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Article *in* Journal of Visualized Experiments · October 2012

Impact Factor: 1.33 · DOI: 10.3791/2771 · Source: PubMed

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Manual Restraint and Common Compound Administration Routes in Mice and Rats

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URL: http://www.jove.com/video/2771
DOI: doi:10.3791/2771

Keywords: Basic Protocols, Issue 67, Anatomy, Medicine, Rodents, training, handling, restraint, injections, oral gavage

Date Published: 9/26/2012

Citation: Machholz, E., Mulder, G., Ruiz, C., Corning, B.F., Pritchett-Corning, K.R. Manual Restraint and Common Compound Administration Routes in Mice and Rats. *J. Vis. Exp.* (67), e2771, doi:10.3791/2771 (2012).

Abstract

Being able to safely and effectively restrain mice and rats is an important part of conducting research. Working confidently and humanely with mice and rats requires a basic competency in handling and restraint methods. This article will present the basic principles required to safely handle animals. One-handed, two-handed, and restraint with specially designed restraint objects will be illustrated. Often, another part of the research or testing use of animals is the effective administration of compounds to mice and rats. Although there are a large number of possible administration routes (limited only by the size and organs of the animal), most are not used regularly in research. This video will illustrate several of the more common routes, including intravenous, intramuscular, subcutaneous, and oral gavage. The goal of this article is to expose a viewer unfamiliar with these techniques to basic restraint and substance administration routes. This video does not replace required hands-on training at your facility, but is meant to augment and supplement that training.

Video Link

The video component of this article can be found at http://www.jove.com/video/2771/

Protocol

1. Safe Restraint and Gentle Handling of Animals is a Key Part of Experimental Procedures

1. This video is designed to be a supplement to hands-on training provided by your institution.
2. Always be sure that IACUC or ethics committee approval is in place before beginning any experimental procedure.
3. Each person working on a protocol should know the details of procedures approved for that protocol, and any others on which they are working.
4. Approach the rodent with confidence and handle the animals gently, but firmly. Both too- rough handling and tentative approaches may result in bites or scratches to the handler or injuries to the animal.
5. When handling animals, there is always the possibility of accidental release or the animal being dropped. Most of these manipulations are best performed over a work surface so that if the animal is dropped or escapes, it is not injured and can be easily recaptured. Follow your institutional policies concerning animals that contact the floor.
6. Never handle animals by the tip of the tail, as this may result in a degloving injury of the tail. Be especially careful with large rats or pregnant mice. Always use the other hand to support the body as you lift by the tail.
7. Sharp needles work best when giving injections. Although needles for laboratory rodents are sometimes used for multiple injections, this is not advised for a number of reasons, the least of which is that the small gauge often used means that the needles dull quickly.
8. Being bitten or scratched is always a possibility when working with animals. If working with a substance or an infectious agent that may cause injuries to humans, take extra precautions, such manipulating animals or agents in fume hoods or biosafety cabinets.
9. Gentle approaches and acclimation to handling before attempting a procedure can pay off in animals that are less stressed by handling.
10. Practice restraint before attempting compound administration, and practice administering substances to control animals before experimental animals.
11. Practicing these techniques regularly instills confidence and confidence results in better handling, less stressed animals, and better scientific results.
12. With any handling technique, if the animal is recalcitrant, try a different technique. The animal (and handler) may also benefit from putting the animal back in the cage and trying again later.

2. Manual Restraint

1. One-handed mouse restraint

1. Lift a mouse by the base of the tail and place it on the cage lid, wire bar cage top, or a similar rough surface.
    1. One handed mouse restraint is usually performed with the non-dominant hand, leaving the dominant hand free for use.
    2. An alternative method allows the technician to use their lab coat or uniform sleeve covering the forearm to position the animal prior to restraint.

2. Tuck the base of the tail between the 3rd and 4th finger, while gently pulling back on the tail. This will cause the mouse to grasp the surface with all four paws and pull forward.
    1. Do not grasp mice by the tip of the tail, especially if suspending their entire bodyweight by their tail. This can cause a degloving injury in which the skin of the tail slips off.

