ARTICLE
A Laboratory Exercise for a College-Level, Introductory Neuroscience Course Demonstrating Effects of Housing Environment on Anxiety and Psychostimulant Sensitivity

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In this paper we describe a lab exercise developed for the Introduction to Neuroscience course at Williams College. One of a series of five labs, this exercise demonstrated several key principles of behavioral neuroscience. In this lab, students explored the effects of post-weaning housing environment on anxiety-like behavior and psychostimulant sensitivity in rodents. The exercise was intended to emphasize the importance and utility of animal models in neuroscience research and to give students hands-on experience with behavioral neuroscience research techniques. Students tested rats reared in social isolation or environmental enrichment for anxiety-like behaviors on the elevated plus maze, and for spontaneous and amphetamine-induced locomotor activity in the open field. They were then asked to analyze pooled class data and prepare a short lab report. Overall, student performance was excellent. This exercise emerged as a class favorite on course evaluations. Interestingly, the first time this exercise was conducted, the effects of environmental enrichment on anxiety-like behaviors and psychostimulant sensitivity were not consistent with those published in previous studies. Key methodological issues that may account for this discrepancy and contribute to successful implementation by other programs are discussed.

Key Words: amphetamine; animal model; anxiety; elevated plus maze; environmental enrichment; open field; social isolation

One of the most important goals of undergraduate neuroscience programs is to spark students' interest in neuroscience research. With this goal in mind, introductory courses should include meaningful laboratory experiences that actively engage students in commonly used neuroscience research techniques. This paper describes such a lab experience, which demonstrates several fundamental principles of behavioral neuroscience, while also giving students a sampling of behavioral neuroscience research techniques.

Ideally, undergraduate lab experiences should allow students to formulate and test their own hypotheses. However, such empirical approaches often require a high level of instructor involvement in the planning and execution of students' experiments, and are better suited for smaller, more advanced classes. For larger introductory level courses, lab exercises that allow students to use established research techniques to replicate robust phenomena may be more practical. This type of exercise can build technical proficiency and confidence, enhance critical thinking and analytical skills, and reinforce key concepts from lectures and readings.

Students in the Williams College Introduction to Neuroscience course participate in a series of laboratory experiences. These labs are designed to demonstrate nervous systems structure and function at the cellular, systems and behavioral levels. One recently developed exercise allows students to explore the effects of early social experience on anxiety and psychostimulant sensitivity in rodents. This topic was chosen because it incorporates several key concepts from behavioral neuroscience, and because the effects of early social experience on rodent behavior are well-documented. This exercise is also relatively inexpensive to implement and requires minimal special equipment.

Early social experience plays a critical role in brain development. Deprivation of social interaction in young animals can result in alterations in brain development that have been linked to a wide array of psychopathologies, including depression, schizophrenia and substance abuse (reviewed in Lapiz et al., 2003). Rats reared in isolation from the time of weaning exhibit heightened anxiety-like behavior (Weiss et al., 2004; Brenes Saenz et al, 2006) and enhanced locomotor responses to environmental novelty and amphetamine (Smith et al., 1997). These behavioral changes persist into adulthood and are associated with alterations in brain monoamine function (Hall et al., 1998; Lapiz et al., 2003).

Conversely, social housing in physically complex environments seems to have opposite effects on anxiety-like and locomotor behaviors. Exposure to such complex housing conditions is typically referred to as "environmental enrichment." Post-weaning environmental enrichment reduces anxiety-like behavior (Santucci et al., 1994; Brenes Sáenz et al., 2006). Furthermore, switching rats from an isolated housing condition to an enriched environment partially reverses the effects of isolation on anxiety-like behavior and locomotor activity (Hellemans et al., 2004).

In this lab exercise, students worked in small groups to test isolated or enriched rats on behavioral measures of anxiety and psychostimulant sensitivity. They learned how

to use the open field and elevated plus maze (EPM) to assess anxiety-like behavior, and observed the locomotor activating effects of amphetamine. EPM was chosen as a measure of anxiety-like behavior based on its use in previous studies that demonstrated anxiogenic effects of post-weaning social isolation (Weiss et al., 2004). Detailed reviews on the use and interpretation of the EPM have been published by Wall and Messier (2001) and Carobrez and Bertoglio (2005). The open field was chosen for its utility in measuring both locomotor activity and anxiety-like behavior, in the form of thigmotaxis. Previous studies have demonstrated that benzodiazepines, which increase open-arm time in the elevated plus maze (Pellow et al., 1985), also decrease thigmotaxis in the open field (Choleris et al., 2001; McNamara and Skelton, 1992). Upon completion of the lab, students were expected to be able to:

