



# PSY310 LABORATORY 3

## THE EFFECTS OF CASTRATION AND HORMONE REPLACEMENT ON SEXUAL AROUSAL IN ADULT MALE MICE (*Mus domesticus*).

Male mice emit 70kHz vocalizations while investigating and copulating with females. These vocalizations are an index of the male's sexual arousal. Experienced males will emit vocalizations to the odor of a female (e.g., urine odors). In contrast, naive males rarely vocalize to female odors. In group housed male mice, the dominant animal usually emits the most vocalizations and is most attractive to females. This fact suggests that the level of testosterone present in his bloodstream determines the amount of vocalization a male makes.

In this lab we will conduct an experiment that will test this hypothesis. Naive males will be given social experience with a female. Following this social experience regime, the males will be tested to verify that they reliably emit USV to a female conspecific. Next the males will be randomly assigned to one of three groups: a group that will be castrated and given an implant containing testosterone propionate (CAST + TP); a group that will be castrated and given an implant containing estradiol benzoate (CAST + EB), or a group that will be castrated and given a blank implant (CAST + BLANK). One week following the surgeries, all animals will be tested for their USV in response to the odor of female mouse urine and an intact female mouse.

The lab report is due at 2:30 PM on Monday, November 19. The lab report will be comprised of the following sections:

- Cover page
- Abstract
- Introduction
- Method
  - Subjects
  - Apparatus
  - Procedure
- Results
- Discussion
- References
- Tables
- Figure Captions & Figures

## **The Effects of Castration on Sexual Arousal in Adult Male Mice (*Mus domesticus*)**

Many rodent species communicate with conspecifics (i.e., other members of the same species) using ultrasonic vocalizations (Nyby & Whitney, 1978). These vocalizations are called ultrasonic because they are above the typical human range of hearing. Most humans are only able to detect sounds with frequencies between 20 Hz and 20 kHz (Pinel, 1992). Bats use these ultrasonic vocalizations (USV) to echolocate prey and navigate in total darkness (Sales & Pye, 1974). Although there is little compelling evidence for echolocation in other rodents, many rodents emit USV during sexual or aggressive interactions between adults. In particular, male mice emit 70 kHz vocalizations during sexual encounters with female conspecifics (e.g., Nyby, 1983). These vocalizations may be elicited by a variety of stimuli associated with the female, but urinary odors are sufficient to elicit an amount of vocalization equivalent to that of an intact female (Whitney et al., 1974; Nyby et al, 1977).

USV are believed to be an index of sexual arousal in males. Like other measures of sexual arousal, USV have been shown to be androgen dependent (Bean, et al., 1986; Dizinno & Whitney, 1977; Nunez, Nyby, & Whitney, 1978; Nunez & Tan, 1984; Nyby, Matochik, & Barfield, 1992). When the level of testosterone (or in some cases metabolites of testosterone) within the circulation fall below a critical threshold, most males cease to vocalize. In this experiment we will test this hypotheses as well as compare the ability of testosterone and estradiol to maintain USV.

### **Method**

#### ***Subjects***

All animals are F1 offspring of AKR/J male and C57BL/6J female mice (Charles River Laboratory: Wilmington, MA) bred in the animal vivarium at Washington College. Subjects are adult (>55 day old) male mice from which ultrasonic courtship vocalizations are monitored in response to experimental stimuli. All subjects are individually housed in 12.5 cm x 17 cm x 28 cm polycarbonate cages, with stainless steel wire lids, and filter barrier tops (Micro-isolator Cages; Lab Products, Maywood, NJ). Social-experience animals are adult male and female mice that were systematically introduced into each subject's home cage prior to the start of behavioral testing. All animals are allowed free access to food (Purina Mouse Chow) and water, and are maintained on a 12 hr. light-dark schedule (lights-on at 0800 hrs.).

#### ***Social Experience Regime***

Each of the subjects in this study have been given daily 3min encounters with a male and a female conspecific in the subject's home cage. The order in which they encountered a male and female mouse was counter-balanced across days. To insure that the animals were not injured, the male social experience animal was removed from the cage once they began to fight.

