory Rats

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This paper describes an exercise in a Systems and Behavioral Neuroscience with Laboratory class, an introductory laboratory class taken by Barnard College students majoring in a wide range of academic topics. The study took place over three weeks, allowing students to assess the effects of caffeine on motor stimulation in laboratory rats. The within-subject design involved injecting rats with three different caffeine doses and measuring five different motor outputs in a standard open field. Students completed four different assignments related to this study, demonstrating acquisition of the stated learning goals. This lab exercise allowed students to learn about basal ganglia neural circuitry and stimulant

pharmacology, to work directly with an animal model, and to generate enough data to perform statistical analyses. Course evaluations suggest that students liked learning about caffeine, a stimulant many of them have personal experience consuming. They also expressed appreciation for working with rats and for learning how to analyze data. This study can easily be implemented at most undergraduate institutions under minimal cost. The wide-ranging effects of caffeine also permit for flexibility in experimental design, allowing instructors and students options for different avenues of investigation.

Key words: caffeine; basal ganglia; motor stimulation; open field; stimulant; rat

Undergraduate students take introductory laboratory classes for different reasons. For many students, the class is in an academic area they major in, and they like the topic. For other students, it may simply be a general education requirement they need to fulfill. Regardless of the intent for enrolling in a class, instructors hope that lab exercises can allow students to study certain topics they are introduced to in lecture courses in greater depth, and that this process not only promotes deeper understanding of a theory but also puts into practice elements of experimental execution and analysis. While upper division laboratory classes and independent research projects, such as a senior thesis, permit students more intellectual choice and ownership in their experimental ideas and design, introductory laboratory classes educate students in the scientific method, fundamental laboratory techniques, and scientific communication. We describe here a laboratory exercise that captures these goals at an introductory level.

Each year, about 60 students at Barnard College enroll in the Systems and Behavioral Neuroscience with Laboratory Class (PSYC 1117). This 1000-level, introductory course can be taken to fulfill a major requirement by students in Psychology or in Neuroscience and Behavior, or it can be taken as an elective by students in other majors. Because a one-year sequence of a laboratory science class is a general education requirement for all Barnard College students, up to one-third of the students in this lab class may be students in the humanities or social sciences. As such, one challenge of this class is to keep the major students stimulated in their chosen field while ensuring that non-majors are similarly engaged. A laboratory exercise dealing with a topic that typical students can relate to in everyday life has the potential to peak interest from all students. In this study,

students used laboratory rats to examine the effects of caffeine on locomotor behavior.

Caffeine, a substance found in coffee, tea, soda, energy drinks, chocolate, and some pain medications, is the world's most widely consumed stimulant (Fredholm et al., 1999). It is not a controlled substance and is commonly consumed by college students seeking a state of alertness. Athletes may also ingest caffeine to aid their speed and endurance (Ivy et al., 1979; Ganio et al., 2009). Recently, one-half of surveyed students reported consuming caffeine, primarily in the form of energy drinks, combined with alcohol, while partying (Malinauskas et al., 2007). Students often sought caffeine to increase energy and vigilance and were not always aware of the potential negative consequences of caffeine consumption, including experiencing headaches, heart palpitations, and jolt and crash episodes.

In this lab exercise, students learned about the physiological basis of the action of caffeine in the brain and body. Over a series of three weeks, they observed and scored rat locomotor behaviors after the rats were injected with three different doses of caffeine. Upon the completion of their data collection, they analyzed the data and drew statistically meaningful conclusions from their empirical observations. They submitted four assignments related to this lab exercise. Based upon a review article they were assigned to read (Fisone et al., 2004), students expected to observe that a low dose of caffeine would be most effective at stimulating motor activity while a higher dose would either have no effect or even suppress locomotion when compared to placebo.

Learning Objectives

Upon completion of the lab, students should be able to:

1. Explain how dopamine, acting on the basal ganglia via

the D₁ receptors in the direct pathway and via the D₂ receptors in the indirect pathway, disinhibits the thalamo-cortical neurons, resulting in motor stimulation.

