# ARTICLE
# The Student Surgeon: A Behavioral Neuroendocrinology Laboratory Exercise in Rats

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This article describes a two-part laboratory module taught at Wheaton College and provides resources to allow instructors to recreate this module at their own institutions. This module introduces students to basic surgical techniques and allows for in-depth discussion of 1) effects of hormones on behavior, 2) ethics of animal use in research, 3) psychopharmacology, 4) experimental design and 5) empirical data collection. This exercise provides students with the opportunity to replicate a classic behavioral neuroendocrinology study while developing critical thinking, experimental design and empirical data collection skills.

*Key words: hormones; ovariectomy; lordosis; sexual receptivity; proceptivity; estrogen; progesterone.*

## INTRODUCTION

I developed this two-part lab in an effort to expose the students in my courses to the practical experience of conducting behavioral research using animal models and collecting empirical data. I use this lab exercise in Psychology 341: Laboratory in Behavioral Neuroscience, an upper-level lab course intended for junior and senior Psychology and Psychobiology majors at Wheaton College.

The course is structured like most lab courses of its kind and is limited to 20 students. In addition to sheep brain dissections and other expected lab exercises, I wanted my students to have an opportunity to actually replicate some of the work they were reading about. Being at a small college with a limited number of research laboratories means that my own laboratory is the primary place to expose students to research techniques relevant to neuroscience. My research focus of behavioral neuroendocrinology inspired the development of this laboratory module.

In rodents, sex behavior typically begins when the male mounts the female. In response to the mounting by a male, the female rodent will display the lordosis reflex (Pfaff et al., 1977; Pfaff et al., 1978). The lordosis reflex is classified by immobility, the female arching her back, raising her head, and the deflection of her tail to one side. Only when the female exhibits this combination of postural changes will the male be able to intromit his penis (Pfaff et al., 1977; Pfaff et al., 1978; Kow et al., 1979). Lordosis, as well as proceptive behaviors (i.e. hopping, darting and ear wiggling) exhibited by the female during mating interactions are easily observed and quantified (Hardy and DeBold, 1971; Erskine, 1989).

It has long been understood that sex behaviors in female rats are dependent on the ovarian hormones, estrogen and progesterone. Estrogen and progesterone levels change throughout the 4-5 day estrous cycle of the female rat and sexual receptive behaviors are only observed following peak levels of both ovarian hormones (Powers, 1970; Butcher et al., 1974). The importance of estrogen and progesterone in sexual behavior is also clearly demonstrated in females who have had their ovaries removed (via ovariectomy). Ovariectomized females do not display proceptive or receptive behaviors and therefore, are unable to mate with males (Boling and Blandau, 1939; Davidson et al., 1968; Hardy and DeBold, 1971; Komisaruk and Diakow, 1973; Butcher et al., 1974). However, sexual behaviors can be reliably reinstated in ovariectomized females by administering estrogen alone or in combination with progesterone (Boling and Blandau, 1939; Davidson et al., 1968; Hardy and DeBold, 1971; Komisaruk and Diakow, 1973; Butcher et al., 1974). There are almost no detectable differences between sexual behaviors facilitated by artificial hormone treatment as compared to sexual behaviors that occur during the natural estrous cycle (Hardy and DeBold, 1971).

This laboratory exercise was designed to recreate a portion of the experiments described in a classic study by Hardy and DeBold (1971) that examined the effects of varying doses of estradiol and progesterone on inducing sexual receptivity in ovariectomized female rat.

The use of small animal surgical techniques such as ovariectomy is ideal for undergraduate physiological lab courses. The relatively simple surgical procedure is easy to perform for students with little or no experience with animals or surgical techniques. The opportunity to perform a surgery is a unique one for undergraduates that can best be offered to students in a laboratory course with a small number of students (I typically conduct the surgery portion of this lab with only 9-10 students at a time). Performing a recovery surgery provides students with some technical skills and a potentially new understanding that they are capable of doing the technical work required of a researcher.