3. Next, firmly grasp the mouse by the scruff with the same hand that is holding the tail. Grasp with the index finger and thumb near the base of the head and extend the grasp down the mouse's back by incorporating the middle and ring fingers.
    1. Be sure to apply just enough pressure, or firmness, to the skin around the neck to prevent the mouse from turning or twisting out of the restraint, but do not pull the skin so tightly that the animal cannot breathe.
    2. Control of the head is crucial. If the mouse can move its head, it can reach the handler's fingers and may bite. This may occur when novice handlers grasp the mouse too far down the back, rather than right behind the skull.

2. Mouse restraint two-handed
    1. Lift a mouse by the base of the tail and place on the cage lid, wire bar lid, or rough surface.
        1. An alternative method allows the technician to use their lab coat or uniform sleeve covering the forearm to position the animal prior to restraint.

    2. Pull gently backwards on the tail and the mouse will grasp the surface with four paws and pull forward.
    3. Next, with the other hand quickly and firmly grasp the mouse by the scruff of the neck (see one handed restraint above).
    4. With the tail in one hand and the scruff in the other, lift the mouse and tuck the base of the tail between the palm and the 3rd or 4th finger of the hand holding the scruff.
        1. As with the one-handed method, firmly grasp the scruff to prevent the mouse from twisting or turning while not grasping so firmly that the animal cannot breathe.
        2. If the mouse is resistant to scruffing, gentle pressure on the mouse's back can allow the hand to move up for a better grasp.

3. Rat restraint; scruffing
    1. Rat scruffing is generally performed two-handed and only in smaller rats. It is not a commonly used technique because rats are less accepting of scruffing than mice, but it is useful in some blood collection situations.
    2. Grasp the rat by the tail with the non-dominant hand and pull gently backwards on a rough surface (as described above for mice).
        1. Be careful to grasp near the base of the tail, as the rat's tail skin can come off if grasped near the tip.

    3. Hold the tail firmly in the hand and approach the scruff of the rat from the rear.
        1. For example, if the rat's tail is in the handlers' left hand, do not approach the rat from the nose to scruff it with the right hand. Instead, reach over the left hand, and approach the scruff from behind.

    4. Apply gentle pressure to the back of the rat, over the shoulder blades, then grasp the scruff close to the base of the skull between the fingers and the palm of the hand.
    5. Control of the head is important to prevent bites. Rat bites can cause serious injury.
    6. Rats may vocalize when restrained in this fashion.

4. Rat restraint; over the shoulder grip
    1. Grasp the rat by the tail with the dominant hand and pull gently backwards on a rough surface (as described above for mice).
        1. An alternative method allows the technician to use their lab coat or uniform sleeve covering the forearm to position the animal prior to restraint.
        2. Be careful to grasp near the base of the tail, as the rat's tail skin can come off if grasped near the tip.

    2. Place the non-dominant hand over the rat's back, approaching from the rear.
    3. Grasp the rat around the thorax with the ring finger, pinkie, and thumb. The rat's head should be between the index and middle fingers.
        1. Do not compress the thorax.

    4. The rat can be held in this manner with one hand, if the body is stabilized against the handler.

5. Rat restraint; under the shoulders grip
    1. Grasp the rat by the tail with the dominant hand and pull gently backwards on a rough surface (as described above for mice).
        1. An alternative method allows the technician to use their lab coat or uniform sleeve covering the forearm to position the animal prior to restraint.
        2. Be careful to grasp near the base of the tail, as the rat's tail skin can come off if grasped near the tip.

    2. Place the non-dominant hand over the rat's back, approaching from the rear.
    3. Grasp the rat around the thorax, right under the shoulder blades. The rat's forearms should be gently pushed up with the thumb and index finger.
        1. The forearms should cross under the rat's chin, preventing it from biting.
        2. Do not compress the thorax.

4. The rat can be held in this manner with one hand, if the body is stabilized against the handler.

6. Decapicone
    1. A Decapicone is a flexible, cone-shaped piece of thin plastic with a hole in one end. The hole is small enough so the mouse or rat can get its nose out of the hole, but not the rest of the body.
    2. To restrain the animal, place the mouse or rat in a Decapicone of proper size.
    3. Push the animal forward until its nose protrudes from the hole in the Decapicone.
    4. Either hold the bag closed around the tail, or use a twist tie to seal the animal in the cone.
    5. The advantage of a Decapicone is that the thin plastic allows for injections through the material.
    6. The disadvantage is that the material does not breathe and animals can become overheated. Only keep an animal in a Decapicone for as long as it takes to perform the procedure.