2. Explain how adenosine, acting on A_{2A} receptors in the basal ganglia, antagonizes the effects of dopamine.
3. Understand that caffeine is a competitive inhibitor of adenosine and can thus amplify the stimulant effects of dopamine.
4. Calculate drug dosing by body weight.
5. Observe and score five different types of common locomotor behaviors exhibited by rats in a standard open field.
6. Perform basic mathematical and statistical calculations on the raw data and graph data summaries using Microsoft Excel.
7. Demonstrate an inverted-U effect of caffeine dosing on locomotor behavior.
8. Write an introduction section, a methods section, and a results section, to effectively communicate the theoretical background and practical design of the experiment and to present statistically meaningful data.

MATERIALS AND METHODS

Student Participants

Participants were 60 Barnard College undergraduate students (all female) enrolled in the Systems and Behavioral Neuroscience with Laboratory course (PSYC 1117). The class was composed of 13 sophomores, 36 juniors, and 11 seniors. Fourteen students were Neuroscience and Behavior majors, 34 were Psychology majors, and the remaining 12 students majored in studies outside Psychology or Neuroscience (one in Anthropology, three in Art History, three in English, one in History, two in Music, one in Philosophy, and one in Theater). The students attended two 75-minute lectures and one three-hour lab each week. The lab portion of the class was divided into three sections, with 20 students in each section.

Animal Subjects

Experimental procedures were performed on a total of 30 adult female Sprague-Dawley rats (CD strain code:001; Charles River, Wilmington, MA). The rats were used in a Psychology of Learning Laboratory course for classical and operant conditioning experiments during the previous semester. They were nine months old at the start of this study and had never received injections of any chemicals or drugs in earlier studies. The rats were housed in pairs in standard clear polycarbonate laboratory cages (45 x 25 x 20 cm), received unlimited access to food and water, and were maintained on a 12-hour light:12-hour dark cycle with lights on at 0800h. Animals were weighed three times each week throughout the study to habituate to handling and to acquire necessary information for proper dosing of the caffeine. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Columbia University Institutional Animal Care and Use Committee (IACUC).

Experimental Design

This was a within-subject design. Each lab section of 20 students observed 10 rats over a period of three weeks. A student always worked with the same lab partner. Each pair of students was assigned one rat and one cubicle for the duration of the three weeks. Each rat received each of three different caffeine doses, administered one dose per week, with the students blind to dosing information. Students observed five different locomotor behaviors: ambulation, grooming, scratching, assisted rearing, and unassisted rearing. The measured, or dependent, variables were the locomotor behaviors. The manipulated, or independent, variables were the caffeine doses.

Testing Environment

Thirty minutes prior to the start of each lab, the rats were transported from the Psychology Department vivarium to the teaching laboratory. The laboratory has a main classroom and 12 cubicles, individually-contained rooms with doors. Each cubicle has an overhead light, a bench countertop, a desktop computer, and two chairs. An open field made of clear plexiglass (45 cm wide x 45 cm deep x 30 cm high; Med Associates Inc., St. Albans, VT) was placed on the bench countertop. One rat was placed inside each open field with a ventilated plexiglass cover and allowed to habituate for a minimum of 30 minutes before experimentation. All procedures were performed during the lights on period (between 9:00 a.m. and 4:00 p.m.) with the cubicle overhead light on. An internet browser was open on the desktop computer with an online stopwatch visible on the screen (<http://www.online-stopwatch.com/>); the online stopwatch allowed the students to keep track of the behavioral observation time. In addition, two timers were placed next to the open field for the students to use for timing locomotor behaviors. It was imperative that the timers have silent control buttons to avoid distracting the rats with beeping sounds when buttons were pushed.