The use of an ovariectomy lab exercise has the added benefit of providing animals to use in a second lab exercise focused on sexual behavior observation. One week after surgery, the ovariectomized animals are given different doses of estrogen and/or progesterone and tested for sexual receptivity with male rats. This second lab provides an opportunity for students to develop a set of research questions, create an experimental design, and participate

in data collection and hypothesis testing using a real animal model of hormone action in the brain. Both the surgery lab and the behavioral testing lab are designed to correspond with their assigned readings relating to the "Methods and Strategies of Research" and "Reproductive Behavior" chapter of our textbook (Carlson, 2010). The students are also required to read Hardy and DeBold (1971) before coming to class.

## MATERIALS AND METHODS

### Subjects

All experimental subjects are gonadally intact adult female Sprague-Dawley rats (n = 16) obtained from Simonsen Laboratories (Gilroy, CA, USA) and housed in the vivarium facilities at Wheaton College. The female rats are approximately 150- 175 g and 50 days old at the start of the study. Upon arrival, all females are group-housed (4 rats to a cage) in stainless steel hanging cages and kept on a 14:10 hour light:dark light cycle (lights ON at 24:00). Sexually experienced Sprague-Dawley male rats (n = 5) obtained from Simonsen Laboratories (Gilroy, CA, USA) are used as sexual stimuli during mating tests and are individually housed. The age of the males is less important than sexual experience (see below), however the males should be at least slightly larger than the experimental females for optimal testing. All animals are given food and water ad libitum and animals are allowed to adjust to the vivarium conditions for at least one week prior to the start of the study. All procedures used in this study adhere to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Wheaton College.

### Ovariectomy

All female rats are ovariectomized through a single midventral incision under Ketamine cocktail anesthesia (Ketamine HCL 100 mg, Xylazine HCL 20 mg and acetopromazine 10 mg/ml (Sigma Aldrich, St. Louis, MO) in distilled water) delivered i.p.. The dose is calculated using the following formula: (Body weight (grams) X 1.5) + 0.1. Metacam, an oral antibiotic, was administered (1 drop/rat orally) just prior to and 24 hours following surgery. Body weights are taken the day of the surgery for accurate dosing. The drugs listed above, especially ketamine, are controlled substances and cannot be obtained without appropriate federal and state licensing. This process can take months to complete and instructors should plan accordingly. Obviously, other methods of anesthesia could be used if available and deemed appropriate. While administering the anesthesia, I discuss the specific drug effects and review the drug classes and related CNS effects. I also demonstrate the methods for assessing level of anesthesia (response to tail pinch and/or toe pinch, monitoring breathing, etc.) prior to beginning the surgery.

The animal's abdomen is shaved and the surgical area (and 1.5-inch square area of abdomen by the top of leg to the midline) is cleaned with iodine or Novalsan solution (Henry Schein, Melville, NY). Instruments are soaked in Novalsan solution and rinsed with sterile water before use. An initial vertical incision to the skin is made using a scalpel, approximately 0.5 inches long. The connective tissue between the skin and the abdominal wall is gently severed using small scissors. Small scissors and rat-toothed forceps are used to make a second internal incision in the abdominal wall that is slightly smaller than the external incision. Caution should be taken to lift the abdominal wall away from the body cavity while making the incision to prevent accidental damage to underlying organs. Using two blunt smooth forceps, the abdominal incision is opened and the uterus is located. Typically, the pink fat surrounding the uterus is easy to locate and can be pulled on to locate the uterus. Caution must be taken to avoid the nearby bladder and intestines. The uterus may also be easily identified by the dark red spider web-like blood vessels attached to the length of the pink uterine tissue. Using a gentle hand-over-hand movement, both horns of the uterus are lifted out of the body cavity and placed on sterile gauze laid out on the abdomen of the rat. Once laid out, the ovaries attached to the far ends of each uterine horn can be seen, often surrounded by a small amount of fat. Sterile absorbable surgical suture (chromic gut, Henry Schein, Melville. NY) is used to securely tie off each uterine horn ¼ inch below the ovaries. The ovaries are then cut off using scissors just above the suture tie-off. If there is no bleeding after the ovaries are removed, the uterine horns are gently replaced in the abdominal cavity. If there is bleeding, an additional tie-off may be made slightly below the first to stem bleeding before returning the uterus to the abdominal cavity. The wound is closed internally with sterile absorbable surgical suture and the external incision is sutured using sterile non-absorbable Vicryl suture (Henry Schein, Melville, NY). Level of anesthesia is assessed before, during and after surgery by monitoring breathing and response to mild tail and ear pinch. The animals are placed on warm heating pads during recovery and are not put back into their home cages until they are fully awake and mobile. All animals are observed two times a day for 48 hours post-operatively to assess pain/distress and health.