1. Articulate the specific effects of isolation rearing on amphetamine sensitivity and anxiety behavior.
2. Explain the mechanism of action of amphetamine and similar psychostimulants.
3. Provide conceptual and operational definitions of thigmotaxis and discuss its utility as a behavioral measure in the open field and the elevated plus maze.
4. Explain the concept of an animal model and discuss the importance of animal models in discovering developmental factors that influence vulnerability to psychiatric disorders.
5. Propose a cellular/molecular mechanism by which isolation rearing produces its behavioral effects.
6. Graph and perform statistically meaningful comparisons of behavioral measures between two rearing conditions.
7. Discuss their results in the context of the existing literature and provide possible explanations for discrepancies between their data and those from similar, published studies.

MATERIALS AND METHODS

Student Participants:

Participants were 63 students enrolled in the Williams College Introduction to Neuroscience course (NSCI 201, also offered as PSYC 211 or BIOL 211). The class was composed of two first-year students, 32 sophomores, 16 juniors and 13 seniors. Because most students in the class were sophomores, many had not yet declared majors. However, among students who had declared majors, Biology (34%) and Psychology (31%) were the most common. The lab portion of the course was divided into six sections, with 10-15 students per section. Prior to their scheduled lab section, students were asked to read a review article summarizing the behavioral, neurochemical and neuroanatomical effects of isolation rearing (Lapiz et al., 2003). They also completed a pre-lab worksheet, which required them to answer the following questions:

1. Define “thigmotaxis” both conceptually (i.e. what does the word mean?) and operationally (i.e. how do we define the concept so we can measure it reliably in the lab?).
2. Why are animal models used in neuroscience research?
3. Based on your readings, what behavioral differences do you expect to observe between isolated and enriched rats? How will we measure those differences in the lab?

At the start of the lab period, students were briefed on health risks associated with handling rodents and instructed in proper animal handling technique (see Heinrichs and Koob, 2006, for an excellent discussion of handling techniques). Students who had concerns about allergies were provided with gloves and lab coats.

Subjects:

All procedures involving animals were approved by the Williams College Institutional Animal Care and Use Committee and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals. Subjects were Long-Evans rats bred from the Williams colony. On the day after birth, PN 1, pups were counted, and litters were culled to a maximum of 12 pups. Litters were then left undisturbed, except for twice-weekly cage changes, until weaning at PN 21.

Half of the male pups in each litter were then assigned to the isolated condition, which consisted of individual housing in standard hanging wire mesh cages (17.8 x 35.6 x 17.8 cm^3). The remaining males from each litter were housed as a group (four to six rats/cage) in an enriched environment (*Figure 1*). The enriched environment consisted of a large wire mesh cage (76 x 46 x 152 cm^3) with multiple levels connected by ramps. Rats in the enriched environment were provided with a variety of “toys” (lengths of PVC pipe, balls, wood blocks, etc). The selection of toys in each cage was changed every four days. Each condition (isolated or enriched) had a total of 24 rats.

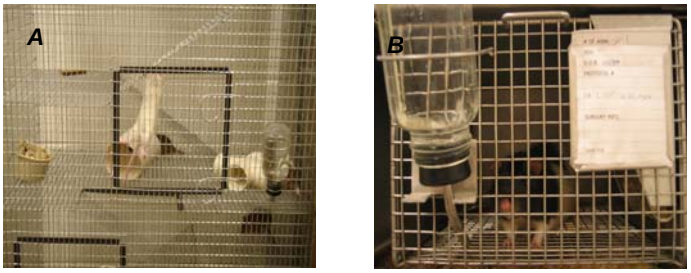

Figure 1. Cages for Enriched (*A: 17.8 x 35.6 x 17.8 cm^3*) and Isolated (*B: 76 x 46 x 152 cm^3*) Housing Conditions.

Rats in both conditions had *ad libitum* access to standard lab chow (Harlan Teklad) and tap water. Rats were maintained in these rearing conditions for approximately six weeks until they were tested by the Introduction to Neuroscience class. All rats were habituated to handling for two minutes per day for four days before the beginning of the lab exercise.

Lab Procedures:

Students performed testing during a three-hour lab period. Rats were transported to the teaching lab about 30 minutes

prior to the start of class. Cages were left in a quiet area of the lab and covered with a cloth drape to allow rats to recover from the stress of transport. Throughout the lab period, overhead lights were turned off, and dim light was provided by several lamps around the periphery of the lab. Students worked in groups of three. Each group was assigned one isolated rat and one enriched rat, but was blind to rearing condition. Rats were identified only by ear punch.

