

NASA Ames Research Center STANDARD OPERATION PROCEDURE  
**PROCEDURE FOR RAT HINDLIMB UNLOADING**

Date of approved revision: September 8, 2004

The hindlimb unloading technique was first approved by the NASA Ames Research Center Animal Care and Use Committee on 12/14/83, the first revision was approved on 8/8/01, and this is the second revision. This SOP revision updates the technique for long-duration studies in mice or rats. The initial approval was based on data showing that indicators of stress (thymus weight, adrenal weight, corticosterone levels) in hindlimb unloaded animals did not differ from those in control animals. Food consumption may decrease for the first few days of the experiment, but the animals adapt to the system quickly as indicated by essentially normal food consumption and stable or increasing body weight. Description of the hardware can be found in the following references: Harper JS, Mulenburg GM, Evans J, Navidi M, Wolinsky I, and Arnaud SB. Metabolic cages for a space flight model in the rat. *Lab Anim Sci* 44: 645-647, 1994; Park E and Schultz E. A simple hindlimb unloading apparatus. *Aviat Space Environ Med* 64: 401-404 1993; Wronski TJ and Morey-Holton ER. Skeletal response to simulated weightlessness: a comparison of suspension techniques. *Aviat Space Environ Med* 58: 63-68, 1987; and Morey-Holton ER and Globus RK. The hindlimb unloading rodent model: a technical review. *J. Appl. Physiol.*, 92:1367-1377, 2002. With proper handling and care, unloading of the hindquarters using the tail traction system provides a minimally stressful system for studying hindlimb unloading and subsequent remobilization in rats.

**SUPPLIES:**

**Required:**

- Gauze or towel soaked with 70% alcohol
- Towel for restraining rat
- Tincture of Benzoin aerosol spray (Professional Packaging Corp. Aurora, IL)
- “Skin-trac” or similar adhesive traction strips (3” x 40”, Zimmer, Inc., P.O. Box 708, 1800 West Center Street, Warsaw, IN 46581-0708, Telephone: 800-348-2759, e-mail: [zimmer.infoperson@bms.com](mailto:zimmer.infoperson@bms.com), Web Site: [www.zimmer.com](http://www.zimmer.com))
- Filament tape (0.75” roll can be purchased at any stationary store)
- Hindlimb unloading cage
- Plastic-backed absorbent paper that fits under the cage to absorb urine
- Plastic tab with attached paperclip loop; the dimensions of the tab are sized to the weight of the rat:
- Gauze for wrapping tail

**Optional:**

- Hair Dryer
- Bitter Apple spray for use with adult rats
- Wooden dowels or Nylabones
- Gauze to wrap rat tail
- Marking pen for rat tail
- *Syringe barrels for long duration studies*

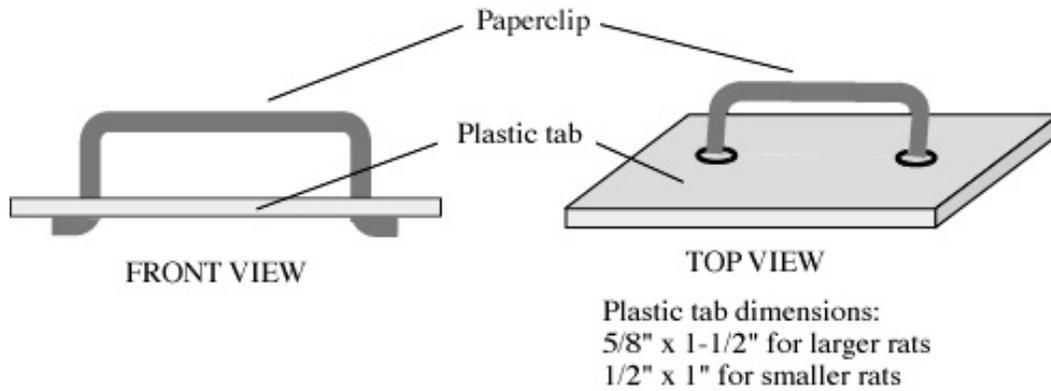


Figure 1. To make a plastic tab, a paperclip is inserted through small holes drilled in a piece of plastic, bent along the underside, and trimmed with wire cutters.

**PREPARATION:**

Prepare strips of the “Skin-trac” (i.e., traction tape) and filament tape. The size of the “Skin-trac” strips depends on the width of the rat’s tail. The strips are cut to about 12” long and 0.25” wide for young animals (about 150gm). The strips should be narrow enough so that two strips do not touch when placed on opposing sides of the tail, and long enough to fold in half with each half covering about 2/3 of the length of the tail beginning at the base of the tail just above the hairline.