To begin the surgery lab, I demonstrate the surgery once for the group. During the demonstration, I identify all of the necessary procedures, instruments and anatomical landmarks. After the initial demonstration, I will provide each student pair with an anesthetized rat and will do another surgery while they follow along. It has been very helpful to have one or two other people who are experienced with the procedure available to help students while I work on my animal. While the ovariectomy procedure would typically take me approximately 10 minutes to complete, I plan on 90 minutes for the discussion, demonstration and student surgeries. This time frame allows me to work with two groups within our three-hour lab time. However, more time would certainly be helpful, especially if the instructor is new to the technique.

### Behavioral Testing

Because rats are nocturnal and display maximal behavioral activity during the dark phase of their light/dark cycle, all behavior tests are conducted approximately three to four

hours after lights off under dim red light. This light will allow the experimenters to see but will not be detected by the animals. All testing will occur outside of the home cages in a glass enclosure with absorbent paper or shavings on the floor.

Male sexual training/screening: Prior to use in experiment, all naïve male rats should be screened for sexual behavior and allowed to mate with a sexually experienced and receptive female rat. A mating interaction between a male and female rat is comprised of a series of brief, discrete mating stimulations when the male mounts the female and quickly dismounts. Each mount attempt can be classified as a mount, a mount with intromission or a mount with ejaculation. A mount is characterized by the male grasping the female from the rear with his forepaws and may be accompanied by pelvic thrusting. An intromission is similar to a mount but the pelvic thrust is longer and results in penile insertion. During an intromission, the female may respond with a more intense and prolonged lordosis response. Ejaculation is characterized by a prolonged intromission with seminal emission. Following ejaculation, the male will usually raise its forepaws before dismount and will begin an extended period of inactivity. Typically, a male rat may ejaculate after approximately 15 minutes of mating with a receptive female, however naïve rats may take longer the first several times they mate.

During the training/screening procedure, each male will be allowed to mate with a sexually receptive female until the male ejaculates, 30 minutes elapses without ejaculation, or 15 minutes elapses without any mounts, intromissions or ejaculations. All male rats are given free access to females two to three times prior to use in the experiment. Any males that fail to exhibit any mating behaviors should not be used for testing. Five sexually experienced male rats would be adequate to conduct this study.

Female behavioral testing: At least, five to seven days after ovariectomy, females are given estradiol benzoate (EB, 5 µg/rat) or the vehicle 48 hours prior to behavioral testing followed by progesterone (500 µg/rat) or the vehicle three to four hours prior to behavioral testing. EB and progesterone are dissolved in USP grade sesame oil and delivered s.c. in a 0.1 ml volume. The experimental groups are: Estradiol Benzoate + Progesterone (EB + P, n =4), Estradiol Benzoate + Vehicle (EB alone, n = 4), Progesterone + Vehicle (P alone, n = 4), Vehicle + Vehicle (Vehicle, n = 4).

Females are removed from their home cage and placed into testing arena with a male. Once both animals are in the testing arena, the students begin recording mating behaviors including mounts, mounts with intromissions and mounts with ejaculation. During the test, the response of each female to each mount is rated on a 4-point scale based on Hardy and DeBold's original 3-point scale (1971). A score of 0 is characterized by a hunched back, a score of 1 is characterized by a slightly flat back, a score of 2 is characterized by an arched back and raised head, and a score of 3 is characterized by a more intensely arched back and a head raised at a higher level (see Hardy and DeBold, 1971 for an illustration).