Open Field

Each group constructed an open field on their lab table by taping together four pieces of foam insulation board (Home Depot) to form a 90 x 90 cm^2 square arena. White lab tape was placed on the lab table top to divide the arena into nine smaller squares (Figure 2). After setting up the open field, one group transported their rats to the EPM testing room, while the others remained in the main lab for open field testing. Rats were left in cages on the table next to the open field for five minutes to habituate. One student placed the rat in the center square, started a 10-minute timer, and recorded total number of squares crossed. A second student counted entries into the center square, and a third counted peripheral square crossings. Hand-held cell counters (Fisher Scientific) were used to count square crossings. Students recorded the number of peripheral and center square crossings, total square crossings, and the order in which they tested their rats (right or left ear punch first) on their group data sheets. These data were used to derive a thigmotaxis score for each rat:

$$T = \frac{\text{peripheral square crossings}}{\text{total square crossings}} \times 100\%$$

Students were instructed to clean the open field with Odor Mute, an enzyme-based product that breaks down organic odor sources (Hueter Toledo, Inc., Bellevue, OH, http://www.gundogsupply.com/odormute-c-concentrate.html) after testing each rat.

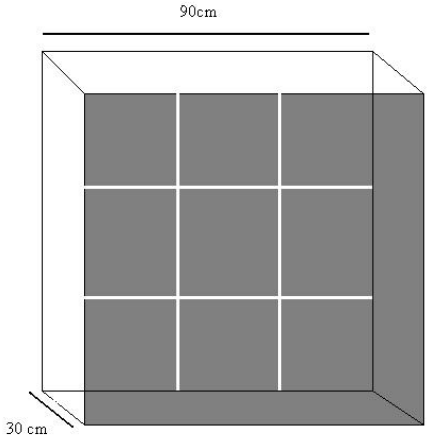

Figure 2. Open Field. Students constructed an open field by taping together four pieces of foam insulation board and marking nine equal sized squares on the lab bench with white tape.

EPM

The elevated plus maze was standard size for adult rats (each arm 40 cm x 10 cm, walls 40 cm high, elevation 1m). All maze surfaces were made of plywood and painted black. Rats were left in cages in the EPM room for five minutes to habituate. One student in each group placed the rat on the maze, while another observed the rat via video monitor and recorded number of open arm entries, number of closed arm entries and time spent in open/closed arms for five minutes. All variables were scored using a simple behavior scoring software program (ODLog, Macropod Software, http://www.macropodsoftware.com). Students were instructed to clean the EPM with Odor Mute after testing each rat. Groups took turns using the EPM until all groups completed it. Each group recorded open and closed arm entries, time spent on each arm and order in which their rats were tested on their group data sheets.

Amphetamine Sensitivity

Because amphetamine is anxiogenic, all groups performed amphetamine sensitivity testing last. The instructor injected each rat with one of three solutions: 0.9 % saline, 1.0 mg/kg d-amphetamine or 7.5 mg/kg d-amphetamine (1.0 ml/kg, s.c.). All rats were returned to their cages for 15 minutes to allow amphetamine to take effect. During this time, students entered their data from the open field and EPM into a master spreadsheet. Each rat was placed in the center of the open field and observed for five minutes. One student counted total squares crossed, and another scored the presence or absence of stereotyped behaviors: grooming, focused sniffing, head bobbing, oral stereotypes, and gnawing on the paws. Each group recorded these data and the order in which the rats were tested on their group data sheets. They then entered their data into the master spreadsheet.

Lab Report

After testing was completed, students participated in a post-lab discussion with the instructor, during which the blind was broken for housing condition. Class data were pooled, summarized and provided to students in an Excel spreadsheet. This spreadsheet contained the mean, standard error of the mean (SEM) and n for each rearing condition on each dependent variable. Each student prepared a three-page lab report, including graphs of pooled class data and a brief discussion of the class results in the context of the existing literature. Because many students in this class had not taken a statistics course, they were instructed in the use of an “eyeball” estimation method for identifying statistically significant group differences, based on overlap of standard error (SEM) bars.

RESULTS

Open Field

Rats reared in isolation or enriched environment did not differ in spontaneous locomotor activity, as measured by total square entries (t_{46} = 1.345, p = 0.1851). There were

no group differences in thigmotaxis scores (t_{46} = 0.6202, p = 0.5382) (Table 1).

