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Introduction

Behavioural Neuroscience Lab Manual

Welcome

Note, test the following: here

This is a simple manual which outlines the content currently being provided by the BNLM project. The content is hosted in GitHub, while using GitBook to generate this website. This site is designed for those who are less tech savvy to have easy access to the documentation. In future updates, easy to use download links will be provided so that individual chapter pdfs and modifiable .doc files may be accessed.

In the mean time, the repository on GitHub has the full pdf format of this work as well as the master word document. Simply follow the following link and follow the instructions to access this information.

BNLM on GitHub

Probably put some sort of index here of the different reports.

Animal Handling

Objectives:

- To learn about animal laboratory safety
- To learn about respectful use on animals in a lab setting
- To learn how to handle a rat properly
- To learn how to give a rat an injection

Introduction:

Behavioral Neuroscience attempts to explain the neural underpinnings of behavior. There are a variety of approaches that can be used to study the neural control of behavior in humans, such as neuroimaging studies and neuropsychological assessments in clinical patients, but these approaches do have limitations as they involve people. Animal models allow for greater experimental control, finer resolution, and more invasive procedures. The animal used in research must always be treated with respect and compassion, and all procedures conducted with the animal must adhere to the guidelines outlined by the Canadian Council of Animal Care (CCAC, 1993). Additionally, all procedures conducted on the animal must have had prior approval of the university's animal ethics committee.

Throughout this course, you will be exposed to a variety of approaches for studying the behavior of the laboratory rat. Proper handling and husbandry of the laboratory animal is essential for both ethical and practical reasons. In this laboratory exercise, you will learn the proper technique for handling a rat, and will gain experience in proper restraint and injection methods. These skills require practice and learning, both on the part of you and the rat. A rat that is handled frequently will become easier to handle and will be less anxious during experimental procedures.

Procedure:

Safety: In this lab, you will learn the proper procedure for animal handling so that risk of injury to both you and the animal is minimized. Accidents are always a risk in any setting, and an animal lab is no exception. Report any accidents to your TA or Instructor and they will ensure that proper emergency responses are initiated. When working with animals, it is always a good idea to make sure your tetanus vaccinations are current (within the last 10 years). The tetanus bacillus is ubiquitous and can make you quite ill if your skin is broken. You should always wear a lab coat when working with animals. Washing your hands before and after handling animals is essential. Wearing latex or nitrile gloves is also required in order to protect both yourself and the rat. Heavy gloves should not be used for animal handling as they increase the risk of injuring the animal.

Habituation: Frequent handling from an early age makes the animals much easier to handle. The best approach when initially handling a rat is to habituate both yourself and the rat to the situation. Place your hand in the cage with the rat, with the backs of your fingers against its flank. Initially it may move away, but just follow it slowly around the cage, and it will quickly learn that your hand presents no threat. This is a good approach for you to also learn that the rat presents no threat, and to allow you to become comfortable with the situation. Slow, deliberate motions are best. Quick, jerky movement can startle the rat, increasing its anxiety (and thereby increasing your anxiety), so every effort should be made to use slow, deliberate movements. If you're uncomfortable, take your hand out slowly and wait for yourself to calm down before trying again.

Lifting the rat:

Initially, the best way to pick up a rat is by the base of its tail. Grasp the tail firmly near the body (never near its tip). Lift the animal out of its cage and place it on your forearm near your body. Continue to hold the tail, as the rat's first instinct is to run. Eventually you will want to transfer the tail to the hand that belongs to the arm on which the rat is sitting. Gentle stroking of the fur will get the animal accustomed to being handled. When the animal is accustomed to being handled, it is possible and preferable to lift the animal by grasping it around the body with one hand (such that your thumb and a finger are behind the elbows), while holding the base of the tail with the other. A gentle, yet firm, grasp that is initiated smoothly is best. Do not hold the rat like this for long periods of time; place it on your arm if you need to walk it to a piece of equipment.

Weighing the rat:

As today's exercise will involve a demonstration of several different injection techniques, we need to know the animals mass before we begin. Set up the balance with the weighing container (don't forget the lid!). Zero the balance, remove the lid from the container, place the animal inside, and replace the lid. Wait for the animal to stop moving, and note the mass to the nearest gram. Due to animal movements, this will only be an approximation, but deviations of ±5g or so are acceptable given the resolution of the volume-scale on syringes.

Injections:

To load a syringe, first use an alcohol swab to disinfect the rubber stopper on the drug vial. Using sterile technique, place a syringe needle of appropriate gauge (22 gauge) on the syringe. Pull in a volume of air equivalent to the volume of fluid to be withdrawn from the bottle. Inset the syringe into the bottle, and inject the air. This procedure prevents the creation of a vacuum in the bottle. Then invert the bottle, and withdraw an excess of fluid. Grasp the base of the syringe with your pinkie finger, and flick the tip of the syringe with your index finger to dislodge any air bubbles. When the air bubbles are at the top of the syringe, expel them back into the bottle by depressing the plunger to the desired injection volume. Air bubbles can be lethal if injected into the animal and will lead to inaccurate measurements of the injected fluid. Use a fresh syringe for each injection.

Intraperitoneal (IP) injection:

Hold the rat by the base of the tail (never the tip) with your dominant hand. With your other hand, firmly grasp the rat around the thorax with your other hand such that your thumb and index finger push on the animal's elbows, forcing its forepaws to cross in front of it. With the paws crossed under its chin, it should be unable to bite you. Let go of the tail. If you are alone, place the rat's hind legs on your hip, and rotating your hand slightly. This will stretch the abdomen and immobilize the hind limbs and the tail. If you have an assistant, you may prefer to use your dominant hand to grasp the legs and tail and have your assistant give the injection. In this case, invert the animal so that its head is pointed down, this will allow the visceral organs to move towards the diaphragm, minimizing the risk of puncturing them with the needle. The needle should be inserted about 5mm in the animal's lower right abdomen (this avoids the bladder and cecum) with the needle-tip angled slightly towards the head. Pull back on the plunger to confirm that you have formed a vacuum. Small bubbles should appear in the syringe (these are dissolved gases coming out of solution under low pressure). If you see these bubbles, then depress the plunger at a steady rate over a couple seconds. If you see blood or some other fluid instead of bubbles, this indicates that you have hit an organ. Dispose of the syringe and start again. NEVER inject this material into the abdominal cavity as it will lead to peritonitis

Subcutaneous (SC) injection:

A subcutaneous injection can be accomplished by pinching the skin just posterior to the rats ears between your index finger and thumb. You should be able to slide your thumb up and down a feel the two layers of skin pass by each other. Your needle will enter the along the midline from posterior surface created by pinching the skin. Insert the needle into the base of the triangle formed when the skin pulled in this manner. Slide the needle deeply in between your fingers, and wiggle the skin on either side of the needle (slide your thumb and finger up and down). The skin should move but the needle should not. If this is the case, the tip is subcutaneous, and you may inject. If the tip moves with the skin, it is intra-dermal and should be repositioned. If possible, have the tip of the needle posterior to your fingers, with the entry anterior to your fingers. Pull back on the plunger to confirm that you have formed a vacuum. After the injection, withdraw the needle, but maintain your pinch briefly. This will help prevent leakage. Rub the bolus produced after releasing the animal to help disperse the drug. This will aid absorption.

Dose, volume, and concentration:

The subcutaneous route allows for about 1ml of fluid for every 100g of body size. The intraperitoneal route can accommodate more, but the 1ml/100g ratio is a good rule of thumb. Ideally, vehicle should be isotonic (0.9% saline), sterile, and of physiological pH (7.3-7.4). Dosage can be calculated as mg or drug per kg of body mass (mg/kg). Typically, your drug is pre-prepared to a certain concentration (mg/ml). To dose the drug properly, you will need to weigh the rat ahead of time. To maintain the same dosage across animals, the volume of your injection must be altered based on the weight of the animal (ml/kg). It is handy to prepare a dosage chart ahead of time where the ml/kg has been calculated for a range of animal weights.

Homework Assignment:

Create a dosage chart for rats weighting between 300 and 400g in 10g intervals. The drug you will be injecting needs to be given at a dose of 25mg/kg, and is supplied at a concentration of 40mg/ml.

References

Canadian Council of Animal Care (1993) Guide to the Care and Use of Experimental Animals, Volume 1 (2nd Ed). http://www.ccac.ca/Documents/Standards/Guidelines/Experimental_Animals_Vol1.pdf

Graham, R. L. (1972). An efficient algorithm for determining the convex hull of a finite planar set. *Information Processing Letters*, *1*(4), 132–133.

Olfert, E. D., Cross, B. M., McWilliam, A. A., & others. (1993). *Guide to the care and use of experimental animals* (Vol. 1). Canadian Council on Animal Care Ottawa.

Objectives:

- To describe appetitive/proceptive behaviors in the male and female rat
- To describe the sequential components of both male and female copulatory behavior
- To quantify a lordosis quotient
- To quantify paced mating

Introduction:

Being a careful observer of behavior is essential to being a skilled behavioral neuroscientist. Changes to the nervous system may only produce small changes in behavior, and therefore it is important to develop skills and techniques to quantify behavior so that these small changes can be detected. A good behavior to start with is sexual behavior, as it is highly stereotyped. Sexual behavior is essential for the survival of a species, and requires careful coordination of both endocrine activity and behavior; each of which is controlled by the nervous system. In this lab you will learn to describe appetitive/proceptive behaviors that precede copulation. You will also learn to describe the very stereotyped components of both male (mounting, intromission, ejaculation) and female copulatory behavior (lordosis).

Procedure:

Preparation:

Rat mating occurs during the night, so the animals will need to be housed in a colony room with a light-dark cycle where the lights go out a few hours before the start of your lab. While male rats will always engage in sexual behavior, female rats are only sexually receptive once every 4-5 days. A variety of approaches can be used to ensure that you have receptive females to work with, which your TA will have performed prior to your lab.

1. Ovariectomized females can be induced to be sexually receptive by giving them an injection of estradiol benzoate (20 pg, SC in sesame seed oil) 48h prior to testing, followed by progesterone (1 mg, SC, sesame seed oil) 4 h prior to testing.
2. Vaginal smears can be taken every day to identify estrus (Jenkins and Becker, 2005)
3. Females can be placed with a male to observe if lordosis appears. Mounting would need to be interrupted immediately so that the female doesn't become pregnant or pseudopregnant.

We want our females to become pregnant, so the first option is not possible. You TA will likely use approach #3, but depending on experience, the vaginal smear approach is better and may be used.

Test:

If paced mating is to be observed, the female must have a "safe" zone where the male can't reach her. Two approaches can be used. Have a chamber where the male can be tethered so that he is restricted to one end of the arena. The female should also have an opaque barrier to hide behind. The male will need prior habituation to the tethering, or he will be more interested in the restraint than the female rat during testing (Jenkins and Becker, 2005). Alternatively, two chambers can be used, connected by a piece of PVC pipe large enough to allow the passage of the female rat, but small enough to prevent entry from the male rat. Alternatively, a barrier with appropriately sized holes can be used to dive the arena in two. Small young females and larger, older males will be required in this case.

Position and turn on the video camera. Place the male in the mating arena. If paced mating is to be scored, the male rat may need a physical restraint. Then place the female in the mating arena. Place the female in the mating arena in her end. If only lordosis quotient and a description of proceptive behaviors are to be scored, simply place the male and then the receptive female into the mating arena. Allow mating to progress for 1 hour. For paced mating, if after 30 minutes the female has not entered the male's side, place her on the male's side to assess sexual receptivity.

From the video you will need to identify mounts (climbing on, but no insertion of the penis, male simply moves off the female), intromissions (mounting and insertion of the penis, male disengages by springing off the female after a pelvic thrust) and ejaculations (mounting and insertion of the penis, several pelvic thrusts, female disengages). You will need to identify lordosis events (immobility, arched back, dorsiflexion of tail, elevation of head).

To calculate the lordosis quotient, divide the number of lordosis events by the number of male contacts, and multiply by 100% (Jenkins and Becker, 2005).

In paced mating, to calculate percent exits divide the number of exits following coital stimulation by the number of coital stimulations and multiply by 100%. To calculate the return latency, simply measure the time until the female returns to the male's area. Separate exits and return latencies by the type of coital stimulation that preceded it (mount, intromission, ejaculation) (Jenkins and Becker, 2005).

For describing proceptive and copulatory behavior, make careful notes about the sequences of events. Look for the female to perform the following: hopping, darting, ear wiggling, and lordosis (Erskin, 1989). For males, look at how he orients to the female, touches her and explores her. Note genital exploration and licking, whether of the partner or of the self. Note the sequence and quantity of mounts, intromissions and ejaculations, as well as postcopulatory behaviors (refractory periods).

Homework Assignment:

1. In one page, describe the proceptive and copulatory behaviors of the female rat. Pay careful attention to the sequence of events. Calculate the lordosis quotient of your rat.
2. For paced mating, calculate the percent exits and return latency for your female rat. Describe how these dependent variables change with increasing coital stimulation (Mounts vs. intromissions vs. ejaculations).

References:

Erskine MS (1989) Solicitation behavior in the estrous female rat: A review. Horm Behav, 23: 473-502. Jenkins WJ, Becker JB (2001) Role of the striatum and nucleus accumbens in paced copulatory behavior in the female rat. Behav Brain Res, 121(1-2):119-28. Jenkins WJ and Becker JB (2005) Sex. In: IQ Whishaw and B Kolb (Eds), The behavior of the Laboratory rat: A handbook with tests. Oxford University Press: Toronto.

Objectives:

- To describe posture and righting behaviors
- To describe ground locomotion
- To describe swimming behavior
- To describe grooming behaviors
- To describe exploratory behaviors

Introduction:

It is the role of the brain to produce behavior. While “behavior” can be broadly defined as “anything that the organism does”, the most obvious behaviors include a motor component. When trying to understand the neural basis of behavior, examining simple motor behaviors is a good place to start. The neural circuits underlying these behaviors are well described and highly conserved in vertebrates. Today you will examine a number of innate behaviors. Pay attention to the sequence of simple movements that make up the overall behaviors. The variety of behaviors examined in this lab is a good survey of the types of behaviors that rats would engage in on a daily basis. Observing the adult behavior today will help you evaluate the development of such behaviors in the later pup-development lab.

Procedure:

Preparation: Read chapters 11, 13, 14 and 16 in the Behavior of the Laboratory Rat book before this week’s lab.

Tests:

Posture and righting:

- Trigeminal righting – righting of the head, triggered by the trigeminal nerve
  - Place the rat on the table either on its back or its side making sure the head is touching the ground. Hold the animal there for a moment, then observe trigeminal righting when the head is released.
- Body Tactile righting – body-on-head righting
  - Righting/rotation that begins at the shoulders
  - Place the rat on its side, without touching it’s head and without its head touching the table (head should be hanging off edge of the table), observe body-on-head righting.
- Body Tactile righting – body-on-body righting –
  - Righting/rotation that begins with the pelvis
  - Place the rat on its side, hold one hand over the rat’s shoulders, the other hand over its pelvis, release the pelvis hand first, observe body-on-body righting.
- Tactile/Proprioceptive Dynamic righting
  - Rotation to face the direction of falling (falling while in contact with the ground)
  - Hold the rat in the bipedal position, pull the rat backwards onto the ground, observe tactile/proprioceptive righting

Grooming

- Place your rat in an empty cage and watch it for 5 minutes. Recorded the frequency of grooming behaviours. Note the sequence of body parts groomed.

Locomotion

- Place your rat at one end of the walkway, and let it run to the other end. Video-taping this behaviour may simplify the analysis
- Note the sequence of limb movements. Identify the stance and swing phase of movement for each limb.

Swimming

- If you have a water maze available, prepare it with warm water and a visible platform. Place the rat in the water facing the platform
- Observe the movements of its limbs and tail during the swim to the platform.
- Removed the rat from the pool and dry it off with a towel.

Exploratory Behaviour

- Place the rat in an open field for 5 minutes
- Note the variety of behaviours exhibited, their sequence and durations. These include thigmotaxis (running along the wall), head movements, rearing, and different patterns of limb movements. Measure the amount of time spent in different regions of the open field. Attempt to determine if the animal has established a home-base.

Homework Assignment:

Choose one of the motor behaviours that were observed in lab. Using information from lab and the textbook, describe the behavior and its components as it is expected to be seen in a normal healthy rat, as well as the observations you made in lab of that behavior. Include a brief description of the neural systems that regulate this behavior, and how this behavior may be altered specific brain damage or a specific drug treatment. Page limit: 2 pages.