Caffeine Injections

A caffeine stock solution (15 mg/ml) was prepared on the day of the lab by dissolving caffeine powder (Sigma-Aldrich, St. Louis, MO) in 0.9% sodium chloride solution. Rats received each of three caffeine doses: 0, 15, and 50 mg/kg caffeine. A small volume injection of saline (approximately 0.3 ml) was used for the 0 mg/kg placebo dose; this volume was selected to match the volume of injection for the 15 mg/kg dose of caffeine. One dose was administered each week by intraperitoneal injection after the rats had 30 minutes of habituation to the open field. The order of drug dosing was randomized within each lab section across the three weeks of experimentation. Because the labs met once per week, the rats only received one caffeine dose each week and were thus unlikely to develop any tolerance to caffeine (Finn and Holtzman, 1986).

Behavioral Observation

Following the 30-minute acclimation period, the rats were injected with a dose of caffeine and students immediately

began recording five different locomotor behaviors for 45 minutes. The 45-minute time period was chosen because caffeine gets distributed throughout the body within five minutes of ingestion/injection and begins to have peak effects after 15 minutes, persisting for one hour (Fredholm et al., 1999). The behaviors were (1) ambulation – moving forward on all four paws, (2) grooming – washing the face or any other part of the body with the forepaws and/or with licking, (3) scratching – raising of hindpaw to touch any part of the body, (4) assisted rearing – body inclined vertically with hind paws on the floor and forepaws on the chamber wall, and (5) unassisted rearing – body inclined vertically with hind paws on the floor and forepaws unsupported in the air. Ambulation, grooming, and scratching were scored by time duration, with students using timers to keep track of the amount of time a rat engaged in each behavior. Assisted and unassisted rearing were scored by event counting, with one event tallied each time a rat displayed the structural topography fitting of the definition for each type of rearing. The 45-minute observation period was divided into nine repeating blocks of five minutes each. Students divided the scoring during each five-minute block in the following manner: 1st minute – one student tallied rearing events; 2nd and 3rd minutes – one student continued to tally rearing events while the other student timed ambulation; 4th and 5th minutes – one student timed grooming behavior while the other student timed scratching behavior. This five-minute sequence of behavioral scoring repeated eight times until the 45 minutes of observation ended. The rats were then returned to their home cages, and the students cleaned and disinfected the open fields with 20% ethanol.

Statistics

Using the cubicle computers, the students logged on via a link to a Google Docs spreadsheet to enter the data for their rat after each week of observation. The data collected from the 10 rats were pooled together for each lab section. After all three observations had been completed, the caffeine dosing code was revealed to the students. They were then able to export the data from Google Docs into an Excel spreadsheet for data analyses. Students calculated group means and standard error of the mean (SEM). Comparisons were made between caffeine doses using two-tailed, paired t-tests with significance defined as $p < 0.05$.

Assignments

Prior to the start of the study, students were asked to read a review article summarizing the known mechanisms of caffeine on locomotor activity (Fisone et al., 2004). They answered a set of pre-lab questions on adenosine, dopamine, and caffeine transmission in the basal ganglia. The first lab started with a discussion of the review article, with a focus on the functional organization of the basal ganglia, dopaminergic disinhibition, and caffeine antagonism of adenosine, leading to psychomotor stimulation. The students were introduced to different types of rat locomotor behaviors and shown demonstrations on how to score the behaviors. After

demonstrating understanding of the study design, students made the first observation of one dose of caffeine. Their next assignment was to write an introduction section for the study. The second lab started with a discussion on caffeine tolerance and withdrawal (Finn and Holtzman, 1986; Fredholm et al., 1999), the effects of caffeine on urination and defecation (Maughan and Griffin, 2003), and the role of caffeine in pain relief medication (Ward et al., 1991), endurance sports (Ivy et al., 1979; Ganio et al., 2009), and neuroprotection from the development of Parkinson's Disease (Chen et al., 2001; Xu et al., 2005). Students then made the second observation of a different dose of caffeine and had to submit a methods section for this study. During the third lab, students started by completing the third and final observation of a dose of caffeine. When they were done, the dosing code was revealed, and they were able to start the data analyses. Their final assignment was to submit a results section, including tables and figures, as appropriate. Each writing assignment section was based on author guidelines set forth by the *Journal of Neuroscience* (<http://www.jneurosci.org/site/misc/itoa.xhtml>).