To quantify the receptive behavior, a lordosis quotient (LQ) and a lordosis rating (LR) are calculated for each animal. LQ is a measure that reflects the percentage of lordosis responses the female rat displays in response to mounts by the male. The LQ score for a female rat is calculated by dividing the number of receptive mounts (scored 2 or 3) received by the total number of mounts x 100. LR is a measure of the intensity of the female's response. The LR score is calculated by adding the lordosis scores (0, 1, 2, or 3) for all mounts and dividing by the total number of mounts. Experimenters will calculate both LR and LQ because LR can provide more information about the degree of receptivity (or lack thereof) observed. For example, a female who exhibits 10 lordosis responses scored as 2 will have the same LQ (LQ = 100) as a female who exhibited 10 lordosis responses scored as 3 (LQ = 100). Yet, the first female would have an LR of 2 and the second female would have an LR of 3, indicating a more intense receptive response.

Typically, a female will receive 10 mounts from a male during a receptivity test. Non-receptive females may take longer to test than receptive females due to loss of interest from the males. Having several sexually experienced males available and moving the female to a different male after each mount should maintain male interest.

Proceptive behaviors may also be observed and quantified by students during this demonstration. When sexually receptive, the female rat moves about the testing chamber with quick, darting movements called hops and darts that are not observed at other times during the reproductive cycle. Although more difficult to observe, students will enjoy seeing the female "ear wiggle." Typically seen just after a series of hops and darts and just prior to a mount when a female "plants" (stops and remains immobile) in front of the male, the female will make many very small, fast, imperceptible head movements that will cause the tips of her ears to shake. These proceptive behaviors signal a female's sexual readiness to the male and are only observed in sexually receptive females. The students can simply count the number of these behaviors they observe and compare the totals between groups.

## RESULTS

The experimental design provides very reliable and predictable results that the students are very likely to correctly predict. Based on the results of Hardy and DeBold (1971), the combination of estradiol and progesterone would be expected to induce high levels of sexual receptivity and proceptive behaviors. The estradiol alone group may display very low levels of receptivity and very few proceptive behaviors while the progesterone alone and vehicle groups were expected to show no receptivity or proceptivity. The following data are based on student scores taken during class. As predicted, we observed a significant difference between the different hormone-treated groups on both measures of sexual receptivity: lordosis quotients ($F(3, 15) = 159.22$, $P < 0.05$) and lordosis ratings ($F(3, 15) = 113.38$, $P < 0.05$). Mean lordosis quotients and lordosis ratings were significantly

greater in the EB + P group as compared to all other treatment groups (Tukey, $P < 0.05$, See Figures 1 and 2). The EB alone group did show some receptive behavior but this amount was not significantly different as compared to the P alone or the Vehicle group (See Figures 1 and 2).

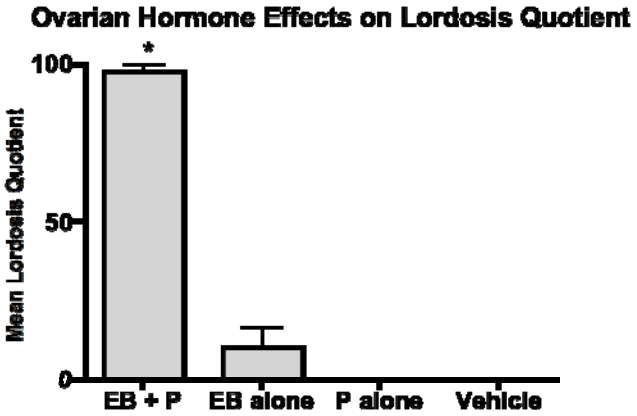


*Figure 1.* Effect of estradiol benzoate (EB) and progesterone (P) on mean lordosis quotient. * = $P < 0.05$.