References:

Aldridge JW (2005) Grooming. In: IQ Whishaw and B Kolb (Eds), The behavior of the Laboratory rat: A handbook with tests. Oxford University Press: Toronto. Golani I, Benjamini Y, Dvorkin A, Lipkind D, and Kafkafi N (2005) Locomotor and Exploratory Behavior. In: IQ Whishaw and B Kolb (Eds), The behavior of the Laboratory rat: A handbook with tests. Oxford University Press: Toronto. Muir G (2005) Locomotion. In: IQ Whishaw and B Kolb (Eds), The behavior of the Laboratory rat: A handbook with tests. Oxford University Press: Toronto. Pellis SM and Pellis VC (2005) Posture. In: IQ Whishaw and B Kolb (Eds), The behavior of the Laboratory rat: A handbook with tests. Oxford University Press: Toronto.

Objectives:

- To describe prehension
- To describe skilled food handling
- To describe skilled forelimb reaching behaviors
- To describe skilled walking behaviors and hindlimb movements
- To describe the sequence of movements

Introduction:

Last week we examined a number of basic innate behaviors. Rats, like humans, can learn a number of difficult behaviors. These skilled behaviors improve with practice. Being able to quantify these behaviors is useful for understanding neural plasticity of the systems underlying these behaviors (e.g., Kleim et al., 2004). Additionally, some general motor behaviors are resistant to some types of brain damage, such as stroke. Skilled behaviors are often more sensitive to detect deficits following some insult to the brain. Two of the most common behaviors are skilled reaching and walking along a horizontal ladder.

Procedure:

Preparation:

Read chapters 15 in the Behavior of the Laboratory Rat book before this week's lab. Rats may need some pretraining on the reaching task, so discuss this with your TA to see haw you can participate in this task. Have the rat food deprived for about 3 hours before the lab so that it is motivated to handle food items. Make sure that it has had experience with the food pellets to be used in the task. Palatable pellets may help motive the rat further.

Tests: **Food Handling**

- Give the rat a piece of rice, a froot loop or a piece of rat chow.
- Observe how the rat holds each type of food and how it brings it to its mouth.

Single Pellet Reaching

- Place the rat in the reaching chamber. Place a number of pellets centered on the platform at the reaching slit. Watch the rat reach for them. Which paw does it use? Observe the order of movements. Videotaping the reaching behavior may make it easier to observe each individual component of the reaching.
- Once the preferred paw is determined, place a pellet to the side of the slit on the same side as its preferred paw, forcing it to use its non-preferred paw.
- After the rat retrieves the pellet, observe the movements that bring the pellet to the mouth.
- Drop a pellet at the back of the chamber to get the rat to turn around to reset. Place another pellet on the platform by the slit while it is turned around.
- If this lab can be done over a number of days, record the percent success each day (number of pellets retrieved, number of pellets retrieved without missing) Horizontal ladder
- Place the rat at one end of the horizontal ladder and its homecage or a dark chamber at the other end. Videotape the rat as it runs from one end to the other
- Observe how it places its paws on the rungs. Count the number of time is misses, slips or needs to reposition its paws.
- Increase the difficulty by randomly pulling out 10 rungs (never more than 2 adjacent rungs) and compare the number of errors and time to cross to trials where no rungs are removed.
- If this lab can be done over a number of days, compare the number and types of errors each day.

Homework Assignment:

Assignment #1: If you can only run a single lab make graphs for the various measures of success on the reaching and ladder tasks across successive trials (reaching) or between the simple and difficult versions of the ladder task. Also describe the sequence of movements involved in food handling, single pellet reaching, and horizontal ladder.

Assignment #2: If the lab can be run over a number of days, prepare graphs for the various measures of success on the reaching and ladder tasks across successive days. Also describe the sequence of movements involved in food handling, single pellet reaching, and horizontal ladder.

References:

Kleim JA, Hogg TM, VandenBerg PM, Cooper NR, Bruneau R, Remple M (2004) Cortical synaptogenesis and motor map reorganization occur during late, but not early, phase of motor skill learning. J Neurosci, 24(3):628-33. Whishaw IQ (2005) Prehension. In: IQ Whishaw and B Kolb (Eds), The behavior of the Laboratory rat: A handbook with tests. Oxford University Press: Toronto.

Objectives:

- To observe and describe a number of maternal behaviors in the female rat.
- Compare the behavior of virgin female rats to the behavior of mother rats with respect to the pups.

Introduction:

Maternal behavior of the laboratory rat is a highly organized behavior that appears spontaneously in postpartum mother rats, but not in males or virgin females (Fleming and Rosenblatt, 1974; Jans and Woodside, 1990). Important hormonal events during pregnancy lead to neural changes that prime a mother for maternal behaviors. Maternal behavior is sensitive to various drugs, and as such, can be an important screen for therapeutic drugs that may alter human maternal behavior. Post partum depression and infanticide can be tragic events for new families, and understanding the hormonal and neural changes that accompany maternal behavior is an essential foundation for decreasing the suffering experienced by some new mothers.

Procedure:

Preparation: Read Chapter 27: Maternal Behavior (Rees et al., 2005) before the lab this week. A few weeks ago you studied sexual behavior in rats. Hopefully this was a fruitful endeavor, providing you with pups to study for today's lab. Collect a cage with a mother and pups, a cage with a virgin female, and 2 clean empty cages. Put on gloves before handling any pups. *Test:* **Observing Undisturbed Maternal Behavior** To start, simply watch the mother in her home cage interacting with her pups. First assess the quality of the nest (see Rees et al. (2005) page 290). Note the following behaviors: body licking, anogenital licking, and nursing. Note the mother's posture. Observe the mother and litter for 30 minutes.

Pup Retrieval Remove the pups to clean cage and place them in a huddle. Remove the mother to a separate clean cage. Wait 5 minutes. Place the mother back in her home cage for 5 minutes and note her behavior. Then place the pups back into their home cage in the corner diagonally opposite the nest. Observe the mother's retrieval behavior and time how long it takes for her to move all of the pups back to the nest. Note any behaviors she performs on the pups in addition to carrying them.

Virgin Female Response to Pups Remove both the pups and the mother back to their clean cages. Place the virgin female in the cage with the nest. Observe her behavior for 5 minutes. Place the pups in their home cage in the corner diagonally opposite the nest. Observe the virgin females response to the pups for 20 minutes. If she attempts to injure any pups, remove her immediately and inform the TA. At the end of the testing session, place the pups and some clean paper towel into the clean cage containing the mother and return the family to the colony room in this clean cage.

Homework Assignment:

- In one page, describe licking, nursing, and pup retrieval behavior of the mother.
- The virgin females' behavior differed from the mothers. What is the biological reason for this difference in behavior? Search the literature for a study that examines the biological underpinnings of maternal behavior, and summarize this study (hypothesis, experimental design, findings and conclusions).

References:

Fleming A, Rosenblatt JS (1974) Maternal behavior in the virgin and lactating rat, Behav Neurosci 86(5):957-72. Jans JE, Woodside BC (1990) Nest temperature: Effects on maternal behavior, pup development, and interactions with handling. Dev Psychobiol, 23:519-534. Rees SL, Lovic V, Fleming AS (2005). Maternal Behavior. In: IQ Whishaw and B Kolb (Eds), The behavior of the Laboratory rat: A handbook with tests. Oxford University Press: Toronto.

Objectives:

- To observe pup development in terms of physical appearance and motor responses.
- To screen pups for developmental milestones.

Introduction:

Many pharmaceuticals and environmental chemicals can alter brain development, altering behavior. Some of these changes can be quite subtle, merely delaying the appearance of a behavior by a couple of days, while others can be quite severe, resulting in persistent morphological and behavioral changes throughout life, such as is observed with Fetal Alcohol Syndrome. As pregnant mothers experience illnesses that must be treated, it is important to understand the effects that various treatments will have on her developing fetus. Understanding the behavioral development of the rat is an important foundation for developing screens for potentially teratogenic substances. Some very detailed screens have been developed (e.g., Altman J and Sudarshan K, 1975).

Procedure:

Preparation: Read Chapter 25: Infancy (Alberts, 2005) before the lab this week. The slab will be spread out over a number of days over a number of weeks. Sharing the workload with your group will make this less onerous. You're TA will inform you when the pups have been born, and you should start screening them on the day after birth if possible. Prepare a chart where you can note either the presence/absence of a trait (eyes open, pinna separated from head, ear canal open, presence of fur), the latency to observe a behavior (righting reflex, orientation on an inclined place, placing reflex), and the quantification of a behavior (distance traveled in 1 minute, duration of a wire hang). You'll need a page for each day, and a column for each pup. Do not worry about tracking individual pups; we are interested in litter effects so merely calculate means and percentages for each trait.

Testing: On each testing day, prepare a small clean cage. Place all the pups in this cage. Test each pup individually for all the traits, and then return it to the mother when the battery of tests is completed before proceeding to the next pup.

Examine the pup to determine if the eyes are open, if the pinna (fleshy part of ear) is separated from the head, if the external auditory meatus (ear canal) is open, and if fur has appeared yet. Score each of these as "Yes" or "No". Weigh the pup.

For the next series of test, start a timer when you place the rat in position, and stop it when the behavior is complete. If the behavior fails to appear score the test as unsuccessful. **Righting Reflex:** Place the pup on its back and time how long it takes until it is back on all 4 feet. Orientation on an Inclined Plane: Place the pup nose-down on the inclined plane apparatus. Time how long it takes to turn around 180 degrees. If it looses its grip and falls to the bottom of the plane, place it nose-down again. If it falls a second time, score the test as unsuccessful. **Placing Reflex:** Gently pick up the pup by the base of the tail near the body and lift it. Time how long until it flays its forepaws outwards (the placing reflex).

For the next series of behaviors, quantify the behavior. **Locomotion:** Place the pup in the middle of the movement mat and start the timer. Count how many gridlines it crosses in 1 minute. **Wire hang:** Place a stack of paper towels under the wire to soften any fall. Gently place the pup's forepaws on the wire and let go. Time how long it can hang. If it falls immediately, replace it once. If it can hang for 30 seconds, stop the test and score it as 30 seconds.

Variations

- Mother rats could be treated with a drug during pregnancy to examine how prenatal exposure to such substances can alter pup development.

Homework Assignment:

- Create developmental figures for each of traits, with age in days along the X-axis, and either percent success, latency, duration or distance along the Y-axis.

References:

Alberts JR (2005) Infancy. In: IQ Whishaw and B Kolb (Eds), The behavior of the Laboratory rat: A handbook with tests. Oxford University Press: Toronto. Altman J, Sudarshan K (1975) Postnatal development of locomotion in the laboratory rat. Anim Behav, 23(4):896-920.

Objectives:

- To observe a pial strip surgery as a model of stroke.
- To test rats with a pial strip of the caudal forelimb area of the motor cortex on a number of behavioral tests.
- To write a full manuscript-style lab report comparing the effects of the stroke on the various behaviors.

Introduction:

Stoke is a leading cause of death among adults. While many patients survive a stroke, they are frequently left severely impaired. Since there is little in the way of regeneration following a stroke, behaviors controlled by the area of brain injured by the stroke must be re-learned. This involves surviving areas of the brain acquiring control of the affected behavior, or the learning of new compensatory behaviors to replace the impaired function. Development of therapies that promote these sorts of brain plasticity could enhance both the speed and extent of recovery (Whishaw et al., 2008). The development of potential therapies depends on useful models of stroke in animals, particularly those that mimic the types of strokes experienced by people. There are a number of animal models of stroke, each with their own advantages and disadvantages (Howells et al., 2010).

Procedure:

Preparation: Read Kolb (2005) before the lab. Your TA will prepare the test animals ahead of time by performing the pial strip surgeries (See Appendices B and C for the procedure). Depending on local regulations, you may be invited to observe and/or participate. The same male rats used in Laboratory Exercises C and D will be used for the surgeries, allowing you to compare the behaviors both before and after the stroke. Some behaviors will be more affected than others.

Testing: Follow the procedures outlined in Laboratory Exercises C and D with your rat. Submit the data to your TA who will collate the data from all groups. This complete data-set will be used for your write-up.

Follow-up: In any surgical intervention, histological confirmation of the effectiveness to the surgery is important. Discuss with your TA opportunities for learning about histological procedures (perfusion, tissue slicing and staining, microscopy) that may be available. For this study, perfusion with formalin, tissue sectioning and a nissl stain such as cresyl violet would be appropriate histological procedures.

Homework Assignment:

- Complete a full manuscript style lab report (See appendix A) of the experiment. Include statistical analysis of the behaviors before and after stroke.

References:

Howells DW, Porritt MJ, Rewell SS, O'Collins V, Sena ES, van der Worp HB, Traystman RJ, Macleod MR (2010) Different strokes for different folks: the rich diversity of animal models of focal cerebral ischemia. J Cereb Blood Flow Metab, 30(8):1412-31. Kolb B (2005) Neurological Models. In: IQ Whishaw and B Kolb (Eds), The behavior of the Laboratory rat: A handbook with tests. Oxford University Press: Toronto. Whishaw IQ, Alaverdashvili M, Kolb B (2008) The problem of relating plasticity and skilled reaching after motor cortex stroke in the rat. Behav Brain Res, 192(1):124-36.

Objectives:

- To observe a 6-hydroxydopamine (6-OHDA) neurotoxic lesion surgery as a model of Parkinson's Disease.
- To test rats with 6-OHDA neurotoxic lesion of the nigostriatal system on a number of behavioral tests.
- To write a full manuscript-style lab report examining how various behaviors are altered in this model of Parkinson's Disease.

Introduction:

Parkinson's disease is complex neurological disorder. Although the causes of this disease are still debated, one common feature appears to be the loss of dopaminergic neurons in the substantia nigra. These dopaminergic cells typically innervate the striatum, and regulate its activity. Loss of these neurons leads to a progressive and profound loss of motor control, particularly with respect to initiation of movement. Animal models that mimic this loss may aid in the development of therapeutic interventions that could decrease the severity of the disease. The most common model of Parkinson's Disease is neurotoxic lesions of the dopaminergic fibers in the medial forebrain bundle or the rostral striatum (Blandini et al., 2008; Deumens et al., 2002).

Preparation:

Read Kolb (2005) before the lab. Your TA will prepare the test animals ahead of time by performing the 6-OHDA neurotoxic lesion surgeries (See Appendices B and C for the procedure). Depending on local regulations, you may be invited to observe and/or participate. The same male rats used in Laboratory Exercises C and D will be used for the surgeries, allowing you to compare the behaviors both before and after the lesion. Some behaviors will be more affected than others.

Testing:

Follow the procedures outlined in Laboratory Exercises C and D with your rat. Submit the data to your TA who will collate the data from all groups. This complete data-set will be used for your write-up.

Follow-up:

In any surgical intervention, histological confirmation of the effectiveness to the surgery is important. Discuss with your TA opportunities for learning about histological procedures (perfusion, tissue slicing and staining, microscopy) that may be available. For this study, perfusion with 4% paraformaldehyde, tissue sectioning and immunohistochemical using a peroxidase stain for tyrosine hydroxylase in the striatum would be appropriate histological procedures.

Homework Assignment:

- Complete a full manuscript style lab report (See appendix A) of the experiment. Include statistical analysis of the behaviors before and after stroke.

References:

Blandini F, Armentero MT, Martignoni E (2008) The 6-hydroxydopamine model: news from the past. Parkinsonism Relat Disord,14 (Suppl 2):S124-9. Deumens R, Blokland A, Prickaerts J (2002) Modeling Parkinson's disease in rats: an evaluation of 6-OHDA lesions of the nigrostriatal pathway. Exp Neurol, 175(2):303-17. Kolb B (2005) Neurological Models. In: IQ Whishaw and B Kolb (Eds), The behavior of the Laboratory rat: A handbook with tests. Oxford University Press: Toronto.

Objectives:

- To learn about a surgical model that elicits feeding behavior.
- To quantify feeding in rats following activation or sham activation of the lateral hypothalamus.

Introduction:

Feeding is one of the most critical behaviors that any organism performs. This behavior must be tightly regulated so ensure against energy deficits when food is scarce. This must be balanced with being encumbered by energy reserves. In today's modern society where food is always plentiful, my members of society are encumbered by their energy reserves (i.e., obesity) which has serious implications for health by increasing the risk of developing a variety of diseases. Understanding the neural mechanisms that regulate energy balance is critical if effective treatments for eating disorders are to be developed. The rat is a good model for studying eating behavior (Clifton, 2005).

Procedure:

Preparation: Rats will need to have a cannula implanted aimed at the lateral hypothalamus. See Appendix B+C for specifics on the cannulation surgery. The coordinates for this are based on Hettes et al. (2010). The guide cannula should be implanted 6.1mm anterior to the interaural line, 1.8mm lateral of midline, and 8.2mm ventral to the surface of the skull. The incisor bar should be set to 3.3mm below the center of the ear bars. An injector cannula that extends 1mm beyond the end of the guide cannula should be used. For the week prior to testing, rats should have ad libitum access to a milk-mash diet (Hettes et al., 2010) consisting of powdered Purina rat chow (500 g), sucrose (400 g), and evaporated milk (354 ml). This will prevent neophobia to this diet during testing.