Cut the paper protecting the “Skin-trac” in the middle of the strip and peel back (but don’t remove) about 1/2 inch of the paper on each side. Thread the tape through the paperclip loop on the plastic tab until an equal amount of tape is on either side. Press to adhere the strip to the plastic tab.

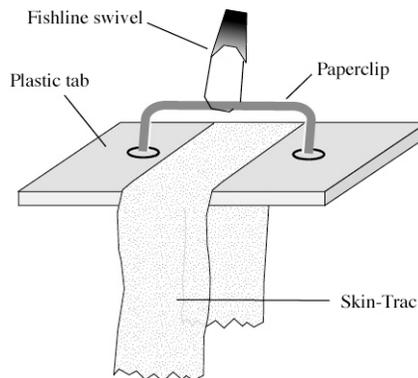


Figure 2. “Skin-trac” strip is threaded through the paperclip loop and adhered to the tab.

**PROCEDURE:**

- Handling of the animals should begin about a week before the procedure. The animals should be acclimated to their cages at least 2 days before beginning the hindlimb unloading. If the animals have been recently shipped from a vendor, then a longer adaptation time is advisable.

- On the day of hindlimb unloading, wrap the rat loosely in a towel (the rat should run into the towel and away from the operator). A second person can assist in keeping the rat in the towel, but as an operator becomes familiar with the procedure, he/she can usually perform this procedure alone. Use minimal restraint to avoid stressing the rat during the procedure. Anesthesia should not be used for the procedure.
- Hold the rat's tail and clean the tail with alcohol, removing all the dead or dirty skin. Allow the tail to dry; drying usually takes one minute or less.
- When the tail is dry, spray the tail with Tincture of Benzoin. When the Benzoin is dry (between one and five minutes), the tail should be sticky. The rat can be placed in their cage while the Benzoin is drying. To speed up this drying, a hair dryer can be used on a **low** heat setting.

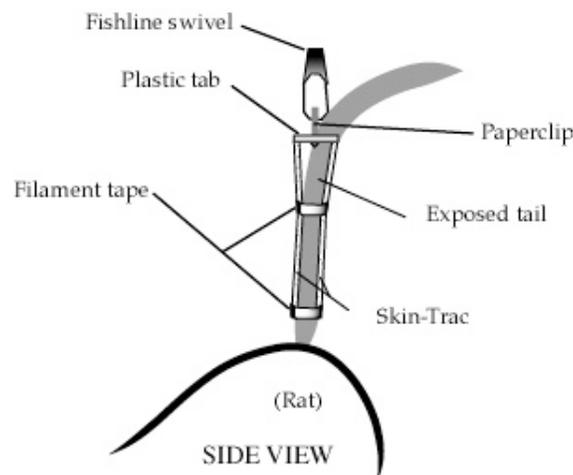


Figure 3. Attaching “skin trac” to the tail

- Apply a strip of “Skin-trac”, pre-attached to the plastic tab (Figure 2), to the tail. Begin by cutting the “Skin-trac” to the correct length for the rat. The length is determined by holding the plastic tab at the base of the tail just above the hair line and determining the length of tape required to extend about 2/3 the length of the tail on two sides of the tail (Figure 3). Cut the tape to that length. Remove the backing from the tape. Attach the one end of the tape to the base of the tail just above the hair line, then attach the other end to the other side of the tail. Gently press the “Skin-trac” to the tail so that it sticks along the tail’s surface. The “Skin-trac” should be narrow enough that the tape on one side of the tail does not come into contact with the tape on the other side of the tail. Make certain that the two sides of the tape do not touch (Figure 3).
- Secure the “Skin-trac” to the tail with two strips of filament tape (~0.25” wide x1.5” long). Place one strip of filament tape around the base of the tail over the ends of the “Skin-trac” and a second strip about half-way up the “Skin-trac” (Figure 3). Make certain that the filament tape is loose enough to allow normal blood circulation, but tight enough that the “Skin-trac” will not peel. After the filament tape has been in place for 2-3 days, either

remove it, cut it in two, or replace it with looser tape to avoid restriction of tail growth. Do not allow the “Skin-trac” or other types of tape to restrict tail circulation.