RESULTS

Ambulation, Grooming, and Scratching

Ambulation, grooming, and scratching behaviors were each sampled for two minutes every five minutes throughout the entire observation session. For each two-minute time block, group means and SEM were calculated. A line graph plotting ambulation throughout the time blocks demonstrates the significant effect that an intermediate dose of caffeine had on sustained movement (Figure 1). This pattern of activity in which the least amount of activity was observed under the 0 mg/kg caffeine dose, the most activity was induced by the 15 mg/kg caffeine dose, and an intermediate level of activity was observed on the 50 mg/kg caffeine dose was obtained for grooming and scratching as well (data not shown), but the effect on ambulation was most pronounced.

Time spent ambulating, grooming, or scratching during each time block was summed for each rat to obtain the total time recorded for each activity across the observation period. Group means and SEM were calculated, and the results are displayed in Figure 2. While both caffeine doses induced more ambulation compared to the placebo dose, the 15 mg/kg dose produced the most significantly elevated effect. Grooming was significantly increased by the 15 mg/kg dose. There were slight increases in scratching behavior on both caffeine doses, though it should be noted that scratching was relatively negligible compared to ambulation and grooming.

Rearing Behavior

Assisted and unassisted rearing events were counted for three minutes every five minutes throughout the observation period. Figure 3 demonstrates that only the 15 mg/kg caffeine dose resulted in elevated assisted rearing throughout the duration of the 45 minutes of behavioral recording. Similar results were obtained for unassisted rearing (data not shown).

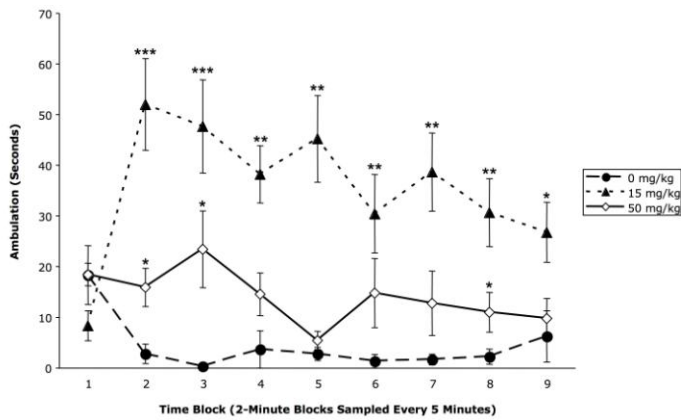


Figure 1. An intermediate dose of caffeine produced sustained ambulation. Data points represent group mean \pm SEM. After the first time block, a 15 mg/kg caffeine dose significantly enhanced time spent ambulating for the entire duration of observation. Asterisks indicate a significant effect compared to the 0 mg/kg placebo dose (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

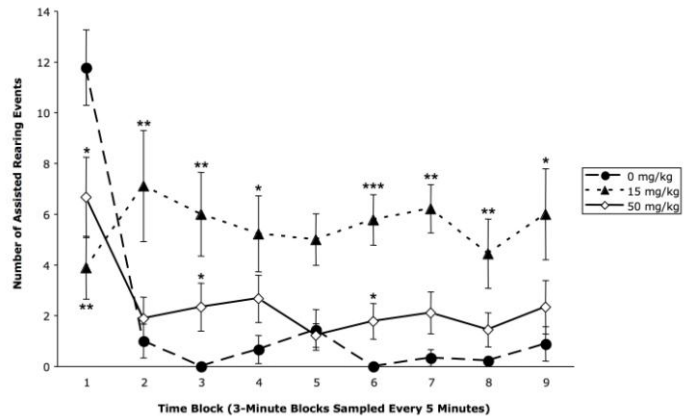


Figure 3. An intermediate dose of caffeine increased assisted rearing. Data points represent group mean \pm SEM. After the first time block, a 15 mg/kg caffeine dose significantly enhanced the number of assisted rearing events for the entire duration of observation. Asterisks indicate a significant effect compared to the 0 mg/kg placebo dose (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