Test: Prepare a solution of RS-AMPA (Tocris Bioscience, Ellisville, MO, USA; catalogue number 0169) in saline. Mix 6.2mg of drug into 1ml of saline (this will give a 33mM solution, or 10nmol in 0.3µl). Warm slightly and vortex to aid getting the drug into solution. Prepare some milk-mash in a dish, and weigh it. Place it in the test chamber (clean empty cage). Load the injector with at least 0.5 µl of either RS-AMPA or saline. Do this by connecting the injection cannula to some polyethylene tubing. Fill the tubing with distilled water. Attach the tubing to a 1 µl Hamilton syringe. Lay the assembly flat and then pull back the plunger to the 0.2 µl mark. This will produce an air bubble inside the injector that will keep your drug from mixing with the distiller water filling the PE tubing. Then place the injector into the drug solution and pull back to the 1 µl mark. Remove the tip from the solution, and watch the tip while pressing the plunger to the 0.8 µl mark. Confirm that a bubble of solution appears. Failure to see this would indicate an incomplete seal in the assembly, or an obstruction in the assembly. If it is confirmed, then you are ready to inject. If this is not confirmed, flush the line with distilled water and load the assembly again. Gently restrain the rat and unscrew the dummy cannula. Insert the injector all the way. Slowly inject 0.3µl the solution over the course of about 1 minute (press the plunger down to the 0.5 µl mark, there will be residual drug in the injector, which prevents the air bubble from being injected). Allow an extra minute for the solution to disperse in the brain tissue before withdrawing the injector. Withdraw the injector and replace the dummy cannula. Place the rat in the testing chamber containing the food-dish of mash. Observe its behavior for 60 minutes. At the end of the test, return the rat to its home cage and weigh the food-dish. Report your weight and injection solution to the TA, who will tabulate the data and supply summary data to the group.

Follow-up: In any surgical intervention, histological confirmation of the effectiveness to the surgery is important. Discuss with your TA opportunities for learning about histological procedures (perfusion, tissue slicing and staining, microscopy) that may be available. For this study, perfusion with formalin, tissue sectioning and a nissl stain such as cresyl violet would be appropriate histological procedures.

Homework Assignment:

- Prepare a graph of the results. Perform a statistical analysis (Student's t-test) to determine if the two groups (saline or RS-AMPA) differed significantly in the amount of food they consumed in the hour following the injection.

References:

Clifton PG (2005) Eating. In: IQ Whishaw and B Kolb (Eds), The behavior of the Laboratory rat: A handbook with tests. Oxford University Press: Toronto. Hettes SR, Gonzaga WJ, Heyming TW, Nguyen JK, Perez S, Stanley BG (2010) Stimulation of lateral hypothalamic AMPA receptors may induce feeding in rats. Brain Res, 1346:112-20.

Objectives:

- To learn about a surgical model that elicits drinking behavior.
- To quantify drinking in rats following intracerebroventricular injection of a dipsogenic substance.

Introduction:

Fluid homeostasis is carefully controlled in animals. Obligatory losses of body water due to respiration and urination require regular consumption of water. The brain anticipates needs and responds to losses in a homeostatic manner. The brain also regulates the tonicity of body water to maintain the correct osmotic pressure across membranes, and to maintain an appropriate blood pressure. Osmoreceptors in the brain and baroreceptors in the heart provide the brain with feedback for that drinking behavior and kidney function can maintain body water volume and tonicity in the optimal range (Rowland, 2005).

Procedure:

Preparation: Rats will need to have a cannula implanted aimed at the lateral ventricle. See Appendix B+C for specifics on the cannulation surgery. The coordinates for this are based on those of Harland et al., (1988). The guide cannula should be implanted 0.8 mm posterior to bregma, 1.5 mm lateral to the midline, and 3.2 mm ventral to the surface of the skull. The incisor bar should be set so that bregma and lambda have the same dorsoventral level. An injector cannula that extends 1mm beyond the end of the guide cannula should be used.

Test: Prepare a solution of Angiotensin II (Sigma Aldrich, St Louis, MO, USA; catalogue number A9525) in saline. The final concentration is 100µg/ml. Since 100µg is difficult to measure, weight out a small amount and mix it at a concentration of 1mg/ml in saline. This will give you a stock solution 10x stronger than is needed. Mix a small amount of this stock at a ratio of 1:9 with sterile saline to give the working solution for injection. For example, mix 100µl of the stock solution with 900 µl to give 1ml of 100µg/ml Angiotensin II in saline. Prepare a test cage. Fill a water bottle, and weigh it. Carefully place it on its holder, avoiding any leakage. This may take practice. Connecting the injection cannula to some polyethylene tubing. Fill the tubing with distilled water. Attach the tubing to a 10 µl Hamilton syringe. Lay the assembly flat and then pull back the plunger to the 0.5 µl mark. This will produce an air bubble inside the injector that will keep your drug from mixing with the distiller water filling the PE tubing. Then place the injector into the drug solution or saline vehicle and pull back to the 10 µl mark. Remove the tip from the solution, and watch the tip while pressing the plunger to the 8 µl mark. Confirm that a bubble of solution appears. Failure to see this would indicate an incomplete seal in the assembly, or an obstruction in the assembly. If it is confirmed, then you are ready to inject. If this is not confirmed, flush the line with distilled water and load the assembly again. Gently restrain the rat and unscrew the dummy cannula. Insert the injector all the way. Slowly inject 5µl the solution over the course of about 1 minute (press the plunger down to the 3 µl mark, there will be residual drug in the injector, which prevents the air bubble from being injected). Allow an extra minute for the solution to disperse in the cerebrospinal fluid before withdrawing the injector. Withdraw the injector and replace the dummy cannula. Place the rat in the testing chamber containing a drinking bottle. Time latency until it begins to drink. Continue to observe its behavior for 30 minutes. At the end of the time, return the rat to its home cage. Carefully remove the water bottle, avoiding any leakage, and weight it again to calculate how much the rat drank. Report your values and injection solution to the TA, who will tabulate the data and supply summary data to the group.

Follow-up: In any surgical intervention, histological confirmation of the effectiveness to the surgery is important. Discuss with your TA opportunities for learning about histological procedures (perfusion, tissue slicing and staining, microscopy) that may be available. For this study, perfusion with formalin, injection of 5 µl of a 1% aqueous solution of Evans blue dye and examining the brain for staining of the ventricular spaces, tissue sectioning and a nissl stain such as cresyl violet would be appropriate histological procedures.

Variations:

- A 2x2 design could be used where the saline vs. Angiotensin II treated rats are given a choice or 2 solutions (water vs. hypotonic saline; water vs. glucose solution)

Homework Assignment:

- Prepare graphs of the results. Perform statistical analyses (Student's t-tests) to determine if the two groups (saline or angiotensin II) differed significantly in the amount of water they consumed in the 30 minutes following the injection, and in their latency to drink.

References:

Harland D, Gardiner SM, Bennett T (1988) Cardiovascular and dipsogenic effects of angiotensin II administered i.c.v. in Long-Evans and Brattleboro rats. Brain Res, 455(1):58-64. Rowland NE (2005) Drinking. In: IQ Whishaw and B Kolb (Eds), The behavior of the Laboratory rat: A handbook with tests. Oxford University Press: Toronto.

Objectives:

- To discover how a complex behavior can be triggered by minute application of specific chemicals to discrete brain regions.
- To learn about an animal model of Obsessive Compulsive Disorder
- To observe how drugs used to treat Obsessive Compulsive Disorder can modify the behavior in this animal model.

Introduction:

Throughout this course we have been using the rat to study behavior. It is important to note that the choice of species to examine can be influenced by a wide variety of factors, one of which is the ability of the species to display the behavior of interest. Sometimes you may wish to examine a behavior which is not ubiquitous in the animal kingdom. One example is the social communication behavior of flank marking in hamsters (Ferris et al., 1984). The neural circuitry of this complex behavior has been well defined (Bamshad and Albers, 1996). This model has found promise as a simple screening tool for Obsessive Compulsive Disorder (OCD). Specifically, antidepressants used to treat OCD inhibit flank marking, while antidepressants that are not indicated for use in OCD have no effect on flank marking (Ferris et al., 2001).

Procedure:

Preparation: Male hamsters will need to have a cannula implanted aimed at the medial preoptic area of the hypothalamus. See Appendix B+C for specifics on the cannulation surgery. The coordinates for this are based on Ferris et al. (2001). The guide cannula should be implanted on an 8 degree declination from vertical. This helps you hit midline structures without damaging the sagittal sinus which sits along midline between the two cerebral hemispheres. The incisor bar should be leveled with interaural line (i.e., set to zero). The coordinates are 1.1 mm anterior to the bregma, 1.8 mm lateral to the midsagittal suture, and 6.5 mm below the dura. An injector cannula that extends 1mm beyond the end of the guide cannula should be used. For the two week prior to testing hamsters should be given daily injections of either 2mg of Clomipramine (Sigma-Aldrich, St. Louis, MO, Catalogue #C7291) or saline vehicle. Randomly assign each hamster to drug or vehicle control, and track this, as they will need the same injection each day. Prepare the drug with to a concentration of 20mg/ml sterile saline, and give 0.1ml to each hamster. Your TA will coordinate this procedure and may make up a duty roster requiring your assistance. Prepare a solution of Arginine Vasopressin (AVP, Sigma Aldrich, St Louis, MO, USA; catalogue number V9879) in saline. The final concentration should be 100μM, or 100μmol in 1000ml. If you have 1mg of AVP, this is equivalent to about 1μmol of AVP, so you would need to dissolve this in 10ml of saline, which is not practical. Instead, make a concentrated stock solution of 1mg/ml (or 1ml of a 1mM solution). This will give you a stock solution 10x stronger than is needed. Mix a small amount of this stock at a ratio of 1:9 with sterile saline to give the working solution for injection. For example, mix 100μl of the stock solution with 900 μl of saline to give 1ml of 100μM AVP in saline.

Test: Prepare a test cage. Connecting the injection cannula to some polyethylene tubing. Fill the tubing with distilled water. Attach the tubing to a 1 μl Hamilton syringe. Lay the assembly flat and then pull back the plunger to the 0.1 μl mark. This will produce an air bubble inside the injector that will keep your drug from mixing with the distiller water filling the PE tubing. Then place the injector into the drug solution or saline vehicle and pull back to the 1 μl mark. Remove the tip from the solution, and watch the tip while pressing the plunger to the 0.8 μl mark. Confirm that a bubble of solution appears. Failure to see this would indicate an incomplete seal in the assembly, or an obstruction in the assembly. If it is confirmed, then you are ready to inject. If this is not confirmed, flush the line with distilled water and load the assembly again. Gently restrain the rat and unscrew the dummy cannula. Insert the injector all the way. Slowly inject 0.2μl the solution over the course of about 1 minute (press the plunger down to the 0.6 μl mark, there will be residual drug in the injector, which prevents the air bubble from being injected). Allow an extra minute for the solution to disperse in the cerebrospinal fluid before withdrawing the injector. Withdraw the injector and replace the dummy cannula. Place the hamster in the testing chamber. Count the number of flank-marking behaviors you observe over the next 30 minutes. At the end of the testing session, return your hamster to its homecage. Report your observations and treatment group to your TA, who will tabulate the results for analysis.

Variations:

- Other drugs could be examined. Desipramine, an SNRI is not effective, while SSRIs, such as fluoxetine, are effective at suppressing flank marking.
- A 2x2design could also be used where on the test day animals are given either AVP or a vehicle control injection.

Follow-up: In any surgical intervention, histological confirmation of the effectiveness to the surgery is important. Discuss with your TA opportunities for learning about histological procedures (perfusion, tissue slicing and staining, microscopy) that may be available. For this study, perfusion with formalin, tissue sectioning and a nissl stain such as cresyl violet would be appropriate histological procedures.

Homework Assignment:

- Prepare a graph of the results. Perform a statistical analysis (Student’s t-test) to determine if the two groups (saline or clormipramine) differed significantly in the amount of flank marking behavior exhibited after the injection of AVP.

Bamshad M, Albers HE (1996) Neural circuitry controlling vasopressin-stimulated scent marking in Syrian hamsters (Mesocricetus auratus). J Comp Neurol, 369(2):252-63. Ferris CF, Albers HE, Wesolowski SM, Goldman BD, Luman SE (1984) Vasopressin injected into the hypothalamus triggers a stereotypic behavior in golden hamsters. Science, 224(4648):521-3. Ferris CF, Rasmussen MF, Messenger T, Koppel G (2001) Vasopressin-dependent flank marking in golden hamsters is suppressed by drugs used in the treatment of obsessive-compulsive disorder. BMC Neurosci, 2:10

Objectives:

- To observe a model of addiction in the rat
- To examine the role of dopamine in this reward model

Introduction:

Rewarding behaviors are thought to be rewarding because they activate the release of dopamine into nucleus accumbens (Wise, 1996). This is true of everyday pleasurable activities (eating yummy food, laughing at a funny joke, having sex), but is also true for drugs of abuse. No matter what neurotransmitter system is directly targeted by a drug of abuse, they all seem to increase activity of dopaminergic cells that project to the nucleus accumbens through the medial forebrain bundle. This has lead to the Positive Reward Model of addiction. One animal model that has been particularly useful in studying addiction is the intracranial self stimulation paradigm (Koob, 2000).

Procedure:

Preparation: The male rats will need to have an electrode implanted into their medial forebrain bundle. See Appendices B+C for the procedure. Implant a bipolar electrode (Plastics One, Roanoke, VA, catalogue # MS303/1-B) at the following coordinates with the skull leveled between bregma and lambda: 2.8 mm posterior to bregma, 1.7 mm lateral to midline, 7.8 mm below dura. Prepare the electrode by cleaning the insulation off of the flat bottom of the wires using a scalpel. Do this with a stereomicroscope.

Test: Set up the test cage equipped with a bar. Connect the bar to one trigger on the stimulation unit. A good protocol is available from Carlezon and Chartoff (2007). Connect the rat to the stimulator through a counterbalanced electrical commutator (i.e., electrical swivel). Set the stimulator (Grass SD88 stimulator coupled to a photic stimulus isolation unit (PSIU6); Grass Instrument Company, Quincy, MA, USA) to the following pulse parameters: 100Hz (1 pulse every 10 ms), 100µs pulse duration, pulse train duration at 500ms. Current should be increased slowly from around 10µA. Depending on your placement, threshold for responding is often around 50 µA, while maximal current that will not elicit aversive responses are usually around 400µA. You want a good responses rate and no aversive responses. Too much current can result in tissue damage, so be conservative while increasing the current, being aware of the upper limit. A current that yields about 40 presses a minute is ideal. With a manual trigger, shape the behavior using successive approximation. First reward the animal when it is in the correct half of the cage, then when it is in that half and facing the bar, then when it is near the bar, then when it is touching the bar, then as presses the bar. Once this is established, allow it to self stimulate for 30 minutes, counting the bar presses. Remove the rat from the box and give it an injection of either saline or the D2 receptor antagonist raclopride (Sigma-Aldrich, catalogue # R121, 0.16mg/kg; Panagis and Spyraki, 1996). Place it back in the box 15 minutes later, and allow it to self stimulate. Count the number of bar presses it makes in 30 minutes. Report your numbers and doses to your TA who will tabulate the results for analysis. *Variation:*

- If time permits, proper thresholding could be used (Carlezon and Chartoff, 2007). This requires more days for training.
- A variety of drugs may be examined to see how they alter responses. Some include lithium chloride, dopamine agonists, and drugs of abuse. See Carlezon and Chartoff, (2007) for a review of some substances examined and their effects on self stimulation.

Follow-up: In any surgical intervention, histological confirmation of the effectiveness to the surgery is important. Discuss with your TA opportunities for learning about histological procedures (perfusion, tissue slicing and staining, microscopy) that may be available. For this study, perfusion with formalin, tissue sectioning and a nissl stain such as cresyl violet would be appropriate histological procedures.

Homework Assignment:

- Prepare a graph of the results. Perform a statistical analysis (2 way ANOVA; treatment x session) to determine if the two groups (saline or raclopride) differed significantly in the amount of bar pressing both before and after injection.