#### ***Surgical Castration and hormone replacement:***

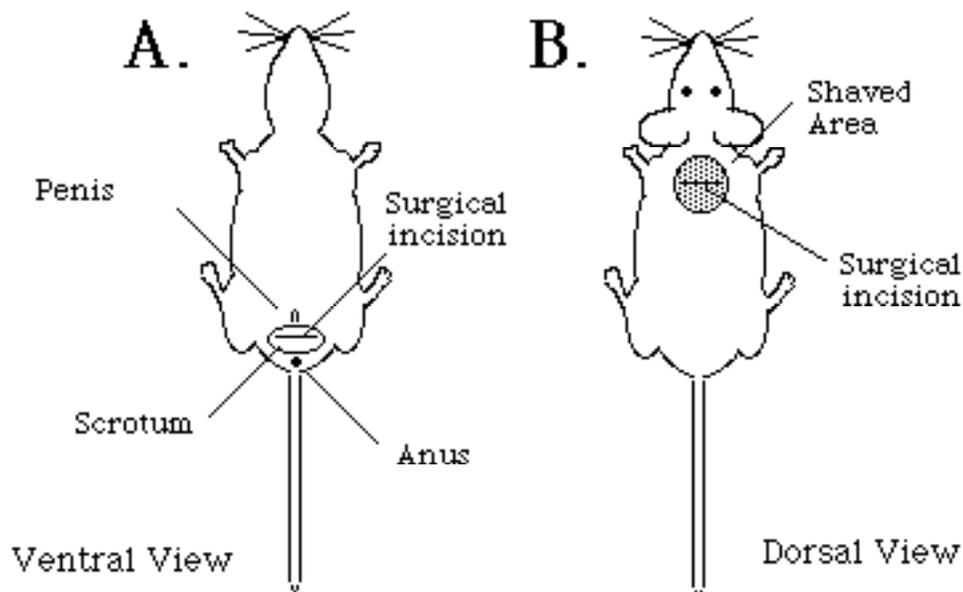
Before you attempt to perform this procedure on your own, the instructor and laboratory assistant will demonstrate the procedure for you. There should be at least one person in each laboratory team who is willing to perform the surgery. The other members of the team will assist. There will always be an instructor and laboratory assistant available to help with your surgeries.

1. Weigh your animal on the balance and record body weight.
2. *Anesthetization:* Using pentobarbital, inject your animal intraperitoneally (i.p.) according to the following formula (0.1cc per 10g of body weight; approx 75mg/kg).

While the anesthetic is taking affect, prepare the sterile surgical tools and supplies several strands of surgical silk approximately 10cm long and tie them in a loose overhand knot. Thread a 20cm long piece of silk through the eye of the surgical needle. Clamp a needle-holding hemostat to the needle so that it also grasps the thread.

- Periodically test the reflexes of the mouse to determine when it is sufficiently anesthetized. First the animal should loose a righting reflex. Thereafter they should become less and less responsive to auditory and tactile stimuli. When the animal has reached an adequate degree of anesthesia to proceed they should be unresponsive to a moderate pinch of their tail and a gentle pinch of their footpad with a blunt forceps

After the animal is deeply anesthetized, use the electric shears to shave the area surrounding the scrotum and a small area on the dorsal surface of the animal's back, near the nape of the neck. Next, use forceps and a sharp pair of tissue scissors (iris scissors) to make a scrotal incision that runs perpendicular to the midline, exposing both testes (See Diagram A). Grasp the scrotum with the forceps, lift the tissue gently forming a "tent" and create a small (2-3mm) incision. Take care not to cut too deeply; you only intend to cut the outer most layer of skin. Make a single incision, rather than several. This will insure that you have two straight edges of the incision to suture once the testes have been removed.



- The testes have a tendency to regress into the body cavity. To get them into the scrotum where they will be accessible, gently rub your finger along the animal's body in a rostral to caudal direction. If this fails, grasp the mouse by the nape of the neck and gently dangle above the table until you observe that the testes have descended into the scrotal sacks.
- Each testis is individually encased in a thin peritoneal membrane that must **first** be cut before the testis can be extruded through the scrotal incision. To cut the membrane, grasp it with a forceps and pull it away from the testis. Using the iris scissors cut the membrane. A very small incision is sufficient. The testis can be extruded from the scrotum by rubbing the animals belly or gently dangling the animal in the fashion described earlier.