7. Acrylic/rigid plastic restrainer
    1. Plastic restraint devices are particularly useful when the animal's tail must be accessed.
    2. These can be purchased commercially or made in the laboratory.
    3. The size should be appropriate for the animal to be restrained--the animal should not be able to turn around in the restraint.
    4. Place the animal in the restraint device by first gently restraining the animal, then releasing it, head-first, at the opening of the device.
        1. It may help to aim the device upward over the cage, as rodents will often scramble up into a secure structure, like a tube.
    5. Place the closure on the end of the device, being careful not to damage the animal's tail, feet, or testicles.
    6. Minimize time spent in restrainers since animals may overheat.

8. Animals may be restrained in other ways as well, such as by wrapping in a small towel, or by simply cupping a hand over the animal. Techniques can be adjusted to meet the needs of the animal and worker. Always take care to avoid bites and scratches and secure the animal from accidental release or falls from heights.

3. Compound Administration Methods

1. This is by no means an exhaustive list and other routes are possible. This protocol seeks to illustrate the most commonly used routes. Other routes may require anesthesia of the animal and post-administration pain relief.
2. Regardless of administration method used, be sure all materials are prepared before restraining animals.
3. Aqueous materials are easier to inject than thicker materials, such as oil-based compounds. Always inject thicker compounds very slowly to avoid dislodging the needle from the syringe.
4. General needle and syringe use considerations.
    1. Always store and dispose of syringes and needles properly.
    2. If you are new to using syringes and needles, practice handling the syringe and injecting before attempting to inject an animal. Ideally, you will be able to confidently manipulate the syringe and needle with one hand, leaving the other for restraint of the animal. A steady hand minimizes needle movement which minimizes tissue damage.
    3. Needles have a point, a bevel, a shaft, and a hub. Syringes have a tip, a barrel, and a plunger (**Figure 1 a and b**).
    4. Needles are sized by gauge and length. The larger the gauge number, the smaller the needle. Small needles are very prone to dulling (from a burr forming on the tip) and should not be used to pierce multi-dose vials (**Figure 1 c**). Always choose the shortest needle that will work to administer the compound.
    5. The needle is attached to the tip of the syringe by the hub. Some syringes have locking tips. Always be sure the syringe is securely attached to the needle.
    6. Needles are best inserted into the animal with the bevel up, especially for intravenous injections.
    7. Never recap needles by hand. This is a common cause of needle stick injuries. Dispose of needles and syringes properly in labeled sharps containers. If needles must be recapped, devices are available (**Figure 1 d**).

5. Intranasal (IN)
    1. Restrain the animal as described above.
    2. Using a syringe or pipettor, place a small amount of the material to be inhaled at the nares of the animal.
    3. Watch for the material to disappear into the nares.
    4. Repeat as necessary until the desired volume has been administered.

6. Intramuscular (IM)
    1. Restrain the animal as described above. Make sure one of the animal's hind legs is free and stabilized for the injection. Restraint may take two people. If the animal can kick during the injection, muscle damage from the needle will result.
    2. The needle should be inserted perpendicular to the skin of the animal. Using an appropriately-sized syringe and needle, insert the needle approximately bevel-deep and inject the material into the animal's quadriceps (the front of the thigh) or lateral thigh muscle mass.
    3. Do not inject into the posterior muscle mass as it is possible to damage the sciatic nerve.
    4. If animals are to receive multiple IM injections, alternate legs.

7. Intraperitoneal (IP)
    1. Restrain the animal as described above.
    2. Tip the animal's nose toward the floor, exposing the abdomen for injection.
    3. Locate the animal's midline and mentally divide the abdomen into quadrants (**Figure 2**). The lower quadrants, especially the animal's lower right quadrant, are the appropriate sites for intraperitoneal injections.
        1. The lower right quadrant is chosen due to the lack of anatomically important structures.

4. Using an appropriately-sized syringe and needle, inject the material into the animal.
5. If animals are to receive repeated IP injections, alternate the site of injection.