References:

Carlezon WA Jr, Chartoff EH (2007) Intracranial self-stimulation (ICSS) in rodents to study the neurobiology of motivation. Nat Protoc, 2(11):2987-95. Koob GF (2000) Animal models of addiction. In: FE Bloom & DJ Kupfer (Eds) Psychopharmacology - 4th Generation of Progress. Lippincott Williams & Wilkins, New York http://www.acnp.org/g4/GN401000072/Default.htm Panagis G, Spyraki C (1996) Neuropharmacological evidence for the role of dopamine in ventral pallidum self.stimulation. Psychopharmacology, 123:280 288. Wise RA (1996) Addictive drugs and brain stimulation reward. Annu Rev Neurosci, 19:319-40.

Objectives:

- To gain experience with a variety of tests designed to probe fear and anxiety.

Introduction:

Models of fear and anxiety are important for understanding the neurobiology of various psychological illnesses and the development of new pharmacotherapies. For instance, diazepam (Valium) was the highest selling pharmaceutical in the US during the 1970's. A variety of tests have been developed for the rat that tap into fear and anxiety. Many of these involve delivery of an electric shock, and we will not be using these tests in this laboratory exercise. While the shocks are very mild and brief (more mild than touching a 9V battery to your tongue to determine if it still carries a charge), there are ways to study anxiety in animals without electricity that are appropriate for an undergraduate lab in behavioral neuroscience. These include the elevated plus maze (Metz et al., 2005), open field (Crawley, 1985) and a startle version of the defensive burying task.

Procedure:

Preparation:

Two of these tests will use the Any-Maze software. You can download this software for free from the supplier to become familiar with its operation (http://www.anymaze.com). Your TA should have the any-maze protocols set up for these tests ahead of time.

Tests:

Elevated Plus Maze For the Elevated Plus Maze test, start the capture program, and make sure the plus maze template has been properly set up. Start the capture program and then place the animal in the maze. Flip a coin to determine if the rat will face an open or a closed maze (head = open, tails = closed). Allow the rat to explore the maze for 5 minutes, counting arm entries (open or closed) and amount of time spent in the arms (open or closed). Any-maze should score this automatically.

Open Field For the open field test, start the capture program, and make sure the plus maze template has been properly set up. The area should be brightly lit. Start the capture program and then place the animal in the middle of the open field. Allow the animal to explore the open field for 5 minutes. Score how long it spends in against the wall and in the open area. Any-maze should score this automatically. The number of fecal pellets and urination occurrences can be counted.

Defensive Burying Task The defensive burying task usually involves delivery of a mild electric shock produced by a novel object (Treit and Pinel, 2005). Anything that startles the rat should also elicit defensive burying. Place the rat's homecage on a low table and remove the cage lid. Push the bedding to one half of the cage, exposing the bare cage floor on the other half. Hold a marble in your hand about 1 meter above bare part of the cage. When the rat is in the side with the bedding, drop the marble. The goal is to have the marble make a loud noise when it hits the floor. Make every effort to avoid hitting the rat! Ideally the rat should be startled by the marble hitting the floor of its cage and then engage in burying behavior. Time how long the rat bury the object, how many burying bouts there are in 10 minutes, how long it takes until the burying begins, how many times it approaches the marble, if it freezes following the marble falling, and if so, how long the freezing lasts. Videotaping the session will help you score this wide variety of behaviors.

Variations:

- If the marble task does not elicit defensive burying, a different marble task can be used. Place a number of marbles (~15) into a cage, evenly spaced, resting on the bedding. Place the rat in the cage. After 30 minutes, remove the rat and count how many marbles are buried. Anxiolytic drugs suppress this burying behavior (De Boer and Koolhaas, 2003)

Homework Assignment:

- Select one of these tasks. Perform a literature search for this task and find a paper that found a change in the behavior follow treatment with a drug. In one page, summarize the paper, its rational, its methods and its findings.
- Select one of these tasks. Perform a literature search for this task and find a paper that examined the neural underpinnings of fear or anxiety. In one page, summarize the paper, its rational, its methods and its findings.
- Fear and anxiety can be learned. Do a literature search to find such a task. Find a paer examining this phenomenon. In one page, summarize the paper, its rational, its methods and its findings.

References:

Crawley JN (1985) Exploratory behavior models of anxiety in mice. Neurosci Biobehav Rev 9(1):37-44. De Boer SF, Koolhaas JM (2003) Defensive burying in rodents: Ethology, neurobiology and psychopharmacology. Eur J Pharmacol, 463:145– 161. Metz GA, Kolb B, Whishaw IQ (2005) Neuropsychological tests. In: IQ Whishaw and B Kolb (Eds), The behavior of the Laboratory rat: A handbook with tests. Oxford University Press: Toronto. Treit D, Pinel JJP (2005) Defensive Burying. In: IQ Whishaw and B Kolb (Eds), The behavior of the Laboratory rat: A handbook with tests. Oxford University Press: Toronto.

Objectives:

- To condition a rat to avoid a sweet flavor using a powerful one-trial conditioned taste avoidance procedure.

Introduction:

Taste is an important sense for rats exploring their world (Spector, 2005). Rats are unable to vomit, and therefore show neophobia for new flavors, consuming only a little to determine if it is safe. If they become ill, they learn to avoid those foods. Conditioned taste avoidance is a powerful learning phenomenon, requiring only one exposure in many cases (Parker, 2003). Anyone who has suffered food poisoning will understand how pervasive this effect can be, as they will often avoid those foods and flavors associated with the illness for a number of years, even if they were previously highly enjoyable foods. The avoidance can be very specific. Because this avoidance behavior is acquired so rapidly and persists for so long, this behavior is a good model for understanding the neural basis of conditioned learning. A popular paradigm has been to pair a novel flavor with an injection of lithium chloride, which induces nausea (Welzl et al., 2001). Conditioned avoidance has direct implications for not only for people experiencing food poisoning, but also for cancer patients undergoing chemotherapy, as they often develop conditioned taste avoidances for foods eaten around the same time that they receive their chemotherapy agents (Jacobsen et al., 1993).

Procedure:

Preparation: Prepare lithium chloride solution (Sigma-Aldrich product code L9650; concentration 100mg/ml of sterile saline, dose 120mg/kg). Prepare a saccharine solution (Sigma-Aldrich product code 109185; 0.15% solution or 1.5g per L of solution. The rats should be habituated to the test chamber for a number of days before hand (5minutes / day for 5 days). The test chamber should be equipped with 2 water bottles.

Test: **Day 1:** 4-6 hours before the lab, remove the waterbottle from the rat's homecage. This procedure is adapted from (Swank et al., 1995). Place the rat in the testing chamber. Put the 0.15% saccharin solution in one of the water bottles, and remove the other waterbottle temporarily. Allow the rat to explore and drink for 30 minutes. Once the rat starts drinking the saccharin solution, put a bottle of plain water at the other location (this procedure ensures that your thirsty rat actually discovers the saccharin solution). Observe its facial features when it drinks the saccharin solution. Measure how much time the rat spends drinking from each water bottle. At the end of the exploration period, remove the rat and give it an i.p. injection of either LiCl (120mg/kg) or saline. Return the rat to its home cage.

Day 2 Place the saccharin bottle and plain water bottle in the same locations as yesterday. Place the rat into the testing chamber for 30 minutes. Note which bottle the rat approaches first. Observe its facial features when it drinks the saccharin solution. Measure how much time the rat spends drinking from each water bottle. Report your treatment condition (Saline or LiCl) and your drinking times per bottle to your TA, who will collate the results from all the students

Homework Assignment:

- Prepare a graph of drinking time for each bottle on day 2 for each group. Run a statistical test (2 way ANOVA, taste X treatment) to explore the findings

References:

Jacobsen PB, Bovbjerg DH, Schwartz MD, Andrykowski MA, Futterman AD, Gilewski T, Norton L, Redd WH (1993) Formation of food aversions in cancer patients receiving repeated infusions of chemotherapy. Behav Res Ther, 31(8):739-48. Parker, LA (2003) Taste avoidance and taste aversion: Evidence for two different processes. Learning & Behavior, 31(2):165-172. Spector AC (2005) Taste. In: IQ Whishaw and B Kolb (Eds), The behavior of the Laboratory rat: A handbook with tests. Oxford University Press: Toronto. Swank MW, Schafe GE, Bernstein IL (1995) c-Fos induction in response to taste stimuli previously paired with amphetamine or LiC1 during taste aversion learning. Brain Res, 673:251-261. Welzl H, D'Adamo P, Lipp H-P (2001) Conditioned taste aversion as a learning and memory paradigm. Behav Brain Res, 125:205–213.

Objectives:

- To learn about different types of spatial navigation, and the tasks that test them.
- To gain experience with the Morris Water Maze and Exploration procedures.

Introduction:

Spatial learning is a complex behavior that has been quite amenable to behavioral neuroscience investigations. Much of the neural circuitry and molecular biology underlying spatial learning has been delineated. There are many approaches to studying spatial navigation, each of which uses different motivations and strategies. T, cross, and radial arm mazes use food rewards in hungry rats, while the Morris Water Maze uses an escape motivation. Finally, the exploration task capitalizes on rats' natural curiosity (Save and Poucet, 2005). In this lab you will gain experience with two different spatial tasks, the water maze and the exploration task. The Morris Water Maze consists of a tank full of opaque water and a platform in the water to serve as an escape from the water. The water maze has a number of different permutations (Morris, 1981; Sutherland and Dyck, 1984). In "cue" learning there is a proximal cue that serves as a landmark for the platform, such as a visible platform or a flag attached to the platform or a cue on the wall inside the maze. In the "place" task the platform is hidden and the only cues are distally located outside the maze, on the walls of the room. In the "matching-to-place" task, the platform is moved to a new location each day, but stays in that spot all day. The rat learns that there is a solution, and that it must locate the platform, and that the platform will be there all day, but will be in a different spot tomorrow. (There is a great resource available on the internet at http://www.watermaze.org) In the exploration task, the animal is presented with a number of objects. After repeated exposure to the objects in the arena, the animal habituates to them (time spent examining the objects declines). When the animal is then exposed to the objects with one or two of them moved, it will increase its exploration in response to the spatial reorganization (Poucet et al., 1986). Small adjustments to the location of one object will yield more exploration of that object, while large relocations of a single object will increase exploration of all objects. There is likely an aspect of object recognition involved in this task as well (see Laboratory Exercise L).

Procedure:

Preparation: The water maze will need to be filled ahead of time, and this could take a while. The water needs to be about 30 centimeters deep. If you only have access to a cold water source, doing it the day before allows the water to warm up to room temperature which is more pleasant for the animal. If warm water can be added to raise temperature to about 26^{238}_{92}C, this would be even better and then water filling could be done an hour or two before the lab begins. The water will also need to be made opaque using powdered skim milk, but as this can develop an unpleasant odor if left too long, this can be done just before the lab begins. Make sure you have some dry towels handy to dry off the rat after each trial. For the Exploration test, collect 4 large, heavy, durable, and distinctive objects to use (e.g., Glass jars, desert bowls, coffee mugs, brass weight). You don't want objects that the rat can damage, move, get caught in, or that will cause injury. Set them up in a square pattern in the middle of the arena (Poucet et al., 1986). These tests will use the Any-Maze software. You can download this software for free from the supplier to become familiar with its operation (http://www.anymaze.com). Your TA should have the any-maze protocols set up for these tests ahead of time.

Test: **Watermaze** For the watermaze test, start the capture program, and make sure the watermaze template has been properly set up. Start the capture program and then place the animal in the water. Do this by supporting it with your hand as you lower it slowly into the water tail-first. Choose a variety of location. Allow it to swim for 60 seconds, or until it finds the platform, whichever occurs first. If after 60 seconds it has not found the platform, carefully grasp it around the middle, lift it up, and place it on the platform for 15 seconds. Allow the rat to sit on the platform for 15 seconds so that it can orient itself. Run 4 or 5 more trials with the rat, allowing it to rest between sessions. Use a different random starting location each time. For the final trial, remove the platform, and allow the rat to swim for 60 seconds. This is the probe trial, where the rat will "tell" you where it thinks the platform should be.

Exploration Test For the exploration trial, place the animal in the arena, and allow it to explore the objects. When it stops exploring the objects, and becomes interested in something else (e.g., it starts grooming), remove it from the maze for a rest. Repeat this a couple of times. For the probe test, do one of the following while wearing gloves:

- move one object slightly (an object-width from its original location)
- relocate one object to a completely different region of the maze
- swap two objects

Give you TA the data to collate.

Homework Assignment:

- Prepare a graph showing time to find platform in the water maze across trials.
- Do a statistical test for time spent in each quadrant in the probe trial.
- Prepare a graph of total object exploration time across trials.
- Perform a statistical test for time spent examining each object following the 3 different treatments (mixed design: 4 objects x 3 treatments)

References:

Morris RGM (1981) Spatial localization does not require the presence of local cues. Learn Motiv, 12:239-260. Poucet B, Chapuis N, Durup M, Thinus-Blanc C (1986) A study of exploratory behavior as an index of spatial knowledge in hamsters. Anim Learn Behav, 14:93-100. Save E, Poucet B (2005) Piloting. In: IQ Whishaw and B Kolb (Eds), The behavior of the Laboratory rat: A handbook with tests. Oxford University Press: Toronto. Sutherland RJ and Dyck RH (1984). Place Navigation by Rats in a Swimming Pool, Can J Psychology, 38(2):322-347.

Objectives:

- To examine rat learning using a test of object recognition.

Introduction:

Object recognition is a model of working memory. It is a useful model for understanding the neural basis of working memory (Mumby, 2001), as it is sensitive to pharmacological, genetic and surgical interventions. Additionally, object memory is useful in understanding memory deficits associated with various dementias such as Alzheimer's disease. This laboratory exercise, describes two object recognition tasks, the Delayed non-matching to sample task and Novel object preference task. If there is a lot of time for this lab exercise (a couple of weeks) and the proper maze available, the Delayed non-matching to sample task can be used. The novel object preference task is more simple can run over a shorter time frame as training and hunger-motivation are not required.

Procedure:

Delayed nonmatching-to-sample *Preparation:* You will need to assemble a large number of objects. These objects should be large enough to cover the food cups, but light enough to be displaced by the rat. The objects should not have any sharp edges that could injure the rat. Also, objects with prominent odors should be avoided. About 50 objects will be needed if you run 25 trials. Rats will need to be trained on the apparatus ahead of time. This can be facilitated if they are a little hungry, so they may need to be maintained at about 95-100% of their free-feeding weight. This procedure required daily weighing and feeding of rats; negligence can be fatal! On the first day weigh the rats. Weigh out an excess of food (~50g) for the rats. At the same time the next day, weigh the food and the rat. The average of the rat weights between the two days will become its free-feeding weight. Whatever the rat ate will become the daily ration of food, to be given after the training/testing is complete. Adjust the amount of food up or down a gram each day to keep the rat in the 95-100% of free-feeding weight range. The following is based on the protocol of Mumby et al. (1990), and assumes you have access to a maze such as those presented by Mumby (2005).

- First, habituate the rat to the choice maze by placing reward pellets in the food cups (VWR, catalog #s CA89067-518; 89067-534; or 89067-518).
- Next, use an object discrimination task where they always are presented with the same 2 objects, and need to displace the same one to get a reward hidden beneath it. Do this over 2 days.
- After they readily perform the object discrimination task, they must be trained to run back and forth so that you can get them to leave the reward area. Do this by alternating the end of the maze where reinforcement is received. Do this over 2 days.
- Finally, if your maze has doors, train the rat in how they operate. Place the rat in the maze, and allow it to move to one end. When it does, close the distal door, and put a pellet in the cup on the other side of the door. When the rat approaches the door, raise it, and as it eats the reward, close the other door and bait a cup behind that one. Do this over 2 days. *Test:* To train the rat on the non-matching-to-sample task, use the following protocol based on that outlined by Mumby et al., (1990):
- Place the rat in the central portion of the maze with both doors closed.
- Place a food pellet in a single cup and cover this food cup with the sample object.
- Place the novel object over an empty cup at the OPPOSITE end of the maze.
- 1 minute later, raise the door between the rat and the sample object food cup.
- Once the rat moves the sample object, move this object to the opposite end of the maze and place it over the other empty food cup while the rat enjoys its treat.
- Raise the distal door, and allow the rat to move one of the objects. Only reward the rat if it moves the novel object. On the first trial ONLY, allow the rat to correct its choice and be reward if it chooses the sample over the novel object initially.
- When the rat eats its reward, remove the objects and close the distal door.
- When the rat finishes eating returns to the central area, close the door behind it.
- Drop a pellet into the central area if the rat does not return there after finishing its reward
- Start the next trial as described above with 2 new objects, one designate sample and one designated novel.
- As the rat acquired the task (21/25 correct on 2 consecutive days), increase the delay between when you remove the sample object and when you raise the door to the choice end to 15s, then 30s, then 60s, then 120s. These may need to be run on separate days.
- For these longer retention intervals, count how many of the 25 trials the rat gets correct.
- Provide the data to your TA who will collate the data and provide summary data to you.