Extrude both testes before proceeding. The testes are oval pink masses, approximately the size

and shape of a very small pea. The testes also can be distinguished from other internal organs by the presence of the vas deferens and seminal vesicles. The vas deferens appears as a very pink, delicate tube-like tissue. The seminal vesicles are a bright white, fat-like substance (However, unlike fat the vas deferens is extremely vascular). The seminal vesicles are usually visible through the thin peritoneal membrane before you make your incision in it. To externalize the testes, gently grasping the seminal vesicles using the forceps and pull. The vas deferens and testicle will usually appear through your incision. If you think this has happened, or you are having difficulty identifying the vas deferens, STOP. Ask for assistance.

Sometimes the intestines may appear through the scrotum. These are typically much darker (light brown to tan) in color than the vas deferens or seminal vesicles. DO NOT pull these through your incision. STOP. Ask for assistance.

After the testis is extruded, tie off the blood vessels that supply the testis and vas deferens with silk suture thread. This takes some practice; you should *ask the lab assistant/instructor to demonstrate this for you*. After both testes have been ligated, remove the testes with the iris scissors. Be careful to cut distal to your ligature. You want to leave enough tissue distal to the knot to insure that the ligature does not unravel.

Using the forceps or a blunt probe, push the extruded tissue back into the body cavity of the mouse.

Gather the tissue of the scrotum together, pulling it up and away from the body. Use the 20cm length of surgical thread and the needle to close the scrotal incision. This takes practice. *Ask the assistant/instructor to demonstrate the technique*. Take care not to pinch the penis or occlude the anus. Failure to close the incision may allow the intestines to "leak" from the abdominal cavity or provide a source for infection. *Ask the assistant/instructor to inspect your suture*. Apply a coat of antibacterial cream to the region surrounding the incision.

Alternatively you may use wound clips to close the incision. However, although these are easier to use than mastering the art of suturing, they can be a source of irritation and possible infection if they become caught on the wire cage-lids as the animal climbs about the cage. After you have applied the wound clip examine the scrotum carefully to determine that the incision is completely closed. *Ask the assistant/instructor to inspect your suture*. Apply a coat of antibacterial cream to the region surrounding the incision.

10. Place the mouse so that the dorsal side is exposed. Grasp the skin of the nape of the neck in the center of the area you had previously shaved (See Diagram B). Pull the skin away from the animal's body and make a small (2-4mm) incision perpendicular to the midline. Using a blunt probe (or the tips of the surgical scissors), run the probe into the incision and below the animal's back, forming a small pocket beneath the surface of the skin. Obtain one of the previously prepared silastic capsules containing testosterone propionate (TP) from the lab assistant/instructor. Insert the silastic capsule into the pocket that you formed in the nape of the animal's neck and close the incision with a wound clip. Examine the incision to determine that there is no opening through which the silastic capsule may be lost. If necessary you may need to use a suture to close the dorsal incision. *Ask the assistant/instructor to inspect your suture*. Apply a small amount of antibacterial cream to the region surrounding the incision.
11. Repeat steps 1-9 using another mouse. Insert either a blank silastic capsule (BLANK) or a silastic capsule containing estradiol benzoate (EB). The assistant/instructor will tell you what type of capsule to implant.
12. Your animal should recover within several hours. Often, they are alert and active within 1hr. They will be returned to the animal colony by the assistant/instructor and their recovery

monitored over the course of the next 48hrs. If there appear to be signs of pain/distress, an analgesic will be administered.

### ***USV Tests***

One week following the surgeries, you will be responsible for collecting behavioral data from two mice. You will determine the amount of USV that these mice emit in response to the odor of urine from female mice, and to a female conspecific. Behavioral tests will occur in Dunning RM36.

*Preparations for testing.* The red lights should be on and the white lights should be off in the test room. Carefully remove the gray rubber cap from the microphone attached to the Bat Detector. Take care that you do not touch the thin membrane on the face of the microphone. Turn the bat-detector on, and adjust the volume so that it is set at the mid point. Check that the large dial on the top of the detector is set at 70kHz. The gain should not be adjusted.

Fill out the top of one data sheet (e.g., Experiment, Experimenter, Date). Let's call this the "*Biopsych Lab 3 USV Experiment*" (see APPENDIX for a copy of a sample data sheet).