8. Subcutaneous (SC, SQ)
    1. Restrain the animal as described above. The animal must be restrained loosely enough so that the skin may be mobilized.
    2. If animals are to be handled routinely after SC injection, do not use the scruff (nape of the neck). Instead, use the skin on the dorsal rump or the flank. If animals are to receive multiple SC injections, alternate sites of injection.
    3. Grasp the skin and gently pull it upwards, making a "tent".
        1. If performing the injection solo, insert the needle and gently tent the skin upwards with the needle to confirm that the needle is in the subcutaneous space.
    4. Using an appropriately-sized syringe and needle, insert the needle at a 30-45° angle into the tented skin, and inject the material. Inject parallel to and away from the fingers holding the skin upwards.
    5. If the injection is successful, a small swelling under the skin will be seen.
    6. After injection, apply gentle pressure to prevent backflow of the material.

9. Intradermal (ID)
    1. For intradermal injections, animals are often shaved so that the skin may be seen.
    2. Restraint of the animal for multiple intradermal injections may be difficult. In that case, chemical sedation may be necessary. The sites for ID injections are the same as those for SC.
    3. Insert an appropriately-sized needle into the skin at a 15-30° angle. The needle will not be inserted very far and the injection should meet with resistance.
        1. An alternative approach is to gently pinch the skin adjacent to the injection site and insert the needle at a very shallow angle. This is useful in mice since it prevents them from moving during the injection process.
    4. If the injection is successful, a small bleb will be seen. It will be paler than the surrounding skin.
    5. After injection, apply gentle pressure to prevent backflow of the material.

10. Intravascular (IV)
    1. The left and right lateral tail veins are the most common vascular access route used in mice and rats.
    2. Other vascular access routes are possible in mice and rats, but generally require sedation and post-injection pain relief.
    3. For a tail vein injection, restrain the animal in a Decapicone or plastic rodent restrainer.
    4. Place the animal's tail under a lamp, or on a protected warming device. This will promote vasodilation, allowing for easier injection.
        1. Do not overheat the animal.
        2. For large male rats, cleaning the skin scales off the tail may allow for better visualization of the vein. Cleaning should be gentle so the skin is not abraded.
    5. Hold the animal's tail by the tip with the non-dominant hand. This will straighten the tail.
    6. Rotate the tail ¼ turn to place the tail veins dorsally for easier injection. The animal has two lateral tail veins and a ventral tail artery (**Figure 3**).
    7. Approach the tail with the needle at a 15-20° angle. Start at the distal portion of the tail.
        1. The veins are shallow and the needle should not be inserted much beyond the bevel.
        2. If the injection is begun as distally as possible, there is more undamaged vein to attempt the injection, should the first try fail.
    8. Inject the material. A successful injection will result in the material entering the vein with no resistance and blanching of the tail vein for the duration of the injection.
        1. Do not aspirate before injecting, as this will collapse the vein.
        2. Gentle pressure on the venipuncture site after injection will prevent bleeding.
    9. In an unsuccessful injection, the material will not flow easily. Instead, the tail skin will blanch or the material cannot be injected at all.

11. Intragastric administration (oral gavage)
    1. Only perform gavage on restrained, awake animals. Anesthesia or sedation increases the risk of aspiration (material inadvertently entering the lungs).
    2. Select an appropriately sized oral feeding needle for use. These needles have ball tips at the end to prevent their passage into the trachea.
    3. Length needed can be determined by holding the restrained animal up and measuring from the corner of the mouth. The ball tip of the feeding needle should reach to the animal's last rib (**Figure 4**). Needle gauge is determined by the weight of the animal.
    4. Restrain the animal so that its head and body are in a straight, vertical line. This straightens the esophagus, allowing for easier passage of the feeding needle.
    5. Insert the ball tip of the needle into the animal's mouth, over the tongue. Once the needle is in place, bring the needle and syringe up, pressing gently against the palate, so the animal's nose is toward the ceiling.
        1. In rats the needle may need to be redirected slightly as it passes the back of the throat. Any tension on the needle indicates the need to adjust position
    6. Continue to pass the needle until the predetermined distance is reached. The needle should pass easily, and the animal should not gasp or choke.
    7. Administer the substance. It should flow into the stomach. If there is resistance or the animal gasps, chokes, or turns blue, immediately stop and remove the needle. Animals that have aspirated may require euthanasia, depending on the compound being administered.

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