Novel-Object Preference *Preparation:* You will need to assemble a large number of objects. The objects should not have any sharp edges that could injure the rat. Also, objects with prominent odors should be avoided. About 20 objects will be needed if you run 10 trials, and you will need at least 3 copies of each. Screen objects ahead of time for baseline amount of exploration evoked. Discard objects that evoke too little or too much baseline exploration (you want all the objects to be mildly interesting, but not too exciting or too boring) Habituate to test arena, won't explore in unfamiliar locations (10-15 minuets over a couple days). Use a pair of identical objects during the habituation stage and do not use these same objects during the actual recognition experiments. You will need to develop an operational definition of object exploration. The following is an example: "A rat was considered to be engaged in object exploration when its head was oriented within 45° of an object and within 4 cm of it. Rearing with the head oriented upward was also included if at least one forepaw was on the object. Climbing over or sitting on the objects was not included" (Mumby et al., 2002). Alternatively, Any-Maze can be configured to score object exploration objectively. *Test:*

- Place a pair of identical sample objects in the arena.
- Place the rat in the arena for 5 minutes
- Remove the rat to its homecage for the predetermined latency (e.g., 1 minute).
- Remove the objects and place a new copy of the sample object along with a novel object.
- Place the rat back in the arena for 3 minutes.
- For each minute, score how long the rat investigates both the sample and the novel objects.
- Run a total of 5 such trials
- Provide the data to your TA who will collate the data and provide summary data to you.

Homework Assignment:

- For the Delayed Nonmatching-to-Sample task, prepare a graph showing the percent correct for each retention interval. Run an ANOVA to determine if percent correct changes significantly over the course of time.
- For the Novel-Object Preference task, calculate average exploration ratios each of the three minutes of the test, and prepare a summary graph. An exploration ratio is the time spent exploring the novel object divided by the sum of the time spent exploring both the novel and sample object (TNovel / (Tnovel + TSample). A ratio of 0.5 would indicate that the objects were explored equally, while a ratio greater than 0.5 would indicate that the novel object was explored more. Run three one-sample t-test to determine if the animals significantly discriminated at each of the one-minute blocks of the test session. Run an ANOVA to determine if the exploration ratio changed over time.

References:

Mumby DG, Pinel JPJ, Wood ER (1990). Nonrecurring-items delayed nonmatching-to-sample in rats: A new paradigm for testing nonspatial working memory. Psychobiology, 18(3): 321-326. Mumby DG (2001) Perspectives on object-recognition memory following hippocampal damage: lessons from studies in rats. Behav Brain Res, 127(1-2):159-81. Mumby DG, Gaskin S, Glenn MJ, Schramek TE, Lehmann H (2002) Hippocampal damage and exploratory preferences in rats: memory for objects, places, and contexts. Learn Mem, 9(2):49-57. Mumby DG (2005) Object Recognition. In: IQ Whishaw and B Kolb (Eds), The behavior of the Laboratory rat: A handbook with tests. Oxford University Press: Toronto.

Learning how to present your results is an important skill to develop. Throughout this course, you will be asked to prepare a number of Manuscript-style Lab Reports. There are a variety of guidelines on how to do this, including the Publication Style of the American Psychological Association (APA, 2009). The APA guide is quiet comprehensive and is a great resource for writing style in general. Few neuroscience journals are published by the APA, so we regularly recommend that our students use the style that the Society for Neuroscience uses for the Journal of Neuroscience (http://www.jneurosci.org/misc/ifa_rscharticles.dtl). Other guides exist as well, such as The Manual of Scientific Style: A Guide for Authors, Editors, and Researchers by Rabinowitz and Vogel (2009); Scientific Style and Format: The CSE Manual for Authors, Editors, And Publishers by the Council of Science Editors (2006) and Scientific Style and Format: The CBE Manual for Authors, Editors, and Publishers by the Council of Biology Editors (1994). The Elements of Style by Strunk and White (2008) is a valuable resource for all writing; scientific or otherwise. The basic format of a research report includes the following sections, based on the style of the Journal of Neuroscience: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, References. Additionally, your report may include tables and/or figures. Following the references you would included a Table Legend, the Tables, a Figure Legend, and finally the Figures.

Title Page:

Your Title Page should include the following:

- The title of your report. This should be informative, as it will be the first thing that people search for information will see, and based on your title they will decide if your paper is relevant enough to their search to go on an read your abstract.
- Your personal information (Name and student number)
- Number of tables and figures
- Number of pages
- Number of words in the Abstract
- Number of words in the Introduction
- Number of words in the Discussion
- Six keywords

Abstract:

Your abstract is a brief summary of the overall manuscript, and is usually limited to 250 words. You want to briefly and clearly set up the topic area and question examined, the goal of the study, the methods, the results and major conclusions of the study. Use full sentences. Avoid using references and subheadings. When you do publish a paper, this is the one thing that people will read to decide if they want to invest the time to read the rest of your paper. Think of it like reading the summary on the back of a novel when trying to decide if you want to spend money on it (except of course that your abstract should give away the ending).

Introduction:

The introduction has two goals: Introduction the topic area, and build a rational for the study. Some journals insist upon strict limits for an introduction. The Journal of Neuroscience has a limit of 500 words (only double the abstract!), while some others allow 1000 words. Check with your instructor on their expectations. Often student papers have a third goal, that is to demonstrate an understanding of the topic area literature. This may require a substantially longer introduction. This can make it very challenging to write an effective introduction, as reviewing the literature and building a case for your study can be hard to combine. The following advice may be useful: Start your introduction with a background paragraph that introduces the area (e.g., learning, feeding, sex). In this paragraph you should move very quickly to research area, so that by the end of this first paragraph, the reader has an idea of where the introduction is headed (e.g., the role of pyramidal cells in the hippocampus for spatial learning). The literature you review in the rest of the introduction should relate back to this first paragraph (e.g., you'd likely want to review the different types of learning, focus particularly on spatial learning; the role of the hippocampus in learning and the cellular anatomy of the hippocampus). For effective writing, try to focus each paragraph on a concept, rather than a study. If you write each paragraph about a study, the document will start to seem like an annotated bibliography, and will fail to build a rational for your study. Focus on the concept, and cite a number of studies that address that concept. Try to organize your paragraphs in a logical fashion so each concept leads logically to the next paragraph. When discussing a paper, particularly a scientific paper, the findings and their meaning that are important. The methods need not be discussed unless you feel that there was a problem with the study and therefore you don't agree with their findings. In this case, just highlight the flaw, and give your point of view. Critiquing the literature is encouraged. Don't believe everything you read. Similarly, you might find in your literature review that two studies found different results, and the reason could be a difference in methodology. In this case it might be worthwhile discussing the different methods, and then make a ruling of which study/result, in your opinion, should be focused on. Your introduction should end with a clear statement of your hypothesis. If you have done an effective job building a rational, this hypothesis should be easy for the reader to guess, as everything up to now should have been leading to this question.

Materials and Method:

The materials and methods section can contain a number of subheadings, that might include: Animals, Apparatus, Drugs, Procedures, Analysis, etc … Sufficient detail should be given so that someone could replicate your study based on reading only your materials and methods section. Often scientists who use the same technique repeatedly will shorten their Materials and Methods section by saying "… was done as has been previously reported (Smith and Jones, 2010)". While this is acceptable in scientific publishing, it is not acceptable for student papers, where your instructors want to see if you understand what you actually did in the laboratory exercise.

Results:

The results section should present your findings. Avoid interpretation of the data. Data need only be presented once, in the text, a table or a figure. You will need to decide while of the three is most effective for your data. If you put the data into a table or a figure, refer to the table of figure here in the results section. Report statistical test here. Include the statistic, the degrees of freedom and the probability that the finding was found by chance alone. Some excellent advice for presenting your statistics has been prepared by the editors of the European Journal of Neuroscience (Sarter and Fritschy, 2008). They recommend keeping statistical statements about main effects and interactions inside brackets to maintain their readability. A good example would be: "The glutamate antagonist impaired learning in all mice (main effect of drug; $F(1,12)=4.65$, $p=0.01$), although it had a more pronounced effect on the knockout mice (significant drug x genotype interaction; $F(3,12)=6.42$, $p=0.001$). Overall, the knockout mice had poorer performance than did the wildtype mice (main effect of genotype; $F(1,12)=3.11$, $p=0.03$)".

Discussion:

The discussion section should start with a clear statement about your major findings. Explain what these findings mean for your hypothesis, and for knowledge in the field. What do we know now that we didn't before you undertook your study? Relate your findings back to the literature. Are your findings consistent with general understanding of the field, or do they change the way we think about the question? Do they resolve any discrepancies in the field? If they are inconsistent with other findings, what are

the possible sources of this inconsistency? Are there any unresolved questions or new questions as a result of your findings? Discuss future directions that the research may take. End the discussion with an overall summary that highlights the significance of your work. Despite these many issues to address, the discussion should be concise. Some journals have word limits on discussions too; for instance the Journal of Neuroscience limits you to 1500 words.

References:

There are a variety of citation styles, and generally these are specified by the journal. Use of a citation manager such as Endnotes (http://www.endnote.com/) or Refworks (http://www.refworks.com/) may simplify building your reference list and track citations throughout your paper, applying the proper style throughout. These software packages have an appreciable learning curve, and should be avoided if the first time you go to use it is the night before you have to hand in your paper. Many university libraries offer course or tutorials in how to use these software packages, and may even have site licenses which make the software available to students for free or at a significant discount. If you will be doing a lot of scientific writing, you should look into such software and the offered courses.

A generic citation style would be to cite the references in the text as follows:

- For one or two authors, give the last names and year in chronological order
  - (Smith, 1990; Jones and Johnson, 2010)
  - Smith (1990) and Jones and Johnson (2010)
- For three or more authors, give just the last name of the first author
  - (Smith et al., 2009)
  - Smith et al. (2009)

For your reference section, list the articles in alphabetical order by the first author's last name. If you have a number of papers by the same first author, list them in chronological order. If there are more than 20 authors, just list the first author, followed by et al. The structure should be:

- Authors' last name then their initials
- Publication year in brackets.
- Title of article
- Journal title, abbreviated. The proper abbreviations can be found here: http://library.caltech.edu/reference/abbreviations/
- Journal volume
- First page number - last page number
- For books, list the publication place and publisher at the end of the entry

Advice for effective referencing:

- Do not use "as cited in". You'll see this in some papers, but it reflects poorly on you. Go to the original reference. If you can't get the original paper (ancient paper, not available at your university, it is written in a language you can't read) then it is acceptable, but you should really avoid it at all costs.
- Don't cite course textbooks. Put in the effort to find the original source.
- It is better to cite the paper that **discovered** the phenomenon you're talking about, rather than someone who merely **talked about** the previously discovered phenomenon in their paper. While it is important to back up your statement with references (in which case both approaches work) it is equally important to give credit where credit is due (in which case only the former style is acceptable).
- Publication styles give you instructions on a variety of ways to cite and quote sources. However, just because there is a correct way to quote a paper, doesn't mean that quoting a paper is correct. In most of neuroscience writing you should rarely, if ever, quote (did you find any quotations in the papers you've read in preparing for your paper?). In all my published work, I have only quoted a paper once, and it was because I disagreed with what the authors stated in their paper. A quote lifted out of a paper and integrated into your work may or may not make sense out of context. It makes your paper hard to read and makes it look unsophisticated in most cases. Approach your paper with the plan of not quoting anything. This is different from a paper in a literature class, where if you were trying to analyze or interpret a piece of literature, you'd need to quote.
- When citing work, consider using the style "Schizophrenia is a devastating disease (Smith, 2001; Jones, 2002)" rather than "Smith (2001) claimed that schizophrenia is a devastating disease, a thought echoed by Jones (2002)". By putting the authors' names inside the brackets, you put the emphasis on the concept. When the authors' names are outside the bracket, it puts the emphasis on the authors. There are times when you might want to emphasize an author (e.g., a leader in the field who's contribution changed the way we think about something; a scientist whose paper is directly relevant and applicable to your central hypothesis), but this is not necessary, and you should approach your paper with the plan of having the authors' names inside the brackets. By doing this, it will help you keep your writing about the concepts, rather than having your paper sink to simply being a summary of a bunch of papers.

Table and Figure Captions:

Following your reference list should be a page that contains all the captions for your tables listed in numerical order. All your tables should be cited in the text in order of appearance. Following the table caption page should be all your tables, each on a separate page. Following the tables should be a Figure caption page that contains all the captions for your figures listed in numerical order. All your figures should be cited in the text in order of appearance. Following the figure caption page should be all of your figures, each on a separate page. Don't feel compelled to include figures and tables just because there is a place for them in the style. Use them if you need them, and leave them out if you don't need them.

Figures

Making a good figure is a bit of an art. If someone has a copy of your paper on their desk and they are interested in what you found, they may first (and in some cases only) look at your figures without reading a thing! Making your figures easy to understand and self explanatory is essential to getting your central message across. Here are some generic pieces of advice:

- Always display the control group on the left and the treatment to the right. We read from left to right, so this way the reader will see the typical situation first, and then see how this is altered by your treatment
- Don't make the reader refer to the figure caption to interpret your figure if at all possible, as many people won't even read these. E.g., for line graphs or scatter plots, include a figure legend within the plot so that the reader knows what the symbols mean. Assume your reader is incredibly lazy, and make it as easy as possible for them to understand what you are trying to show.

References:

American Psychological Association (2009) Publication Manual of the American Psychological Association (6th Ed). American Psychological Association: Washington, DC Council of Biology Editors (1994) Scientific Style and Format: The CBE Manual for Authors, Editors, and Publishers. Cambridge University Press: Council of Science Editors (2006) Scientific Style and Format: The CSE Manual for Authors, Editors, And Publishers (7th Ed). CSE Books: Rabinowitz and Vogel (2009) The Manual of Scientific Style: A Guide for Authors, Editors, and Researchers. Academic Press: Sarter M, Fritschy JM (2008) Reporting statistical methods and statistical results in EJN. Eur J Neurosci, 28(12): 2363-2364. Strunk W and White EB (2008) The Elements of Style: 50th Anniversary Edition. Longman:

Sample scoring rubric for a lab report

A) Abstract	5
Are all the essential components present?	
Is the language clear?	
B) Introduction	20
Is the topic area introduced in an effective manner?	
Is the research question or area apparent early on in the introduction?	
Is the literature reviewed in an effective manner?	
Is the focus on concepts rather than individual findings?	
Has a rational for the study been built?	
Are there clear hypotheses and predictions?	
C) Materials and Methods	15
Are all method components present?	
Is there sufficient detail?	
D) Results	20
Are findings presented in a clear manner?	
Are results repeated unnecessarily?	
Effective use of visual presentation of findings	
Are all results presented?	
Are statistics presented?	
E) Discussion	20
Clear statement of findings	
Interpretation of results	
Are findings related back to the literature?	
Are shortfalls and flaws addressed?	
Is there an overall conclusion that summarizes the study effectively?	
F) Style	10
Is the primary literature cited effectively and appropriately?	
Has publication style been followed?	
Are figures clear?	
G) Strength of writing	10
Lack of spelling and grammatical mistakes?	
Well organized intro and discussion?	
Interesting an engaging?	
Evidence of creative input and interpretation as opposed to simply a review of the literature?	

Many behavioral neuroscience studies involve surgery on the animal. Surgery can allow the investigator to make strong statements about specific systems in specific regions of the nervous system. With lesions, an investigator can look at the necessary and sufficient properties of various regions of the brain. With a cannula or electrode implantation, the investigator can investigate various was of stimulating or suppressing activity in a certain region of the nervous system. Other surgeries allow for chronic implants for monitoring body systems, or delivering drugs over a long period of time. Finally, surgeries can used to control levels of hormones in the animal's body (e.g., castration, ovariectomy, adrenalectomy, etc…).

Surgery is a serious endeavor, which should only be performed by, or under the close supervision of, a trained professional. Close and careful supervision is important to ensure that the animals are well treated. Listen to instructions and advice of your trainer, and leave the room if you feel uncomfortable. Surgery is stressful to watch and experience. No one will fault you if you need a time out. Please respect the surgeon and the animal. Do not get in their way or distract them. Be aware that there are very strict laws that regulate the use of animals in research, and these laws may stipulate how surgery may be performed, and by whom it can be performed. Before using an animal in research, and certainly before performing surgery on an animal, you should check institutional regulations as well as local and federal laws that are applicable to your project. The guidelines in this book conform to the guidelines of the Canadian Council of Animal Care and the policies of the University of Calgary, but may not be sufficient for your institution.