*Animal handling & testing.* You will be told which subjects you are responsible for testing. Begin by retrieving one of these animals from the colony room (RM 27). Before you walk from the colony to the testing room, flip the water bottle in the cage so that water does not leak into the cage and soak the bedding.

Record the animal's number on the data sheet provided. Place the cage under the microphone and listen for USV. Allow the animal to habituate to the testing environment for 1min. However, if the animal vocalizes during the habituation period, make a note of this in the margins of the data sheet, and do not proceed unless 2min have passed in which the animal has not vocalized.

Take a cotton-tipped swab and the syringe with urine and inject 0.1ml of urine onto the cotton. (Urine will be collected using metabolism cages during the night preceding your tests and supplement with freshly voided urine if more is required). Place the urine soaked tip into the test tube provided, and break off the end of the swab that you've handled.

Dump the contents of the test-tube into the animal's cage.

Place the animal's cage beneath the microphone, start the stopwatch, and record the amount of ultrasonic vocalizations that you hear during a 3-minute trial.

Each 3-min trial is divided into 36 5-second epochs or bins. Place a check in each 5-sec bin where you heard the male vocalize. Place a horizontal line (dash) in each bin where you heard no vocalizations. See the sample data sheet provided in the APPENDIX).

At the end of the 3-minute trial,

- return the subject to the animal colony,
- turn the water bottle around so that the mouse can get water,
- insure that the wire top is properly seated on the cage,
- replace the filter-top on the cage.

Get the next mouse and repeat the testing procedure.

Clean-up. When both animals have been tested and returned to the animal colony,

- lock and close the animal colony door.
- Turn the bat detector off.
- Place the data sheet in the folder provided.

- Turn the lights in the test room off.
- Lock and close the door to the testing room.

### ***USV to Intact Female Mice***

Within the next 24-48hrs, you will test the same subjects for their response to an intact adult female mouse. You should employ the same testing procedure used to test the response to female urine, however in these tests you will place a female in the subjects home cage and monitor USV. In addition to monitoring USV, use another stopwatch to time the latency for the male to mount the female. You can tell that the male is attempting to mount the female if he approaches her from the rear, attempts to climb on her hindquarters, and rapidly palpates (rubs) her flanks. Record the mount latency in seconds. If the male does not attempt to mount the female, the mount latency = 180 seconds.

### **Results**

The data from all of the groups will be pooled with data collected by students in prior years and distributed in class. Each group will be responsible for analyzing the data. You should use both descriptive and inferential statistics to summarize the data and make comparisons between the three groups. Programs for statistical analysis of the data are accessible on most OIT computer classrooms and the laboratory computers in the Psychology Department. Another statistical program, InStat, is available on the biopsychology lab computer. If you are not familiar with the statistical packages available (e.g., StatView, SPSS), I recommend that you use InStat to analyze your results. InStat has help screens that will help you in select and interpret the appropriate statistical test for this experiment.

**IMPORTANT: DO NOT WAIT TILL THE LAST MINUTE TO DO THE STATISTICAL ANALYSIS. IF YOU NEED ASSISTANCE MAKE ARRANGEMENTS WITH ME IN ADVANCE TO PROVIDE HELP USING THE STATISTICAL PACKAGES AVAILABLE IN CAMPUS COMPUTER LABS AND/OR THE BIOPSYCH LABORATORY COMPUTER.**

### **Discussion**

Each student is responsible for writing his or her own results and discussion sections for your laboratory report. We will review the statistical analysis of the results in class and discuss the outcome and conclusions, but when you write the laboratory report you should do so independently. A part of the discussion should include comparisons with findings obtained in earlier experiments, suggestions for revising the study, and suggestions for future studies.

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- Whitney, G., Alpern, M., Dizinno, G. and Horowitz, G. (1974). Female odors evoke ultrasounds from male mice. *Animal Learning & Behavior*, 2, No. 2, 13-18.

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*In addition to the sources listed above, the following articles relating to ultrasonic vocalizations are on reserve in the library. These also may be used to help write the introduction and discussion of your lab report. feel free to use additional sources if you desire.*

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- Maggio, J.C., Maggio, J. H. and Whitney, G. (1983). Experience-based vocalization of male mice to female chemosignals. *Physiology & Behavior*, 31, 269-272.
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Additional details regarding this laboratory exercise may be found online in the Journal of Undergraduate Neuroscience Education (JUNE),

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