- Wrap gauze around the tail to hide the “Skin-trac” and to deter the rat from peeling off the tape. The tail is critical for thermoregulation in the rat, so do not wrap the entire tail--only wrap the area covered with “Skin-trac” primarily around the base of the tail.
- With the marking pen, make a line at the bottom of the “Skin-trac”. If a significant gap develops between the line and the “Skin-trac”, then the tape is too loose and soon will no longer remain attached to the tail.
- The rat is now ready to be attached to the fishline swivel that hangs from the unloading device on top of the cage (Figure 1C in Morey-Holton, E.R. and R. K. Globus. The hindlimb unloading rodent model: Technical Aspects. J. Appl. Physiol., in press). The unloading device consists of two sets of bearings connected by metal rod and a fifth bearing that rolls along the metal rod; the fishline swivel is connected to the fifth bearing. The unloading device should move along the top of the cage with minimal friction, so that the animal can easily move throughout the cage using its front paws.
- The body of the rat must make about a 30° angle from the cage floor to assure that the back feet of the rat do not touch the grid floor. The hind legs should be manually extended to assure that the toenails can not touch the floor. The angle and height should be adjusted daily. The cage sides should be adjustable to increase or decrease the height of the unloading device. Make sure that the animal can reach food and water after making the adjustments.
- Absorbent paper, cut to fit under the cages, is placed there to absorb urine unless using a metabolic cage. The papers are changed as needed, but not less than twice/week. If paper is not appropriate, then normal bedding material can be placed in a tray under the cage.
- Control animals should be kept in identical cages if possible. Control cages do not need the unloading device, but do need a top cover to assure that the rat does not escape. Extra flooring grids make excellent cage tops as they permit air circulation. A filled water bottle or similar weight placed on top of the cage may be necessary for larger animals.
- A lingering problem for long duration studies has been the integrity of the tail harness. The traction tape may slip over time, and both rats and mice have a tendency to chew on the traction tape, which can lead to release from HU. Dr. Marjolein van der Meulen of Cornell University found a simple solution to minimize this problem. The modification involves using a plastic syringe barrel to cover and protect the base of the tail where traction tape is applied. A plastic syringe barrel is selected to fit over the traction tape without constricting the tail. Then, both ends of the syringe barrel are removed and the barrel is cut horizontally into 1-1.5" cylinders (Figure 4). The cylinders are slit longitudinally so that the barrel can be opened and slipped over the tail. The barrel is positioned over the traction tape at the proximal end of the tail distal to the anus and attached to the traction tape with small strips of medical paper tape to hold it in place. This minor modification minimizes the number of

animals that slip out of the harness device and prevents removal of the tape by chewing. The modification was originally designed for mice and also works well for rats.

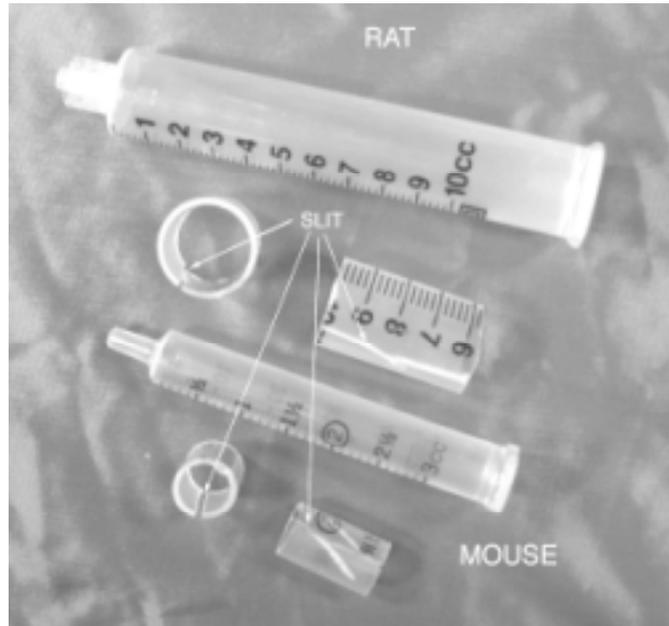


Figure 4. A simple modification for HU using a tail guard for long-term studies.

#### **ADAPTATION TO HINDLIMB UNLOADING:**

Rats readily adapt to hindlimb unloading as evidenced by the disappearance of porphyrin from around the nose and eyes, resumption of normal food and water consumption (if they were curtailed upon hindlimb unloading), resumption of growth in younger animals (if it had been initially impeded), and normal adrenal steroid levels, all within one to three days after hindlimb unloading. Plasma corticosterone levels, measured by two ARC investigators, doubled the first day of hindlimb unloading and were at ambulatory control level by day 2 (R. Grindeland, E. Morey-Holton, unpublished observations) and a similar pattern has been shown for corticosterone excretion (Ortiz, R.M., et al Influence of centrifugation and hindlimb suspension on testosterone and corticosterone excretion in rats. *Aviat., Space, and Environ. Med* [1999] 70:499-504).