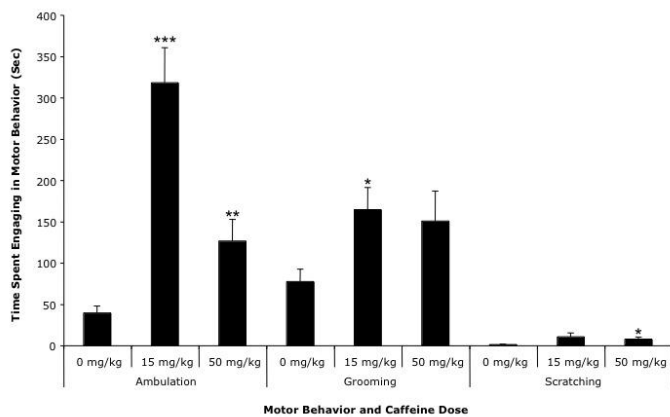


Figure 2. The effect of caffeine on ambulation, grooming, and scratching. Data shown represent group mean \pm SEM. Asterisks indicate a significant effect compared to the 0 mg/kg placebo dose (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

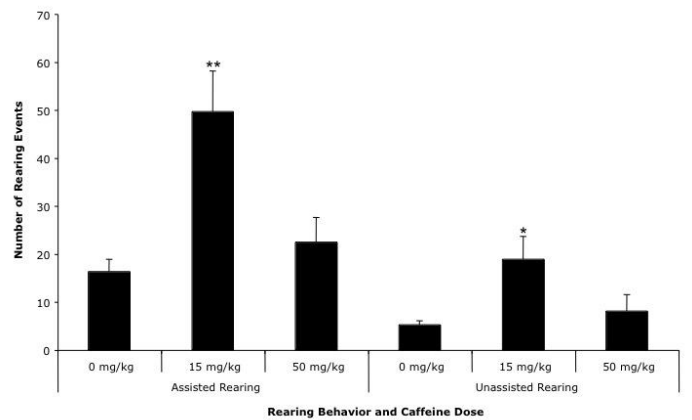


Figure 4. The effect of caffeine on rearing behavior. Data shown represent group mean \pm SEM. Asterisks indicate a significant effect compared to the 0 mg/kg placebo dose (* $p < 0.05$, ** $p < 0.01$).

The total number of rearing events was summed across the nine observation time blocks, and group means and SEM were calculated (Figure 4). The rats displayed more assisted rearing compared to unassisted rearing. Only the 15 mg/kg caffeine dose produced significant increases in rearing behaviors, with the effect on assisted rearing more pronounced than the effect on unassisted rearing.

DISCUSSION

Observations on Caffeine

The rats had at least 30 minutes to habituate to the open fields before they were injected with caffeine (or placebo), and student observations were immediately recorded. The habituation period served to minimize motor activity due to environmental exploration. By having the students plot activity by time (Figures 1 and 3), they were able to conclude that the first time block, representing the first five

minutes of observation, often bore similar levels of activity regardless of caffeine dosing. This can be presumably attributed to the rats' response to being picked up and injected. After this initial response, rats injected with the 0 mg/kg placebo dose were mostly inactive, and some even fell asleep for a large portion of the observation period. With no caffeine in their system, this response seemed reasonable, given that the experiments were carried out during their inactive period. The rats on the 15 mg/kg caffeine dose did not fall asleep and maintained a fairly constant level of motor activity throughout the 45 minutes of observation. They ambulated and groomed significantly more compared to the control dose, and they also reared more frequently. This intermediate level of caffeine was the only dose to promote sustained locomotion. On the 50 mg/kg caffeine dose, students noted that the rats did not walk or rear as often as they did on the lower dose of

caffeine. However, the rats did not fall asleep on the high caffeine dose. Rather, they were often self-confined to a corner of the open field, with eyes wide open, sometimes bulging. They also displayed lateral twitching of their heads, a motor response that was not included in the list of activities for the students to score. A small fraction of the rats, about 15%, made sudden vertical leaps while on the 50 mg/kg caffeine dose. This observation underscores the significance of using open fields with lids for these experiments. Students commented that the amount of urination and defecation emitted by the rats greatly varied from week to week, with a seemingly positive correlation between increasing caffeine dose and increasing urine and fecal output. They were able to link the physiological effects of caffeine discussed in the lab with the rats' physiological responses. For future labs, it might be useful to have students count the number of fecal boli and carry out a correlation analysis for a dose response curve.