Rearing Condition	Total Square Entries Mean (SEM)	Thigmotaxis Score Mean (SEM)
Isolated	66.75 (7.23)	99.91% (0.99)
Enriched	54.17 (5.93)	90.75% (1.19)

Table 1. Open Field Results. Isolated and enriched rats did not differ on total square entries of thigmotaxis score.

EPM

Rats reared in isolation spent significantly more time on the open arms (t_{39} = 2.092, p = 0.0429, two-tailed) and significantly less time in the closed arms (t_{39} = 2.382, p = 0.0222) than did rats reared in an enriched environment (Figure 3).

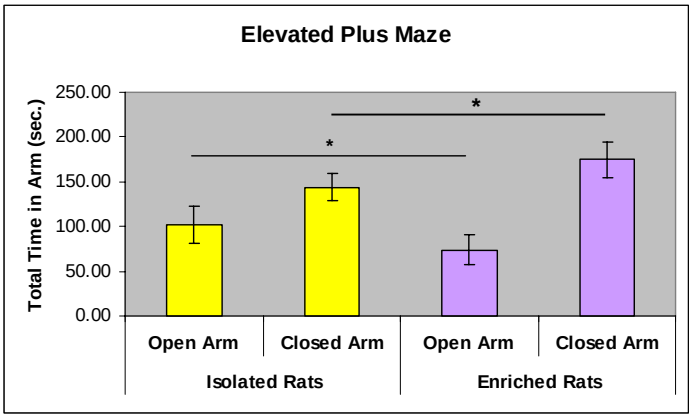

Figure 3. Elevated Plus Maze. Isolated rats spent significantly more time on the open arms (*p<0.05) and less time on the closed arms (*p<0.05) than did enriched rats.

Amphetamine Sensitivity

Amphetamine increased locomotor activity, as measured by total square crossings, in both groups of rats ($F_{2,41}$ = 21.006, p < 0.001) (Figure 4). There was no effect of rearing condition on amphetamine-induced activity ($F_{1,41}$ = 0.030, p = 0.864), nor was there a significant interaction between amphetamine dose and rearing condition ($F_{2,41}$ = 0.226, p = 0.799). Bonferroni's post-hoc tests were performed with data collapsed across rearing conditions. Both doses of amphetamine significantly increased locomotor activity compared to saline, but 7.5 mg/kg amphetamine did not significantly increase locomotor activity relative to 1.0 mg/kg. Though students were provided with standardized operational definitions for each category of stereotyped behavior, stereotypy scores were extremely variable and were not analyzed.

DISCUSSION

Student Outcomes:

Learning objectives 1, 3 and 4 were addressed using a pre-lab worksheet with questions related to the pre-lab reading assignment (Lapiz et al., 2003). Nearly all students were able to predict the behavioral effects of isolation rearing, define thigmotaxis, and discuss the utility of animal models in neuroscience research. The mean score on the pre-lab worksheet was 3.79 out of 4.0 possible points (94.8%), and the median score was 4.0.

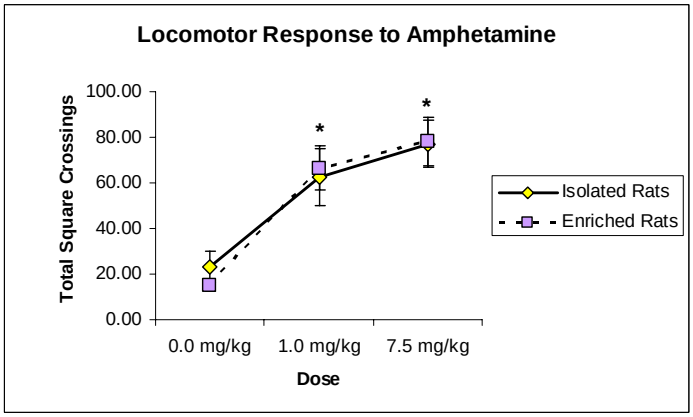

Figure 4. Amphetamine Dose Response. Amphetamine significantly increased square crossings in both groups of rats (*p<0.05 vs. 0.0mg/kg). The magnitude of the amphetamine effect was the same in both groups.

Learning objective 2 was addressed in a brief post-lab discussion with the instructor. The instructor drew a diagram of a typical monoaminergic synapse (Figure 5), with post-synaptic receptors and pre-synaptic transporters. Students were then asked how psychostimulant drugs increase synaptic monoamine levels. In all sections, at least one student volunteered and provided a correct mechanism (i.e. blockade or reversal of presynaptic transporter function).