#### **ADDITIONAL INFORMATION:**

1. The age of the rat is important in the weight response expected. Young, growing rats (<200g) should not lose weight although they may gain weight more slowly than pair-fed controls (definitely slower than *ad lib* controls). Hindlimb unloaded animals never stop eating although their food consumption for the first day or two can drop by as much as 50%. Adult rats (>200g) will probably lose weight initially and then stabilize. Older adult rats (>400gm) may have difficulty adapting to hindlimb unloading; if adult males are used, smaller strains, e.g., Fisher 344 rats, should be considered.
2. If adult male Sprague-Dawley or Wistar rats (~400gm) are used, then the following precautions are recommended:

- a) the rats should be handled several times a day for at least a week before the experiment begins,
  - b) "Skin-trac" should be placed at the very base of the tail (unlike the younger animals, the older animals appear to do much better when the "Skin-trac" begins at the very base of the tail),
  - c) be very careful with the Tincture of Benzoin as it appears to be less well tolerated by the adult animals and any tincture sprayed on the hair should be removed,
  - d) from the beginning, the tails can be coated with Bitter Apple or Chew Guard or equivalent (obtained from a veterinary supply company) to discourage the animal from chewing on the "Skin-trac". The animals must be removed from the cage for this procedure because the rat will avoid any cage area sprayed with bitter apple and will not eat any food with any of this substance. The animal must be held until the spray dries so that the spray is not spread; the spray contains alcohol which probably will not be well tolerated if the animal has any abrasions, but the spray dries quickly,
  - e) any tail with abrasions should immediately be treated with antibiotic cream and zinc oxide; only the abrasion and no other part of the tail should be coated with these substances, i.e., a minimal amount of compound should be used.
3. The temperature of the room in which the animals are housed should be maintained at about 76°F (24°C). This temperature is the approximate thermoneutral zone (that ambient temperature at which the animal neither gains nor loses heat) of the rat in an oxygen-nitrogen environment. This temperature is important for singly housed animals.
  4. Provide wooden dowels or Nylabones for the rats to chew on. The piece of dowel should be about 1.5" long and 3/4-1" diameter. These items minimize chewing up the plastic grid on the bottom of the cage. If the rat chews holes in the cage, it can get under the cage bottom or even out of the cage.
  5. If the animal is allowed to move on an X-Y axis and rotate 360° so that it has access to all areas of the cage, then it will adapt more readily. The pulley system/swivels should impart minimal drag so that the animal can move easily and without effort.
  6. The unloading device is located at the top of the cage and rides along two parallel sides of the cage. If animals should not exert muscle forces against the sides of the cages, then large binder clips can be placed at the end of these cage sides to prevent the animal from reaching the cage sides.

#### **CAGE CLEANING:**

The cage is disassembled for cleaning. In the preliminary bath, each part is washed with hot water and if necessary is scrubbed to remove any visible organic matter such as dried feces, urine, or food pellets. Then the parts are completely submerged in a disinfectant solution for at least one half-hour for plastic pieces and a shorter time for the metal pieces. The metal pieces should be dried when removed. The plastic pieces for the cages are rinsed, reassembled, and allowed to air dry.

## **MONITORING ANIMAL HEALTH AND WELL-BEING DURING THE STUDY:**

1. Animals must be inspected daily by the Attending Veterinarian and/or Registered Veterinary Technicians to assure the health of the animals. In addition, animals should be inspected carefully twice a day by the investigator or his/her staff to ensure that any animal slipping out of the traction tape (and, thus, weight-bearing) is discovered as soon as possible so that it can be hindlimb unloaded if desired, with a minimal exposure to re-loading, or removed from the experiment. Criteria for removing a rat from the study should be agreed upon by the investigator and the Attending Veterinarian before the study begins; criteria include, but are not limited to, tail status, weight, appearance, and inability to remain attached to the unloading device.
2. Specific points of inspection:
  - A. Overall appearance and activity. During the initial days of hindlimb unloading, porphyrin may appear around the nose and eyes in varying degrees. Typically, it disappears or starts to disappear by the third day.
  - B. Evidence that the animals are eating, drinking, and able to move freely.
  - C. The most important part of the inspection is the tail, from both a health and science perspective. If the tape on the tail is too loose, then the end of the “Skin-trac” will move away from the ink mark at the base of the tail. When this happens, filament tape should be replaced and tightened slightly. Typically, the end of the tails in hindlimb-unloaded rats are slightly engorged and pinkish. If the end of the tail is too engorged, or bluish, immediately loosen the filament tape and check to make sure that “Skin-trac” tape is not impeding circulation in the tail.
  - D. Periodic weighing: Either construct a tripod with a hook at the top, or a ring-stand fitted with a crossbar and hook, for weighing hindlimb-unloaded rats. Either device should fit onto a balance. The rat is gently removed from the unloading device and attached to the hook so that the animal’s forelimbs touch the balance while the hindlimbs remain unloaded. Weighing should not take more than a minute. With careful handling, and weighing as described, the rat should not bear weight on its hindlimbs during the procedure. Body weights add a significant parameter to the visual health assessment.
  - E. Male rats should be checked daily for sperm plugs. If there is a sperm plug in the penis, it should be expressed manually from the urethra.