Surgical Area

The surgery room should be well ventilated with appropriate scrubbers on the ventilation system for any gaseous anesthetics in use. Ideally, it should be in an out-of-the-way place with minimal traffic and noise. The area should have a door which should be kept closed to prevent the unprepared and uninitiated from witnessing something they would rather not see. The surgical field should be made of a non-porous surface which can easily be cleaned and disinfected, such as stainless steel. Adequate lighting should be available, ideally in the form of a boom-mounted or a fiber-optic lamp. This will allow you to adjust the angle of the light source. One advantage of a standard incandescent lamp is that it will keep the surgical area warm. A homeothermic surgical pad can be used to help keep the body temperature near the normal physiological level during the surgery, as the animal's ability to thermoregulate during surgery is compromised by the anesthetic. An area removed from the surgical area should be provided for preparing the animal for surgery (injections and hair clipping). This helps keep the surgical area sterile.

Standard supplies which are always handy include: 0.9% saline, 70% ethyl alcohol, distilled water, gauze pads, paper towels, and cotton swabs. Of course, many specific tools will be needed for each surgery, but having the above mentioned supplies at hand is a good idea. The surgeon should be wearing a clean lab coat whenever working with animals. When there will be an open wound, gloves and a facemask are essential. After washing your hands with a disinfectant soap, don a pair of latex gloves. I have found for stereotaxic surgery that it is best to wash and put your gloves on after you get the animal in the stereotaxic frame, but before you make the initial incision. The animal's fur seems to stick to the latex, which makes the task of getting the animal in the ears bars that much more arduous. So long as you put your gloves on and disinfect the incision site before making the incision, sterility can be maintained.

Oxygen and Ventilation

Maintaining the animal on oxygen will help with recovery. As most anesthetics decrease respiration, so increasing the percentage of oxygen in the air which the animal breaths will help maintain an adequate level of oxygen in the blood. For rat surgery, the anesthetic machine should be an open system as opposed to the more typical Magill circuit used on larger animals. (the Magill circuit has a valve, and the animal must have sufficient lung capacity to force this valve open to expel the exhaust air, and rats do not have sufficient lung capacity). Many open circuit systems have optional scavenging units which create an active draw circuit and collect the exhaust anesthetic. The O2 flow should be set at 1L/min, with the isoflurane set to 2%. An animal will begin to look blue (cyanosis) if its blood-oxygen level drops too low.

Tools The tools required will depend on the particular surgery being carried out. The following is a list of commonly used tools:

- Scalpel blade and holder: The blade used should be sterile, and either a #10 (curved) or #11 (straight edge). Never touch the blade with your fingers, use a clamping device to hold the blade when placing it on the holder.
- Hemostats: These locking tools are classically used clamp off blood vessels (hence their name) but are indispensable for grasping and holding tissue. Because they lock closed, they can act as a third hand.
- Forceps: These are ideal for grasping and manipulating tissue or equipment. They come in various sizes, curved or straight, tissue-grasping or normal.
- Needle holders with scissors: these will hold suture needles and cut suture thread.
- Scissors: For cutting tissue only.
- Spatula: For separating bone and muscle, or for delivering and shaping dental acrylic.
- Suture needle and thread: There are many different types of each available. Smaller is better for small animals. Monofilament sutures are preferred as braided silks have spaces that may harbor microorganisms. Catgut or absorbable sutures should be used for internal sutures, while a more durable thread can be used for external sutures. If the suture is in a place when the animal can chew on it, a hidden stitch should be used, or even better, surgical staples (Michel clips).
- Surgical drill and bits: For drilling bur holes for the placement of implants or anchoring screws.
- Jewelers' screws and screwdrivers.
- Dental Acrylic All tools should be cleaned prior to surgery. A bead sterilizer is a good choice, and a few minutes at 220°C will kill most pathogens. Alternatively, a disinfectant bath of gremaphine, Zepherian's solution or 70% ethyl alcohol are quite good at cleaning the tools and will kill bacteria in minutes, although bacterial spores will survive such treatment (Waynforth, 1980). For completely aseptic sterile tools, more extreme measures are needed. Tools can be autoclaved (180°C, 15 lbs/square inch for 15 minutes). However, many facilities are not well enough equipped to have multiple sets of tools such that you can have a separate autoclaved set for each surgery (particularly if you do six or seven a day). Cleaning your tools with a bristle brush and clean water, followed by sterilization in the bead sterilizer or an antiseptic solution is sufficient for most rodent surgeries. If you find that your animals are getting infections, then obviously you'll want to re-evaluate this step, and proceed with more stringent sterile cleaning of your tools. Tools should be left to air dry at the end of the day after a final wash. They should be placed on clean paper towel and hemostats and scissors should be left open to air in drying and prevent rusting. A small tray is often helpful for transporting the tools from the sink to the surgical table. If you are autoclaving your tools, make sure they are encased in sterile packaging so that they their sterility can be maintained until you need them.

Pre- and Post Anesthetics

Various drugs can be used as a pretreatment prior to surgery. The use of any of these depends on the particular surgery and anesthetic being used. Be aware that drugs do interact, and in some cases certain combinations should be avoided. As well, animals will require various other medications during or following surgery. They will be described below as well.

Analgesics

Many anesthetics have no analgesic properties beyond those of obtained during general anesthesia. Although it seems like they should go hand in hand, they do only while the anesthetic is having an influence. As well, any tissue damage early in the surgery can lead to central sensitization, which may lead to a stronger sensation of pain after surgery (Yashpal, Katz, and Coderre, 1996). It has been suggested that a pretreatment with an analgesic will minimize pain when emerging from the anesthetic. Such an analgesic will have an effect prior to tissue damage (i.e., incision), and will ideally, still be active when the anesthetic has worn off. Such a pre-anesthetic would

go a long way towards minimizing suffering. The major hurdle in this case is that some analgesics can have detrimental effects when given before surgery (e.g., some may interact with many anesthetics to decrease respiration, while others may impair) so careful, informed, evidence-based decisions should be made in consultation with a knowledgeable veterinarian. Butorphanol and Buprenorphine are two safe opiates that are partial agonists at the μ-opioid receptor and thus cause less respiratory depression than full agonists such as morphine, but yet produce excellent analgesic effects. The ideal situation would be where pain is manaded through multimodal analgesia, where an opiate, an anti-inflammatory, a local anesthetic and a general anesthetic are used in combination to abrogate pain through a variety of mechanism (Lichtenberger and Ko, 2007) A strong opiate should be given post-operatively. Buprenorphine is ideal as only a very small dose (0.1-0.5 mg/kg) is needed, and its duration of action in rats is much longer than the other opiates (~ 12 h). Other drug which work well include Butorphanol (0.05-2.0 mg/kg), Morphine (10 mg/kg) or Codeine (25-60 mg/kg) (Jenkins, 1987). μ-receptor agonist (such as morphine) may be a better choice than ∓-agonists (such as Butorphanol and Buprenorphine) post surgically for management of somatic pain associated with skin and deep tissue damage (Lichtenberger and Ko, 2007). ∓-agonists appear more suited to visceral pain (Lichtenberger and Ko, 2007). Buprenorphine has been reported to induce abhorrent eating behavior in rats, whereby the rat will consume its sawdust bedding, leading to damage of the gastrointestinal tract (Jacobson, 2000) so care should be used with this drug, particularly following a gastrointestinal surgery. Non-opiate drugs are available, and can be given in the following doses: Aspirin, 100 mg/kg; Acetaminophen, 110-330 mg/kg; Ibuprophen, 10-30 mg/kg (Jenkins, 1987; Flecknell, 1984). These generally provide more mild analgesia than the opiate analgesics, but are relatively safe and long acting (Lichtenberger and Ko, 2007). For long-term pain management, it has been suggested that animals be treated for 48-72 hours after surgery (Flecknell, 1984). This presents a problem for a lab with a small staff doing surgery on a large number of animals. One approach has been to deliver drugs in drinking water. Cooper, DeLong and Gillett (1997) determined that acetaminophen in drinking water up to doses of 600 mg/kg/d did not provide any measurable analgesia. Buprenorphine (2.9 mg/kg/d) did work in their paradigm (paw withdrawal reflex) when delivered in drinking water. Both drugs did work when delivered parentally. Based on these results, acetaminophen, if used should not be given in the drinking water, as it is unlikely to provide any pain relief. Cooper, et al. (1997) hypothesize that the concentration of acetaminophen which would be needed in the drinking water to provide analgesia would make the water unpalatable and thus would decrease the amount of water consumed, and thus would decrease the amount of drug consumed, leading to insufficient analgesia. This is a concern as water consumption is generally decreased after surgery. Cooper, et al., (1997), and Flecknell (1984) recommend the use of Buprenorphine for pain management as its effects are long lasting. Lidocaine can be used as a form of analgesia (it is a topical anesthetic, which acts by blocking voltage gated sodium channels, and thus prevents action potentials). A 5% (0.5 g in 10 ml) solution in sterile saline should be used, and can be applied with a cotton swab, or syringe. It is recommended that it be injected prior at the surgical site prior to an incision. As well, it can be applied prior to suturing a wound. Another topical anesthetic which could be used is Benzocaine (20% solution).

Antibiotics

“An ounce of prevention is worth a pound of cure.” Clean, aseptic surgeries are the best way to prevent infection. If a procedure appears to be infection prone, antibiotics can and should be given. Baytril is a common broad spectrum veterinary antibiotic. It can be given S.C. 12 hours prior to surgery, and again 12 hours after surgery (I.P. delivery is not recommended, as this could lead to rapid metabolism by the liver, decreasing the duration of action). This pretreatment prevents any bacteria from colonizing the wound. A dose of 5 mg/kg is recommended for rodents. A good topical antibiotic for use on wounds is the gel form of chloramphenicol, which can be used in conjunction with Baytril. A small amount can be placed on a cotton swab, and then be applied to the wound. For long term treatment, tetracycline can be used. Tetracycline is supplied in a drink crystal form in different concentrations. Treatment lasts 7-10 days. Fresh tetracycline drinking water should be supplied at least every second day.

Anesthesiology

Anesthesia is defined as a reversible, drug-induced loss of awareness and sensation. There are many choices when it comes to anesthesia. Each technique has its own benefits and disadvantages. Your choice of anesthetic will depend on the type of surgery and the species being used. **All surgeries must be performed under complete general anesthesia.** Under no circumstance should an animal ever have the conscious experience of surgery.

Assessing depth of anesthesia. There are four stages of anesthesia (Trevor and Miller, 1992). The first is **Analgesia**, where the subject experiences analgesia and followed by amnesia. The next stage is **Excitement**. Excitement is characterized by delirious behavior. Struggling, incontinence and vomiting can occur, although this is not a concern in rats, which cannot vomit. The reason for the appearance of this stage is that cortical function is depressed, and thus refinement of motor control no longer exists. Amnesia is experienced in this stage. The third stage is **Surgical Anesthesia**, which is the target stage for our purposes. It is characterized by the loss of many reflexes (described below). However, anesthesia does not mean absence of all movement, simply the lack of awareness and sensation. For example, the corneal reflex is maintained. The fourth stage is **Medullary depression**, which is characterized by the loss of the corneal reflex and the cessation of respiration, followed soon after by death. When assessing the reflexes, it is best to hold the animal in you hand. This will allow you to feel any response by the postural skeletal muscles.

Tail flick Pinching the tip if the tail elicits a response in the animal, which may be withdrawal of the tail or movement of the limbs and torso. **Eyelash reflex** Tickling the anterior corner of the eye elicits an eye blink. **Pedal reflex** Pinching the toe or foot elicits a response in the limb being pinched. The animal will withdraw the foot and may curve its back to better withdraw the limb. **Ear reflex** The animal will shake its head when the ear is pinched with your fingernail. I find this the most conservative test, as responses in the first three reflexes may be absent when this one is maintained. Complete absence is not necessary, but it should be substantially depressed. N.B., the ear can be moved merely by the shaking of you hand while you pinch. It is important to distinguish between the reflex and movement caused by touching the ear. **Corneal reflex** When the cornea is swabbed with a saline soaked cotton swab, the animal will blink its eye. This response should never be absent unless the animal has been too deeply anesthetized. However, it is not necessary to check this reflex. While its presence may be comforting to the surgeon, it may cause trauma to the cornea. Furthermore, there is often little that can be done if it is absent.

Common anesthetics **Sodium Pentobarbital (Somnotol)** This barbiturate was probably the most commonly used anesthetic for rodent surgery, but has fallen out of favor due to the fact that proper dosing can be challenging, leading to frequent overdoses. When used properly, it induces deep anesthesia. It is easy to deliver (i.p.), acts quite quickly (5-10 min), and anesthesia lasts for about 60 minutes. It acts on the GABAA receptor, which inhibits neural activity. The reticular system, which maintains consciousness, is turned off, and thus a deep state of anesthesia is reached. This system controls many important functions, such as respiration, and thus it is possible to overdose an animal. The major disadvantage is its EXTREMELY narrow therapeutic index. That is, the effective dose is quite close to the lethal dose. Care must be taken to carefully weigh the animal prior to delivery of the aesthetic, as well as to carefully fill the syringe. A dose of 40mg/kg is recommended for rats (CCAC, 1993), although a survey of the published literature suggests that this may be insufficient, and doses of ~65mg/kg may be needed. For hamster, a dose of 90mg/kg is recommended (CCAC, 1993), but again, higher doses are often used in the literature (100-120mg/kg) for some stains (i.e., LVG hamsters require more sodium pentobarbital than the HSD hamsters). Given high individual variability, care should be taken when using this drug, and it is better to underestimate the dose needed and then titrate up to surgical anesthesia. For some animals, the dose provided will not be sufficient to achieve anesthesia. This is due to individual variability. A supplement of ~10mg/kg can be given 10-15 minutes after the initial dose. A second supplementary dose can be give after another 10-15 minutes if anesthesia is insufficient. More than two supplementary doses of Somnotol should be avoided is possible, as it is possible that one or more of your injections was placed in a tissue which from which the Somnotol would be absorbed slowly, and hence a large quantity could be present, despite the lack of anesthesia. This could lead to an unexpected overdose. Commonsense should dictate whether or not a supplementary dose is necessary. A topical anesthetic or gaseous anesthetic may be sufficient if the animal regains a degree of consciousness near the end of the surgery. S.K. Wixson and colleagues (1987a,b) evaluated pentobarbital, ketamine and Fentanyl-droperidol (Innovar-Vet) for their respective advantages and disadvantages. Pentobarbital was found in their study to have many undesirable properties, including total lack of analgesia during

sedation. Although they had no animals overdose from pentobarbital, they ranked it second worst for mortality, behind Innovar-vet that had a 6% mortality rate at one dose! They also ranked pentobarbital second worst for depth of anesthesia, a finding likely due to the fact that they used a dose of 40 mg/kg, far below the more typical dose of 65mg/kg. This low dose may also have been responsible for the amount of responsiveness to the noxious stimuli.

Ketamine Ketamine is a dissociative anesthetic that is similar to PCP. It interferes with the excitatory effects of glutamate at the NMDA receptor. Being quite lipophilic, ketamine reaches the CNS quite quickly and anesthesia is obtained within 5 minutes. Ketamine is a wonderful anesthetic that has a large therapeutic index. It is very difficult to overdose an animal with this anesthetic, as it actually stimulates the cardiovascular system and does not depress respiration. Often ketamine is co-administered with a tranquilizer such as Xylazine. For rats a dose of 90mg/kg Ketamine and 5-10mg/kg Xylazine is recommended (CCAC, 1993). Anesthesia lasts for about 60 minutes. For hamsters, some labs have found success with doses of 120 mg/kg Ketamine, 20mg/kg Xylazine, and 0.02mg/kg Acepromazine, although the effects are quite variable in this species.

Isoflurane Isoflurane is a gaseous anesthetic. It is a good anesthetic for short surgeries, or maintenance of anesthesia induced by injectable anesthetics. Simple procedures such as castration, ovariectomy, or abdominal implants may be most easily performed this way. The animal can be placed in an induction chamber and Isoflurane can be introduced or, alternatively, a low dose of Somnotol can be used to induce the animals prior to introduction of Isoflurane. It should only be used in a well ventilated room, and/or with a proper scavenging device. For induction, 2-5% Isoflurane mixed with oxygen is appropriate, while 0.5-2.5% is appropriate for maintenance. Recovery is extremely rapid if isoflurane is used alone. This makes it quite a desirable anesthetic for simple procedures. There is little risk of overdose with isoflurane when used this way. An active draw anesthetic machine should be used for rodents. The flow rate of oxygen should be set at 1 liter/min, while the amount of isoflurane can be controlled by the knob on top of the vaporizer.
Although a single dose of an injectable anesthetic is typically sufficient for anesthesia in rodents for surgeries lasting up to 60 minutes, Isoflurane is a good emergency anesthetic should the animal emerge from anesthesia near the end of the surgery. It is undesirable to give extra injectable anesthetics near the end of a surgery, given that an animal can overdose easily, and this will prolong recovery. Isoflurane is a simple and quick alternative.