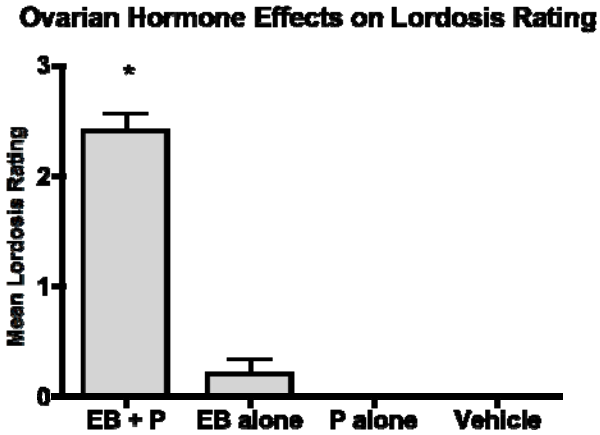


*Figure 2.* Effect of estradiol benzoate (EB) and progesterone (P) on mean lordosis rating. * = $P < 0.05$.

## DISCUSSION

I have found that the success of this lab depends on staging it properly within the rest of the semester. It is useful to prepare the students in advance for what could be a stressful laboratory exercise for some. On the first day of class, I let the students know that we will be doing a lab that involves use of animals and then initiate a semester-long discussion of ethical use of animals in research. That day, we discuss animal research as one part of a historical introduction to the field and mention that we will spend more time thinking about specific concerns regarding animal use in research throughout the semester. I also make it clear to students that participation in surgery lab is not mandatory but students must discuss concerns with me before opting out of the lab. Students may also choose to attend the lab but not participate. Each year, I have maybe one or two students who are concerned and on average, I may have one student who chooses not to participate.

Because we are working with live animals, not prepared specimens, the students are often concerned and curious about the guidelines for working with animals in research and teaching. I use the opportunity to inform the students about proper ethical concerns and procedures for using animals in research. I provide information sheets about animal use at our institution and give details about our Institutional Animal Care and Use Committee's (IACUC) procedures. The students leave this portion of the course understanding the approval process for animal use by the IACUC and have access to contact information if they have questions or concerns they do not feel comfortable bringing up in class.

Timing the exercise in relation to other labs or specific course content is also important to consider. It is helpful for the students to have had some experience with surgical instruments. Therefore, conducting this set of lab exercises after our lengthy sheep brain dissection module allows for the students to become comfortable with handling scalpels and forceps. I also find that this lab provides a great opportunity to review basics of the Psychopharmacology and Research Methods chapters we have already covered. Therefore, having covered those chapters prior to participating in this lab will enhance the student's understanding of the discussion.

I have been using this lab exercise for six years now and I can easily say that is the highlight of the course for most students. I do not collect specific quantitative data on individual lab exercises but this course is always rated very highly by students (4.76 out of 5) and they report learning a great deal of information (4.83 of 5) and are exposed to new insights and ideas (4.33 of 5). Students are allowed to provide written comments on their course evaluations and I frequently see excited reports from students about the "rat sex lab." In the space where students are asked to comment on the most useful aspects of the course, students often comment on how much they took away from this lab module. The following are examples of comments from student evaluations in previous years: "I loved doing the surgery lab. It was amazing!" "The surgery was great- I didn't think I would be able to do it but I did." "The rat surgery was the best thing I have done at Wheaton." The anecdotal evidence for the success of the lab with students has often come years after graduation. I receive at least one email a year from alumni who say they are doing work that reminds them of our class and the surgery lab and are grateful to have had experience. As of yet, I have received no negative feedback regarding these lab exercises. Even students who disapprove of animal research leave the course with an understanding of the carefully considered process we employ and the rationale for this type of research.

As an instructor, I am pleased with both the proximate and ultimate results of this laboratory module. It elaborates on several aspects of the content covered in the course but it also provides the active participation in research that I wanted to bring to the classroom. By bringing the students into my own lab, I hope that these students will begin to associate research with the people who do the work and will not see research as just numbers or dates in a book. Although it is unclear if this lab is the direct catalyst for this

change, I have seen an increase in the number of students in the course who perceive research as a career path. This change is important because the number of graduates who choose to apply to Masters and Ph.D. degree programs in science has historically been low at our institution. This laboratory module provides the opportunity for students to be researchers and experience what an academic researcher does outside of the classroom. My students leave with a clear understanding that research is an endeavor that is separate from teaching, yet the two forms of scholarship continually inform each other for the scholar/teacher.

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