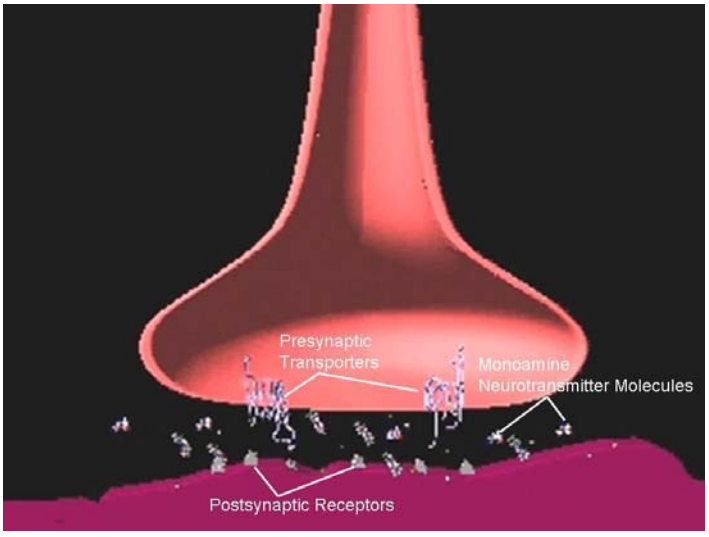

Figure 5. Typical Monoaminergic Synapse. Adapted with permission from the Multimedia Neuroscience Education Project (Zimmerberg et al., 1998).

The remaining learning objectives were addressed in the lab report. Students were instructed to prepare a short

report (no longer than 2.5 pages), consisting of results and discussion sections. The results section consisted of graphs of data from two of the three behavioral tests and a narrative description of results. The discussion section included answers to post-lab questions, comparison of class results to those in the existing literature, and discussion of any methodological errors that may have affected the outcome of the experiment. A similar report was required for each lab exercise, and all were evaluated using the same rubric (Table 2).

Element	Point Value
Format: Clear Results and Discussion Section; Length: Deduction if over 2.5 pages	1 (8%)
Results: Appropriate number and labeling of figures; Narrative presentation of results	3 (25%)
Discussion: Places results in context of existing research; Addresses discussion questions; Addresses experimental errors	4 (33%)
References: Credit given for all sources, including group data; Appropriate citation and reference format	2 (17%)
Quality of writing: Demonstrates understanding, Clear, Organized, Concise	2 (17%)
Total Points	**12 (100%)**

Table 2. Lab Report Evaluation Rubric. These criteria were used to evaluate all lab reports for the Introduction to Neuroscience course.

Though most students were able to produce appropriate data graphs with error bars (learning objective 6), many asked for technical assistance creating these graphs from the Excel spreadsheet. In this regard, instructors may find it helpful to provide students with instructions on generating graphs from spreadsheets in Excel or other platforms. Such instructions could be included as an appendix to a lab manual. Alternatively, if time and computer resources permit, students could create graphs together as part of the lab exercise. Most students were successful in identifying the significant group differences and drug effects outlined above by applying the SEM error bar overlap method.

In the discussion section of the lab report, students were asked to discuss their results in the context of existing literature (learning objective 7). Interestingly, the first time this exercise was conducted, the effects of environmental enrichment on anxiety-like behaviors and amphetamine sensitivity were not consistent with those published in previous studies. In fact, EPM results were opposite those reported in the literature (Weiss et al., 2004), with isolated rats exhibiting lower anxiety (i.e. more open arm time) than enriched rats. Several students suggested that this discrepancy might be due to repeated handling of the rats prior to testing. Indeed, repeated handling has been reported to reduce the effects of social isolation on anxiety (Holson et al., 1991).

Repetition of the experiment with a 2 (isolated vs. enriched) x 2 (handled vs. non-handled) design showed that handling did, in fact, have opposite effects on anxiety-like behavior in isolated and enriched rats (manuscript in preparation). That is, handling *increased* anxiety-like behavior in enriched rats, but *decreased* anxiety-like behavior in isolated rats. It is likely that this handling effect contributed to the unexpected results students obtained. Therefore, extensive handling of rats before testing should be avoided when implementing similar lab exercises. If safe handling of rats by students is a concern, a more docile strain, for which significant effects of post-weaning social isolation have also been documented (e.g. Sprague Dawley) could be used.