General Techniques

Aseptic surgery and the sterile field Minimizing the risk of infection required the proper implementation of aseptic technique and the maintenance of a sterile field during the surgery. Anything in surgical area can harbor microorganisms, and should be cleaned with a disinfectant or sterilized with an autoclave. Anything not sterilized should be considered non-sterile. Two major sources of microorganisms during the surgery are the surgeon and the animal. Since neither of you can be autoclaved, disinfectants and sterile barriers should be used. It is important to be aware that anything that enters a wound should be sterile, and that anything this object touches from the moment it is sterilized until the completion of the surgery should also be sterile. Anticipate what you will need to handle during the surgery, and make sure that it is sterile before you touch it. For instance, while surgical blades and syringe needles inside their packaging are sterile, the packaging itself is not, and therefore handling the packaging would contaminate you. For stereotaxic surgery, the sterile field can be established after the animal is in the frame, as manipulating the animal into place requires handling its body. After the animal is in place, wash your hands, put on sterile gowns, masks, hairnets and gloves. Place sterile drapes over the animal except for the area to be incised. At this point, only touch sterile items, or surfaces that have been disinfected. Have a sterile platform/area for surgical tools.

Preparing the animal. Once the animal is anaesthetized, the area of the body which will be incised must be prepared. Small animal clippers should be used to shave the area of interest. Care should be taken near sensitive tissue (i.e., ears, eyes and vibrissa). The following tips may be useful:

- Hold the stationary part of the clipper against the skin. Holding the moving cutting blade against the skin will cause abrasions.
- Hold the clippers tilted forward at about a 45° angle.
- Hold the skin taut, especially for hamsters, which have loose skin. Perform the shaving away from the surgical field. After you have finished clipping, take the animal to the surgical area and disinfect the area. Use a small gauze pad or cotton swab soaked in a disinfectant (Xenodine, Germaphine, 70% alcohol) to clean the area. Start in the center of the area and work your way outwards, scrubbing firmly. A cotton swab works well here, as you can rotate the shaft between your fingers such that the swab is rolling away from you, as you draw the whole thing towards you. This really cleans all the dirt, oils and hair clippings from the area. If the surgery is prolonged (as it is during stereotaxic surgery) the eyes should be covered with ophthalmic ointment, mineral oil or Vaseline to prevent them from drying out.

Incising Using a 25 gauge needle, inject a small amount of lidocaine (2-5%) along the incision line. Allow about a minute for this to take effect. For making the incision, a #10 blade would be most appropriate for most small animal surgery. A #11 blade may be useful for abdominal surgery, but the cutting technique would be different than that described here (although the present technique would work for abdominal surgery as well). To make an incision, hold the scalpel like a pencil. Pull the skin taut and press the scalpel through the skin (a slight rocking motion of the blade may help). When you are through the epidermis, the skin can be grasped with your free hand immediately adjacent to either side of the blade. Draw these fingers away from the cutting edge, pulling them apart as you go, in a “Y” like motion, where the blade is at the intersection of the “Y” and the path of your fingers starts at the intersection and follows either arm of the “Y”. The stem of the “Y” represents the path the incision will follow. This technique is a little tricky, and a simple incision where the blade is drawn towards you will suffice. For this technique, it is best to use the ring finger on the hand holding the scalpel, and the index finger on the non-dominant hand to hold the skin taut as the blade is drawn towards you. The incision should be performed in a single smooth motion so that the incision has a smooth edge.

Suturing There are various options available to the surgeon when it comes to close the wound, each option having advantages and disadvantages in various situations. The classic technique involves the use of a suture needle and thread, although metal wound clips and super-glue are becoming more popular. There are many types of needles available, in different lengths, diameters and curvatures. They differ also in how they cut skin. Atraumatic needles have a point which resembles a classic sewing needle and have a circular cross-section, whereas the traumatic needle has a triangle cross-section with cutting edges along the shaft of the needle in addition to its point. The atraumatic needle is used for soft tissue (e.g., the intestinal wall), while the traumatic needle is used for tough tissue (e.g., skin). Needles are either supplied with an eye through which to thread the suture material, or with pre-bonded suture material. The advantage of the pre-bonded suture material is that the hole created in the tissue is smaller than with the treaded needle. Suture material comes in various thicknesses. Size is indicated by a number, with a smaller initial number indicating a thicker suture material. 3-0 to 5-0 is a good size for rodents. There are various types of material which can be used. The synthetic absorbable suture material is known as vicryl. This material should be used for sutures which are to left inside the animal, such as suturing the peritoneal wall. Non-absorbable sutures are either made of silk, or some synthetic material such as polypropylene (e.g., Prolene), or nylon (e.g., Ethilon). Silk comes as either twisted or braided. The twisted silk appears to be the most supple of all the non-absorbable suture material, although unbraided seems better for wound healing. Generally, suture material is left in place in experimental animals, unless infection occurs. The animal will eventually groom out the sutures, but the wound would be healed by the time this occurred. If the sutures are irritating to the animal, they should be removed as soon as the wound is healed. There are many different types of suture styles. One thing which they all should have in common is being closed with a square knot (also known as a wreath-knot). To tie one by hand, you would hold a thread in each hand, pass the left thread over the right one, and twist it through the loop formed (completing on half-hitch), then pass the thread which is now on your right over the left and twist it through the loop formed (completing a second half-hitch). You will be left with the threads on each side passing out of the loop side by side. A third half-hitch can be made by repeating the first step. This will strengthen the knot. This is not how we will tie the knots, but you can do this to understand how the thread will lie. Ideally, this technique should be completed without touching the needle or thread with your hands, but when learning, the animal will appreciate speed rather than “proper technique”, provided that you are fairly clean when doing the suturing. It takes practice to master the proper technique, so don’t expect to master it on your first try. To make a simple knot, you should grasp the threaded needle by the shaft with a pair of hemostats or needle drivers. Make sure that they are locked closed, as this will make it easier for you to manipulate the needle, as you won’t have to worry about maintaining pressure on the needle while moving it. If the needle is curved, have the curve pointed upward. Using forceps, grasp either one or both edges of the wound to

hold it still while you use a flick of the wrist to force the suture needle through one or both layers. Doing both edges of the wound at the same time is faster, but you will have greater control over where the needle passes if you do one layer at a time. In some cases it is simply too hard to do both at the same time. Ideally you'll want a suture every 1½ to 2 mm along the wound, with the thread passing through the skin about 1-2 mm from the incision edge. One the needle is through both edges of the incision, grasp it with another pair of hemostats or needle drivers, and disengage the first pair. Draw the needle and suture material through the skin until only about 4 to 5 cm of thread remain on the side of entry. If the needle is a threaded rather than a bonded kind, clamp both the thread entering and leaving the eye with the needle drivers. If you were to hold the needle, any attempt to manipulate the suture material would result in you simply sliding the needle off of the suture material. The suture material should now be head in hemostats in your non-dominant hand. Hold the other pair of hemostats in your dominant hand horizontal over the animal, perpendicular to the wound. Pass the suture material from proximal edge of the free-hemostats over top of it down and around the distal edge. Then use the free hemostats to grasp the free end of the suture material, pass the loop of suture material off of the end of the hemostats and cross your arms to tighten the knot. Let go of the short end of the suture material, and hold the free hemostats (located in you dominant hand) horizontal gain, and this time reverse the direction of the suture material, starting with the distal edge, over top, and under the proximal edge. Again grasp the free end of the suture material, and pass the loop over the end of the hemostats. This time, pull your hands apart, each to its respective side. Repeat the initial step to create a third throw, thereby strengthening the knot. The excess suture material should be cut down to 3-5 mm in length. Too long and there will be too much for the animal to catch and rip, while too little could lead to the knot unraveling. Alternatives exist to this simple knot. A continuous stitch is completed more rapidly, but is inappropriate if an implanted cannula is emerging from the skin. One major disadvantage of the continuous stitch is that if it is broken or torn, the whole stitch will unravel, while an interrupted stitch would only lose the stitch that was damaged. In some cases, sutures are not appropriate, and wound clips should be used. Rodents will chew on any visible thread and can easily re-open a fresh wound. This is an issue for many location, such as abdominal surgery. In this case, wound clips would be best. The two edges of an incision are held together with special forceps and the clip is applied with a special tool that bends the metal and causes it to bite into the tissue. These clips should be removed (again, with a special tool) after one week. For mice, thread or clips may cause too much tissue damage. Their skin is very thin and fragile. Cyanoacrylic glue is the most efficient option. The two edges of the incision are brought close together and a thin bead of glue is run along the wound. The glue dries quite quickly, and care must be taken not to glue forceps to the animal. In addition, glue should be used sparingly to prevent it from running into the eyes, ears or other vital areas.

Post operative care A quiet area should be available for recovery. This area should be warm, and easily accessible to watch the animal. Depending on how invasive the surgery, you may want to leave the animal in recovery for a couple days so that you can monitor for infection and quality of recovery. An animal emerging from an anesthetic will have an impaired ability to thermoregulate due to loss of muscle tone. There are many ways to keep the animal warm. We typically place its recovery cage near a heat lamp during surgery so that it is already warm when the animal is placed in the cage. Rather than leaving the directly under the heat lamp when the animal is recovery, which may lead to the animal overheating, the cage is placed to one side, such that it is kept warm. Wrapping the animal in paper towel, like a burrito, will help it remain warm, much like a blanket would keep you warm at night. Place some food pellets into the recovery cage so that the animal does not have to stand to eat, a task that can be difficult while under the influence of a barbiturate or opiate. For surgeries that are very invasive, a palatable, high calorie mash can be provided. Use equal parts sucrose and ground rodent chow, mixed with vegetable oil until it has the consistency of cookie dough. Animals should be monitored regularly (hourly) until they are conscious, and then periodically (daily) until they are fully recovered. Analgesics should be given based on the dosing regimen for the specific drug that is being given, typically every 6-12 hours until recovery is complete. Animals should be monitored for any sign of pain (decreased appetite, lethargy, aggressiveness) or infection. Confirmation of urination and defecation should also be observed.

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The stereotaxic frame is designed to hold the animal's head perfectly still, and in a reproducible way such that a brain target can be reliable located in 3-D space with reference to some measurable location. The frame allows for manipulation of the probe in an anterior-posterior direction, lateral direction and dorsal-ventral direction. An implant can be placed on an angle from vertical, and the head can be tilted up or down from. Co-ordinates should be reported with reference to some structure, nose bar level, and angle of implant. All will affect the accuracy of the surgery. Typically, bregma is used as an initial landmark on the skull. The skull of a rodent is formed by four bone plates, each separated by sutures (interface of the edges of two bone plates.) There are two parietal bone plates, separated by the sagittal suture, a frontal plate and an occipital plate. Bregma is the point at which the frontal plate meets the anterior edges of the parietal plates along the sagittal suture (i.e., where the coronal suture meets the sagittal suture). The intersection of the sagittal suture with the suture along the anterior edge of the occipital plate (lamdoidal suture) is known as lambda. Areas of the brain are typically defined as being x mm anterior/posterior to bregma, and y mm lateral to midline. For depth, the target is either z mm below the skull surface or dura. Dura is the more accurate of the two, as skull thickness is more variable than the size of the brain. Care must be taken not to damage dura when drilling through the skull. If dura is destroyed, skull surface can be used, and the target depth can be modified by 1 to 1.5 mm for rats, and 0.6 to 1 mm for hamsters. These numbers will depend on the age of the animal and the site of the burr hole. These adjustments should be modified based on experience. A surgeon should be able to collect data from surgeries in which dura was not damaged, and thus determine the mean thickness of the skull in a given cohort of animals at a certain site. Alternative reference points are lambda, interaural zero and a removable zero bar. In the latter two cases, the head of the animal must be perfectly centered relative to the frame. If you are going to use interaural zero as a reference, this must be measure before the surgery. Place the ear bars close together and at the same lateral position and then p-lace your probe precisely between the tips of the ear bars. The position of the nose/tooth bar should be specified (x mm above or below interaural level). The fulcrum of the head in the stereotaxic frame is around the interaural line, and the angle of the head is determined by the position of the nose bar. Typically the head is leveled, that is, it is positioned such that bregma and lambda are at the same dorsal/ventral position with reference to the frame. This position is typically 2 mm below the interaural level for hamsters and 3.3 mm below the interaural level for rats. Always report whether you leveled the head, or if not, at which level the nose bar was set. Stereotaxic co-ordinates are meaningless without information on the tilt of the head. When doing stereotaxic surgery, it is often handy to have a riser of some sort for smaller animals. We use a 1 cm tall block wrapped in paper towel for hamsters. Towel should be place under a rat so that its body is not in contact with the metal base of the frame. A heating pad that can fit inside the stereotaxic frame would be even better than paper towels, as it is even better as it actively keeps the animal warm.

Vernier Scale Precise measurements are accomplished with the stereotaxic frame by the use of Vernier scales that allow for readings as precise as 0.1 mm to be made. One side of the scale (located on the moving bar in each case) is marked off in centimeters (numbered) and millimeters (unnumbered, but marked). Most co-ordinates for rodents are reported in millimeters, and thus you should record your measurements this way (e.g., 4.25 cm = 42.5 mm). There is a second scale opposite the first and attached to the non-moving aspect of each scale. This second scale is divided into 10 equal units, and the whole scale spans 9 mm.
To read the Vernier scale, locate the zero on the small scale. Determine which mm division on the large scale it is in front of. For example, if it is between the third and fourth small division after the 4 cm mark, this would be 4.3 cm or, as you will record, 43 mm. To determine the fraction of a millimeter for the given measurement, the number will come from the small scale. It will be the number of the line **on the small scale** that lines up perfectly with a line on the large scale. This is very important. The tens and ones measurements are determined by the position of the small scale's zero, and are read from the large scale. The tenths position is determined by finding a line on the small scale which lines up perfectly with a line on the large scale, and **is read from the small scale**. There currently is a web site with a java applet which simulates a Vernier scale. I highly recommend practicing reading the Vernier scale, as misreading it will lead to a misplace surgery. The URL is:
http://www.upscale.utoronto.ca/PVB/Harrison/Vernier/Vernier.html

Ear Bars The first step in stereotaxic surgery, once the animal has been prepared, is to place it in the ear bars. This is probably the hardest step in stereotaxic surgery, and also one of the most important steps. If the animal is not place in the ear bars correctly, the co-ordinates will not be accurate. **Proper and repeatable placement is essential for accurate stereotaxic surgery.**
There are two main types of ear bars for rat or hamster surgery; the standard rat ear bars Kopf model # 957), and the atraumatic ear bars (either Kopf model # 951, or # 955). The standard ear bars are quite pointy, and are the easiest to place in the ears reliably. Unfortunately, they frequently damage the tympanic membrane and the bones of the middle and inner ear. This can lead to some balance disturbances or middle ear infections. The ear bar should be disinfected between each use in case damage is done, so that infectious agents are not introduced to the middle ear. The atraumatic ear bars come in a variety of forms, either similar to the standard, yet more blunt, or tipped with a wide point. These ear bars are less likely to cause damage, yet they are error prone. It is substantially more difficult to place the animal accurately and reliably in these ear bars, and the head is prone to slipping out of them. Essentially, you should gauge the needs of the surgery at hand. If the need is simply to hold the head still so that a platform can be fixed to the skull, the atraumatic ear bars are best. If precision is essential, the standard ear bars can be used with extreme care to avoid damaging the tympanic membrane. Placing the animal in the ear bars is difficult. It is difficult to see what is being done. Essentially, the left ear bar should be fixed in place at its anticipated final position. Holding the animal in your right hand, place it such that the tip of the bar is inside the ear canal opening. Reach around with your free hand and grasp the head, thumb on top, index and middle fingers on the lower jaw. Lay the body down so as to free your right hand of its bulk and work only with the head. Holding the head level, it should be drawn up and back slightly, allowing the tip to fit into the ear canal which goes into the skull of the rodent on a downward and forward angle. When this bar is in place, rotate the nose ever so slightly towards the left side of the frame, this will place the free ear canal at an angle such that the second ear bar can clear the external auditory meatus and slide easily into place in the ear canal. As then ear bar in inserted past the outer ear, rotate the nose back to midline. This procedure for the second ear is most easily accomplished if the second bar is just loose enough to slide horizontally without much vertical movement.
When placed correctly, the head should be able to swing up and down quite easily, and there should be no lateral movement of the nose. However, a little lateral movement may merely indicate that the ear bars are not in far enough, rather than indicating that you have missed. Each of the ear bars should be close to the same lateral position (as measured by their individual scales), although this is only necessary if an external zero point is being used, such as a removable zero point.

