

A - 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 $\pm 5g$ 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 rat's 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 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 intradermal 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 \(2nd Ed\)](#)