Only a small proportion of students were able to propose a cellular or molecular mechanism whereby isolation rearing might exert its effects on behavior (learning objective 5). Those who did generally focused on decreased dopamine transporter expression as an explanation for the increased sensitivity to psychostimulants found in other studies. Few addressed mechanisms that might account for the effects of isolation rearing on anxiety behavior. A more thorough pre-lab discussion of the cellular and molecular mechanisms underlying anxiety might have improved student outcomes on this learning objective.

Overall performance on the lab report was high. The mean score was 10.9 out of 12 possible points (90.8%). The median score was 11 (91.7%). The range was 9.25 – 12. Despite the discrepancies between class data and reports in the literature, students enjoyed this lab exercise. It was mentioned more frequently as a positive experience than were any of the other lab exercises on end-of-semester course evaluations.

Implementation

This exercise would be relatively easy and inexpensive to implement for any undergraduate program with basic animal care and testing facilities. We used the elevated plus maze to assess anxiety. The maze itself can be built from inexpensive materials available at any hardware store, as described by Pellow and File (1985). The open field used in this exercise to assess spontaneous and amphetamine-induced locomotor activity and thigmotaxis was built from inexpensive and reusable foam insulation board. Our students did not observe any group differences in open field behavior. However, trends were in the expected direction (i.e. increased locomotor activity and thigmotaxis for isolated vs. enriched rats). A longer test duration or longer habituation of the animals to the lab environment prior to testing may have allowed for detection of group differences on these measures. The open field can also be modified by adding a small, enclosed start box to one corner. In this version of the open field, anxiety is operationalized as latency to leave the start box (Paré et al., 2001). In a subsequent repetition of this experiment, we found that isolated rats had significantly longer latencies to leave the start box than did enriched rats (data not shown).

We bred rats for this exercise in our animal colony. This approach required extensive planning and preparation and significant animal care costs. Significant behavioral

effects of social isolation and enrichment have also been demonstrated for isolation beginning a few days after weaning (Brenes et al., 2008). Therefore, time and cost savings could be achieved by purchasing juvenile rats (22-25 days old) and placing them in enriched or isolated housing upon arrival.

When rats are placed in the isolated or enriched environment, they must be given an ear punch, or some other permanent mark. This allows students to distinguish between the rats, while remaining blind to rearing condition. To achieve maximal behavioral effects of environmental enrichment, a wide variety of enrichment items ("toys") must be provided. It is recommended that each large cage contain at least one toy per rat, and that the toys be made of different materials (plastic, wood, paper, etc.). A few toys should also be removed every four days, cleaned and rotated to a new cage, so that the rats experience a changing array of stimuli.

Instructors should consider the behavioral characteristics of the strain of rats selected for this exercise. We used Long-Evans rats because they were readily available in our breeding colony. However, due to concerns for the safety of the students and the animals, we felt it was necessary to habituate the rats to handling before they were tested in class. We later discovered, however, that repeated handling has opposite effects on anxiety-like behavior in isolated and enriched rats (article in preparation). Using a more docile strain, such as Sprague Dawley, is therefore recommended. Many of the behavioral effects of isolation rearing and environmental enrichment have been established using Sprague Dawleys (Bowling et al., 1993; Green et al., 2003; Weiss et al., 2004; Brenes Sàenz et al., 2006;), and this strain is typically easier for novice experimenters to handle.

One difficulty that arose during the first run of this exercise was the extreme variability among students' ratings of amphetamine-induced stereotyped behaviors. This was likely because students were not adequately trained to recognize these behaviors. The lab manual provided operational definitions for each behavior, but students were often unsure, and frequently asked the instructor or teaching assistants to confirm their judgments. We recommend that square crossings in the open field be used as a measure of amphetamine sensitivity, as our students were able to score this measure more reliably. However, if a measure of amphetamine-induced stereotypy is desired, we recommend that the instructor train students to recognize these behaviors before they begin testing rats on their own. This could be accomplished by viewing a brief video recording of a rat exhibiting these behaviors and scoring the behaviors as a class.

This lab exercise demonstrated a number of important concepts in behavioral neuroscience, including the effects of early experience on anxiety, dose-response relationships for drug effects, and the utility of animal models in neuroscience research. It also introduced students to commonly-used measures of rodent anxiety-like behavior and locomotor activity. We discovered some methodological issues during the first run of this exercise that affected the outcome of the experiments. We have proposed solutions to those problems to minimize frustration and maximize learning. Overall, students enjoyed this exercise and were successful in achieving learning outcomes, as indicated by high performance on lab reports.

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