Nose / Incisor Bar The nose bar help stabilize the skull. It is important that the skull be held firmly in place for two reasons. First, a loose head moves, and it is impossible to measure coordinates accurately on a moving head. Second, a moving head will vibrate more in the ear bars and nose bar. If this occurs, there is the risk of unnecessary damage to the animal, in the form of ear trauma or damage to the upper palate and teeth. Do not worry about cutting air flow by clamping down too hard with the bar over the nose, as the nasal cavity is within the skull, not between the skull and the skin. However, do not tighten it so hard that there will be tissue and bone trauma. You should not be able to slide a thin spatula between the bar and the animal's snout. To place an animal in the tooth / nose bar (Kopf model #920), first correctly place it in the ear bars. Using a spatula, open the mouth but gently pushing down on the lower jaw. Slide the tooth bar forward until it is just under the top incisors. Rest the top incisors on the tooth bar, and press the lower jaw and tongue down. With this done, it should be possible to slide the tooth bar between the upper and lower incisors. Carefully push the tooth bar into the mouth and let the top incisors fall through the hole in the tooth bar, then gently push the tooth bar away from the animal until the inside edge of the tooth bar hole touches the inside surface of the top incisors. Without letting go of the tooth bar at this point, tighten the tooth bar so that it no longer moves back and forth. If you let go first, it will move forward from the weight of the animals head, and will not end up in the correct location, potentially altering the tilt of the head, and thus invalidating your surgery coordinates. A note of caution, be careful moving the tooth bar into or out of the mouth. The incisors must be clear, or you will break them. Always make slow controlled movement and watch what you are doing. One the animals head is fixed in place, establish your sterile field and prep both yourself and the incision area for surgery. This includes washing your hands, donning sterile surgical gear (hair and face mask, gown, gloves), draping the animal in sterile drapes and disinfecting the surgical site. Draping the animal is challenging when it is in a stereotaxic frame, so consult your veterinarian for advice on an acceptable and appropriate technique.

Implantation

Using the stereotaxic frame, it is possible to place an implant in a precise location in the brain. An excellent protocol with video is available (Geiger et al., 2008; http://www.jove.com/index/Details.stp?ID=880). An excellent guide is also available (Cooley and Vanderwolf, 1990). A variety of implants are used in behavioral neuroscience. The two most common would be an electrode and a cannula. A cannula is a tube which allows material to be removed from, or delivered to, a specific area of the body. For neuroscience research, an indwelling cannula can be positioned such that a very small amount of drug can be delivered to a discrete region of the brain. Commercially available cannulas and electrodes are available from Plastics One (Roanoke, VA). There are three components to a cannula. First, there is the implanted guide cannula. Typically, this should be implanted such that its tip is some distance from the target structure (we typically use 1 mm). A dummy cannula or dummy cannula is placed in the guide cannula after surgery and between injections. This prevents fluid from seeping into and thereby clogging the guide cannula. The third component is the injection cannula, which has a collar to prevent it from proceeding too far into the brain. Both the dummy and injection cannula obtrude from the end of the guide cannula by 1 mm. This is essential, as if they were to end flush with the guide, it is likely that the fluid would flow back up the cannula. If they were to obtrude by more than 1 mm, a small bend or kink in the injection cannula could lead to it missing its target. The dummy cannula should be the same length as the injection cannula, so that tissue near the target structure is not damaged for the first time when doing your experimental injection. Before placing the cannula in the holder on the stereotaxic frame, place a small stylet (wire with a hook) into the cannula to fill the space inside the cannula during surgery. Cannulas may need to be cut to length before the surgery, and can have their tips beveled to aid passage through the brain tissue. Electrodes may need preparation prior to implantation. Insulation may need to be removed, and the tips may need to be separated. They may also need to be cut to length, in which case the insulation should be examined to ensure that it has not cracked. Once the animal is placed in the frame, make an incision (see above). The fascia (connective tissue) can be blunt-dissected with cotton swabs. Place two swabs close together, press down firmly and pull them apart, laterally a few times, and rostral / caudal a few times. The skin can be retracted with hemostats (rats) or bulldog seraphines (hamsters) by clamping them onto the fascia. Avoid clamping the muscle or the skin, as this will produce extraneous tissue damage and impair healing of the wound. For indwelling electrodes and cannulas, room must be created on the skull for the head cap that will be created. This is very important when the subject is a hamster, as they have quite small skulls compared to a rat. This can be done with a metal spatula, or the soft shaft of a sterile cotton swab. Start along midline at the anterior end of the incision and move laterally until you catch the edge of the muscle. With firm controlled pressure, ease the muscle off of the skull. Work your way toward the posterior end of the incision. Only move what you need to, as this muscle is what the animal uses to raise its lower jaw. It is attached vertically at the caudal portion of the skull, so the animal will still be able to eat, even with this removed, but there is no sense in making it any more difficult for the animal than necessary. This may not be necessary for rats, depending on how medially the implant will be placed. Once the incision is retracted, and the muscle prepared, dry the skull with a cotton swab that has been dipped in 70% alcohol. This will dry the skull quite quickly, making the suture extremely easy to locate. Use a metal spatula to quickly and firmly scrap any bleeding points on the skull. This will clog the small openings with bone, and stop the flow of blood. It is essential that the skull be clean and dry. Mount your implant in the holder on the stereotaxic arm. Manipulate the arms to place the tip of your implant on bregma (if that is your landmark). This is often easier to do with the aid of a small jeweler's magnifying glass or stereomicroscope. Record its location, and calculate your target. Raise the implant up sufficiently far so that it will clear the skull (1 or 2 mm), and move the cannula to its A/P and lateral targets (a burr hole must be drilled before the D/V coordinate can be reached). Record the D/V coordinate of the skull, even if dura is the landmark for the D/V coordinate. Raise the implant up a bit and make a mark on the skull with a pencil or a fine tipped waterproof marker. Raise the implant up high enough so that it will clear the edge of the wound and move it laterally to get the horizontal bar of the frame out of your way. The head cap will be secured to the skull by placing 4 jeweler's screws into the skull. The heads of these screws will be imbedded into the dental acrylic. Mark 4 point for the screws, and ensure that they are sufficiently far apart from one another and the implant target such that each screw head will not touch another or the implant. If possible, have screws to either side of the implant, both in front and behind. Do not place a screw on a suture or near midline. There is a blood vessel known as the sagittal sinus directly below the sagittal suture. If this sinus is damaged, there will be a profuse amount of bleeding. Using a drill bit slightly smaller than the screws, drill a tiny hole for each screw. Angling the burr holes towards the center of the brain will give the final head cap more stability than if the holes are precisely vertical and parallel. Before your first attempt to drill, practice touching the skull with the drill while it is not turned on. Become aware of the sensation of touching the skull. Try raising it and lowering it a few times, making very small controlled movements. Repeat this with your eyes closed, and really concentrate on the feedback. This is the sensation that you will try to maintain during drilling. Practice small movement, as the skull is only about 1 mm thick. When drilling, press down only hard enough to maintain that sensation, and when you feel it give a little, withdraw the drill.

To drill, hold the drill in your dominant hand and support your wrist of hand with your other hand. Each hole should be drilled so that it is tilted slightly towards the center in such a way that all the screws point in separate directions. This will make it more difficult for them to come out. For hamsters and rats, there will be two stiff points in the drilling, one at the outer surface of the skull, and one at the inner surface of the skull. There is a soft section of bone between these two layers. You may feel the drill give even when you have not completed the hole. This sensation would be from entering this soft layer of the skull. Before placing the screw, ensure that you are through both layers. This can be done by probing the burr hole with the drill when it is not turned on. If minimizing damage to the cortex is critical to your surgery, shallow holes and screws can be used that do not completely pass through the skull. The head cap will be weaker and may come out, but the cortex will be intact. As you have a choice of where the screws will go, but don't have a choice as to the location of the burr hole for the implant, it is recommended that the screw holes be drilled first, in case the cannula hole must be near a location which is prone to bleeding. Should you get a lot of bleeding when drilling the burr hole, you will at least have already completed the task of implanting the screws. Once all four screw-holes are drilled, the screws can be placed in the holes using a small pair of curved forceps and a small jeweler's screwdriver. The head should not be flush with the skull, as it needs to be embedded in the dental acrylic. However, the majority of the shaft should be in the skull, as the further it is into the skull, the better it will hold. To drill the implant burr hole, lower the drill until it touches the skull. Then, without much further downward pressure, work in a circular motion, carving a hole sufficiently large to accommodate the implant without having it touch the sides at all. This fine drilling is often accomplished more easily with the aid of a small jeweler's magnifying glass or stereomicroscope. Carve away the bone until you can see the dura. When the burr hole is drilled, return the implant to it lateral and A/P coordinates, and assess whether or not it will fit into the hole without touching the sides. It is essential that it not touch the sides, as this will cause a small deflection, which translates into an increasingly large error the deeper the implant is inserted. Once it has been determined that the implant fits freely into the burr hole, lower it down into the hole until it makes contact with dura. A small depression in dura will be observed. Stop here, and record the DV coordinate if dura is being used as a landmark for depth. Prior to inserting the implant to its D/V coordinate, raise the implant up a bit and puncture dura with a sterile sharp needle. This is necessary as dura is quite tough. If it were not punctured, it would stretch and break and cause a lot of trauma in the process. If a fine electrode were being implanted, it is more likely that the electrode would bend rather than dura break, which may ruin the electrode, and would most certainly ruin your coordinates that were based on a straight electrode. Once dura has been punctured, stop any bleeding with cotton swabs. Dry the skull and clean it of blood and bone chips. This is essential to ensure that the dental acrylic forms a good firm bond with the skull. Proper clearing of bone chips is also essential for the animal's comfort, as if these remain in the wound, they will irritate the animal. After this, lower the implant to its target slowly (a speed of 1mm per 10 seconds is good). Do not lower it too fast or you may overshoot your target. Once the implant is in place, clean the skull again, being careful not to touch the implant. A Kim-wipe, twisted to a point between your gloved hands, can be used to soak up any fluid under the screws and implant. The dental acrylic (Dentsply repair caulk) should be applied in three coats. The first coat should be quite runny, and must be applied quickly. Mix the acrylic with its solvent in a Petri dish. Do not mix the acrylic much (tilting the dish a couple of times is sufficient) as this will start the curing process, making the acrylic more difficult to work with. Using the pointy end of a metal spatula, apply the acrylic generously to the area between the screws and cannula. This runny coat will fill all the crevices on the skull, and form a solid hold. Shape it with the long edge of the spatula, scooping it towards the center of the skull and away from the tissue. Allow it to cure a little bit to make this task easier. Allow it to dry such that it will not dent when poked with the spatula. Once it is dry, apply a larger, less runny coat. This coat will form the body of the head cap and should come up the sides of the cannula about half way. Ensure that the holder is not embedded in the acrylic, or you will be unable to remove it at the end of the surgery. Also ensure that enough of the implant is above the acrylic such that the dummy cannula or dust cap may still be threaded all the way onto the guide cannula. When this coat is dry, remove the implant-holder. This will give you better access to the head cap for the final coat. This final coat should be extremely runny (mostly solvent). It should be dabbed on in small amounts where the head cap is rough. It should not be shaped, as this will create more rough edges. The purpose of this coat is to smooth out the head cap so that it will not irritate the animal. A rough spot will cause such irritation and the animal will groom at the spot, possibly opening the wound and introducing infection. While the final coat is drying, the seraphine clips or hemostats holding the wound open can be removed. The tissue can be

rehydrated with generous amounts of saline. A 5% lidocaine solution can be applied to the skin to minimize pain during suturing. When the head cap is dry, the wound can be suture (as described above) with sutures both rostral and caudal to the head cap. Ensure that the wound is loose enough around the head cap that should it come detached from the skull, that it will be able to fall out of the head, rather than be held in the head in by the skin. If it can not come out when detached, the implant would move around inside the brain, causing massive damage to the central nervous system. Prior to removing the animal from the ear bars, remove the stylet inserted during surgery, and replace it with the proper dummy cannula for cannula implants, and simply attach a dust cap for electrode implants. Loosen the nose bar, and carefully remove the tooth bar. Then, while holding the scruff off the animal, loosen and remove one ear bar. This is sufficient to allow you to remove the animal. Wrap it in paper towel to aid in thermoregulation and place it in a warm cage near a heat lamp. When it has recovered enough to be mobile, give it an injection of an opiate, such as buprenorphine. Follow-up with proper post-operative care and monitoring.

Devascularization Lesion of the Forelimb Area

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The Devascularization Lesion, or pial strip, is a simple model of stroke.  The procedure for producing a stroke of the forelimb portion of the motor cortex is as follows:
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- Anaesthetize the rat.
- Shave the top of the rat's head.
- Place the rat in the stereotaxic frame.
- Disinfect the scalp.
- Inject lidocaine along the incision line
- Incise the scalp along midline, and retract the tissue.
- Mark an area of the skull on the side contralateral to the preferred forepaw. This area will run from 4 mm anterior to bregma to 1 mm posterior to bregma and from 1–4 mm lateral to midline.
- Using a dental drill, shave down the skull in the area marked. Since dura is adhered to the inside of the skull, simply cutting out a bone flap would rip dura and lead to a lot of bleeding. Carefully shaving away the skull minimizes the damage, and makes it easier to see what you're doing.
- Use a fine needle (26-30g) and bend the tip to about 60 degrees. Use this to puncture dura in an area with little vascularization. Next, use some fine forceps to lift dura at the puncture. Then, using fine spring scissors, carefully remove the dura exposed by the bone-window.
- With a sterile saline-soaked cotton swab, gently rub away the vasculature over the cortex.
- Fill the bone window with sterile, saline-soaked, gelfoam to protect the brain from further damage.
- Close the incision with sutures or wound clips.
- Place the rat in a proper recovery location and provide appropriate analgesics and post-operative care.

Stereotaxic Injection – 6OHDA

Neurotoxic lesions are useful models for understanding how specific brain regions regulate behavior. Neurotoxic lesions allow you to eliminate specific cell-types, or to lesion cell bodies but leave fibers of passage intact. The following section will describe the procedure for producing a lesion of dopaminergic cell fibers in the striatum as a model of Parkinson's Disease. This lesion protocol is based on that described by Ben et al. (1999). Prepare the neurotoxin 6-hydroxydopamine (6-OHDA) fresh just before the surgery. Prepare it to a concentration of 4 µg (salt weight) / µl in sterile physiological saline containing 1% ascorbic acid. Four 2 µl infusions will be performed, so you will need at least 8 µl. Control rats should receive infusions of 2 µl of physiological saline containing 1% ascorbic acid at the same coordinates (sham lesion). Unilateral lesions can be used if the deficits produced by the bilateral lesions are too severe to permit behavioral testing. See the "Implantation" section above for more details on stereotaxic surgery.

- Anaesthetize the rat.
- Shave the top of the rat's head.
- Place the rat in the stereotaxic frame.
- Disinfect the scalp.
- Incise the scalp along midline, and retract the tissue.
- Clean the skull so that bregma can be visualized.
- Mount a Hamilton microsyringe in the stereotaxic frame using a microsyringe holder.
- Manipulate the stereotaxic arms to place the tip of the microsyringe over bregma, and not the coordinates for bregma.
- Injections will be placed at the following locations relative to bregma and skull surface: | | Site 1 | Site 2 | Site 3 | Site 4| |---|---|---|---|---| |AP| +1.7 mm |-0.92 mm |+1.7 mm| -0.92 mm| |ML| +2.8 mm |+4.0 mm |-2.8 mm |-4.0 mm| |DV| -5.6 mm |-5.5 mm |-5.6 mm |-5.5 mm|
- Mark the four locations on the skull and using a dental drill make 4 small burr holes large enough to accommodate your microsyringe.
- Using a small sterile syringe tip, puncture dura to facilitate entry of the microsyringe.
- Slowly lower your microsyringe into position, descending ventrally at about 5mm/min.
- Infuse at a rate of 0.5 µl/min. When each infusion is in complete, leave the microsyringe in place for an additional minute to aid infusate diffusion.
- Fill the burr holes with sterile bone wax.
- Hydrate the incision tissue with sterile saline containing 5% lidocaine.
- Close the incision with sutures or wound clips.
- Place the rat in a proper recovery location and provide appropriate analgesics and post-operative care. Depending on the severity of the lesion, the animal may have pronounced motor impairments, so careful monitoring of eating and drinking is critical for the days following the lesion.

References:

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