By summing the observed behaviors across the nine time blocks, students were able to obtain a measure of total psychomotor responses. The column graphs they generated (Figures 2 and 4) clearly demonstrated the inverted-U response to caffeine, results that are consistent with the published literature (Nehlig et al., 1992; Svenningsson et al., 1995; El Yacoubi et al., 2000; Fisone et al., 2004). They were also able to conclude that caffeine did not universally affect all behaviors in the same way. Ambulation and assisted rearing showed the greatest enhancement by caffeine while grooming and unassisted rearing were increased to a lesser extent. Scratching behavior was minimally observed under all doses administered.

Student Outcomes

A set of pre-lab questions assessed student understanding of learning objectives 1, 2, and 3. An in-class discussion of the review article (Fisone et al., 2004) helped to clarify the mechanisms of how caffeine stimulates motor activity and provided the informational foundation for the students to write an introduction section. Learning objectives 4 and 5 were addressed with a methods section assignment, and learning objectives 6 and 7 were addressed with a results section assignment. The student average on the four assignments ranged between 85-92% (Table 1). The pre-lab questions and the introduction section had lower averages compared to the methods and results sections, suggesting that demonstration of understanding of the multi-synaptic neural circuitry of the basal ganglia and the output to the thalamus and cortex was more challenging for the students to master. Students commented that the concept of disinhibition was confusing when the functional organization of the basal ganglia was comprised of both excitatory and inhibitory neurons and when the neurotransmitter dopamine could either activate or inhibit neurons, depending on whether it bound to D₁ or to D₂ receptors, respectively.

The pre-lab assignment consisted of 10 questions based on the review article (Fisone et al., 2004). The specific questions varied from year to year, but they generally encouraged students to think about what types of

foods, drinks, and medications contain caffeine, how caffeine is absorbed by the body, and where and how caffeine acts in the brain to stimulate a motor response. Examples of pre-lab questions are listed here: (1) Upon ingestion, how is caffeine distributed throughout the body? (2) Caffeine is an antagonist to which endogenous molecule in the brain? What does it mean to be an antagonist? (3) In reference to the endogenous molecule you named above, list all the receptors for this molecule. Does caffeine bind with equal affinity to all of these receptors? If not, which of these receptors does caffeine have the greatest affinity for? Where in the brain are the receptors located? (4) At which dose does caffeine exert a peak effect on locomotor activity? What happens when the caffeine dose exceeds the peak dose? (5) If neuron A hyperpolarizes neuron B, what happens to neuron B?

Assignment	Grades
Pre-Lab Questions	84.9 ± 14.8
Introduction Section	84.9 ± 21.4
Methods Section	90.9 ± 18.2
Results Section	91.8 ± 6.1

Table 1. Summary of grades earned by students (n=60) on 4 separate assignments related to one study. Numbers shown represent the group mean ± one standard deviation. The maximum grade possible for each assignment was 100.

The methods section was assigned after the students completed two observations of behavior in the open field. Students were given a guide for writing lab reports and instructed to provide enough details in their methods section such that an informed neuroscientist could replicate their study. They were also advised to read primary research articles on similar topics. Overall, the students did well on this assignment, scoring above a 90% average.

The most in-class guidance was provided for the results section assignment. After students completed their final behavioral observation, about 90 minutes of the remaining lab period were devoted to helping the students analyze their data and generate and format their graphs. Even though a guide to creating graphs using Excel was provided to all students, many found that they still had specific questions about particular processes, such as how to add Y-error bars, how to format the X and Y axes in a specific way, and how to add asterisks to indicate significant findings. These tasks were further complicated by the different operating systems and different Excel versions the students used. Some students carried out the analysis on the lab cubicle computers while others preferred to work on their laptop computers they brought to class. Fortunately, the lab instructor and student teaching assistants were proficient in using Excel in both Macintosh and PC format and in versions dating from 2004 to 2010 and could provide useful tips to the students as they completed their data analysis. The in-lab time devoted to helping the students with this assignment had positive results, as their average results section grade was 92%, much greater than it was in the previous year, when no in-

class time was provided for the assignment and the students averaged only 80% on their results section.

In addition to the four assignments on this caffeine experiment, students had one more opportunity to demonstrate their understanding of the study. At the end of the semester, a lab final exam was given to test the students on their understanding of the theoretical and practical information about all labs performed throughout the term. Questions related to this particular study composed one-third of the exam. The caffeine study took place in the middle of the semester, after the students completed 3 weeks of sheep brain neuroanatomy and before they embarked upon a month-long unit on female reproductive behavior. Thus, this caffeine lab allowed the students to incorporate the anatomical information they learned in the neuroanatomy unit into a systems-level study and prepared them for an additional study on rat behavior at the end of the term. The individual writing assignments also prepared them to carry out data analysis more independently in the subsequent study and gave them experience for writing components of a lab report, critical for their last assignment, which was a full length manuscript-style lab report on female reproductive behavior.

Student Feedback

Many students remarked on how interesting it was to learn about the effects of caffeine on the brain and the body, particularly because it is a substance the majority of students have personal experience in consuming. The differences in the rats' responses to the 15 mg/kg dose and the 50 mg/kg dose made lasting impressions on some of the students, with several students admitting that this study promoted changes in their own behavior to reduce caffeine intake.

Anonymous student evaluations for the lab course have been generally favorable. Students majoring in Neuroscience seemed to take interest in the hands on work with the rats and the in-depth examination of the neural circuitry of the basal ganglia. Students taking the class to satisfy their general education requirement appreciated the application of the experiments to real life experiences.

Selected student comments below were given in response to the evaluation prompt, "The most valuable part of the lab was..."

"The caffeine experiment was interesting."

"the experience of working with rats and writing lab reports."

"seeing how substances affect the brain and thus behavior through observing rats."

"Learning about the different neural circuits was really cool, especially with the caffeine study. It really made me feel like a neuroscientist when we went through how the different neurons inhibit or disinhibit each other. This lab was really interesting from start to finish."

"learning how to use Excel."

Each year, a couple of students anonymously report negative reactions to the lab. The following evaluations were given in response to the prompt, "The least valuable part of the lab was..."

"Recording rat behavior. I know doing menial tasks like that is part of what science is all about, but I still felt as though I was wasting my time."

"I understood why every component of the lab was in place. I thought watching a rat for 45 minutes went on forever, but I think it is unavoidable."

"It would be nice to have more labs with rat observations. Maybe we could do a caffeine tolerance experiment."

Based on the student evaluations, it would be worthwhile in future labs to distinguish the difference between the final product of a scientific study, such as a research article, and the day-to-day events that take place, often over many years, that contribute to the publication of results. This may prepare students to appreciate that redundant tasks are a part of experimental investigation and are thus necessary components toward achieving better understanding of the natural world. Students could also be made aware of the constraints of a weekly lab course. It would be difficult, for example, to conduct an experiment on caffeine tolerance because drug tolerance typically requires at least seven consecutive days of caffeine exposure (Finn and Holtzman, 1986; Fredholm et al., 1999), a schedule that is impossible for the students to implement in a course that meets only once a week.

Implementation

The lab exercise we report on here utilizes standard laboratory rats to observe locomotor behaviors in response to caffeine injections. This study can be conducted at any undergraduate institution with access to a basic animal facility and a teaching laboratory classroom. Moreover, as no surgeries are required and the distress caused to the animal is minimal, only a category "C" protocol is required for IACUC approval. For this laboratory exercise, the minimum requirements include rats, caffeine, open fields, and timers. Caffeine is inexpensive, is not a controlled substance, and is readily available through a standard laboratory supplier, such as Sigma-Aldrich. While we used free-standing open fields, access to open fields connected to system software might allow for even more precise and comprehensive measurements of activity.

Students expressed concerns that variations in scoring might produce inaccurate data, and they were right to be curious about this. We discussed the common practice of recording rats in behavioral assays and having blind observers score behaviors by watching the recordings. Given that we did not have access to 10 video recorders nor the time to record the rats and then subsequently score by reviewing the recordings, the students carried out the scoring by real-time observations. This gave them the

advantage of a full 360° view of the rats, which is not always feasible when animals are recorded from only one viewpoint. Furthermore, the students learned about the systematic sampling of behaviors, as each behavior was only recorded for two to three minutes every five minutes. Some students were initially frustrated by the timing of the behavioral sampling, as they observed that their rat scratched during the time they were scoring ambulation and then stopped scratching during the time they were scoring scratching. These initial frustrations wore off over time as they saw that the equal intervals of sampling were indeed reflective of their rat's averaged behavior over time, and to their relief, the data obtained for their own rat was fairly consistent with those of their classmates' observations of the other rats. Because this study utilized a within-subject design, each rat served as its own control, and even differences in the scoring techniques of students could be corrected for if each student always observed the same rat from week to week.

An additional learning objective that teaches students about the importance of blind observation could be incorporated into this exercise. All students in the lab were blind to dosing information for the entire duration of the study. However, if some students carried out the same observations with knowledge of the caffeine doses, one could run a comparison on the difference in the magnitude of the reported results between the blind and the informed groups. This has the potential to illustrate to students how observations and data reporting can be influenced by a lack of blindness.

Our students observed the following five locomotor activities: ambulation, grooming, scratching, assisted rearing, and unassisted rearing. There are other behaviors they observed but did not score, such as an awake versus sleep state, lateral head motions, and the amount of defecation. These measurements might be useful to incorporate into future labs. We chose to focus on the physical effects of caffeine because they were the easiest to observe and to score. It would be interesting to assess the cognitive effects of caffeine since it is well known that caffeine induces changes in attention and vigilance (Nehlig et al., 1992; Fredholm et al., 1999). One such experiment could assess the effect of caffeine on performance on visual attention tasks (Broersen and Uylings, 1999). Another possibility could be to test the rats on novel object recognition, a common cognitive task (Botton et al., 2010). Students may find the mental outputs even more interesting than the motor outputs, given that many of them report consuming caffeine for its cognitive effects.

While the lab exercise reported here is well suited to classes of about 20 students per section, the same topic could be adapted for larger or smaller classes. In classes that exceed 100 students that do not break out into smaller sections, an instructor could cover the same introductory background and methodological information. In lieu of live observations of rats in a lab setting, students could watch pre-recorded videos of rats under different doses of caffeine while recording their observations. Conversely, in smaller, upper level undergraduate classes, students could expand their discussion to explain the neurobiological

underpinnings behind their observation that caffeine altered certain behaviors to a greater extent than others. Depending on the scheduling flexibility of an advance lab, students could carry out experiments on tolerance and withdrawal, and if the department has access to specialized equipment and software that can monitor stereotypic movements, the study can be expanded to include a comparison of caffeine to other stimulant drugs, such as amphetamines. Furthermore, a parallel study could be conducted on human subjects. With approval from the Institutional Review Board, an experiment could be designed to test the effects of caffeine consumption of physical exercise by measuring both performance and endurance.

In summary, this lab exercise provided the practical experience of conducting experimental studies on behavioral neuroscience in lab rats, and it also introduced a systems neuroscience topic on dopamine, adenosine, and caffeine transmission in the basal ganglia (Fisone et al., 2004; Xie et al., 2007). The experiment generated enough data for the students to carry out meaningful statistical analyses, and the assignments prepared students for writing manuscript-style lab reports. The learning objectives for the study were met, as students performed well on their assignments, and evaluation feedback suggests that many students found the lab interesting and educational. This study can be easily implemented at nearly any undergraduate program as described in this paper, or the experiment can be modified to examine the effects of caffeine on other aspects of psychostimulation, thus offering a great degree of flexibility in experimental design.

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