

Review Article

A Review on Handling & Restraint of Small Animals in Preclinical ResearchAmol W There^{1,*} and Lalit G. Pund²¹Department of Pharmacology, School of Pharmacy, G H Raison University, Pandhurna, Madhya Pradesh- 480337, India²Shri Sachhidanand Shikshan Sanstha, Taywade College of Pharmacy, Mahadula, Koradi, Kamptee, Dist. Nagpur, Maharashtra**ARTICLE INFO**

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ABSTRACT

The effectiveness in scientific results is commonly obtained by adopting skilled handling and restraint techniques, which are imperative to maintain the homeostasis or adaptive state. The demonstration is designed to impart fundamental knowledge and hands-on experience in handling and restraint of laboratory animals, especially mouse and rat using descriptive and illustrative procedures. This review addresses the guidelines for handling and restraint of mouse and rat, which are usually employed in preclinical research. The cardinal objective of the review is to instill confidence in study personnel so that practicing these techniques result in reduction in stress to the animals and refine scientific outcomes. Exclusively, these techniques have been applied to the study personnel, personnel of animal house facilities, and veterinary care.

Keywords: Animal handling; Preclinical research; Mouse; Rat.

Introduction

The safe and effective handling and restraint of the mouse or rat is an essential humane technique during conduct of preclinical research. The initial methods used for animal lifting from its cage are by tail, a tube, enrichment device, and grasping around the body. The scruffing and other methods are used for restraining the mouse and small rat. An improper handling and restraint can agitate the animals and may likely to bite, which may cause pain and infection. Sometimes, calming animal prior restraint is necessitated, and for this purpose chemical method can be used, such as inhalant anesthetic, isoflurane or sevoflurane. Commonly, the light anesthesia can be delivered using induction chamber, but this involves prior handling of animal from its cage. Therefore, proper handling and restraint techniques are utmost important. It can be accomplished by adopting the basic competency and humanely working with mice and rats, which result into the minimization of the animal stress and facilitate the efficient scientific outcomes.

Definition

Stress: The effect of physical, physiologic, or emotional factors (stressors) that induce an alteration in an animal's homeostasis or adaptive state.

Distress: The effect of stimuli that initiates adaptive responses that are not beneficial to the animal—thus, the animal's response to stimuli interferes with its welfare and comfort.

Fear: An unpleasant emotional experience caused by an awareness of a threat of danger.

Aversion: A desire to avoid or retreat from a stimulus.

PPE: It is defined as the personnel protective equipment, which includes gloves, face mask, head mask and apron, shall be donned during the conduct.

IAEC: Institutional Animal Ethics Committee

Material

Species: Albino Mouse/Albino Rat
Sex: Male and/or Female
General anesthesia: Isoflurane
Equipment: Animal cage, mice and rat restrainer.
Method: Illustration.

Procedure

Even very less handling of mouse and rat can produce stress. The animal cage changing and non-invasive procedures may result in to altered physiological parameters, such as increase in heart rate and blood pressure, including serum corticosterone levels. These fluctuations can be continued for up to several hours. Restraint procedures used for drug administration and blood withdrawal also result in to change in the animal's physiology, which significantly affects the scientific data. To avert such changes, the competent handling and restraint of the mouse/rat must be necessitated.

Essential requirements for precise handling of animals:

- It is indispensable that the protocol involves non-invasive, but painful or invasive procedure must be approved by IAEC or ethics committee.
- Personnel involved in study must be well-versed in procedures.
- Gentle but firm handling is needed, whenever restraint animals, otherwise reckless handling

results in scratches on handlers and also injury to animals.

- Handling animals with the tip of tail must be avoided, this may result in a degloving injury to the tail. Make use of the other hand for body support when handling by tail.
- While injection, sharp needles can be used.
- Animals can be acclimated to handling which minimizes stress.
- Restraint of animals can be practiced before substance administration.
- Adopting all these considerations may result in minimized stress to animals and refine scientific findings.

Restraint Methods: Mouse

Scruffing technique (refer fig. 1)

Mice are most often restrained using the scruffing technique. Mouse can be held by the tail (possibly above the tip of the tail) with the help of thumb and index finger (if right-handed person) and placed on a rough surface or stainless-steel grill. The mouse tail can be pulled back to ensure the grip by the mouse on the grill. The left hand can be used from the rear back and move gently towards the nape of the neck. Hold the scruff of the mouse at the base of the skull with the help of thumb and index finger, and restraint the dorsal skin with the help of middle finger against the base of the thumb. The animal can be firmly restrained by fixing the tail with the help of little finger as shown in figure.

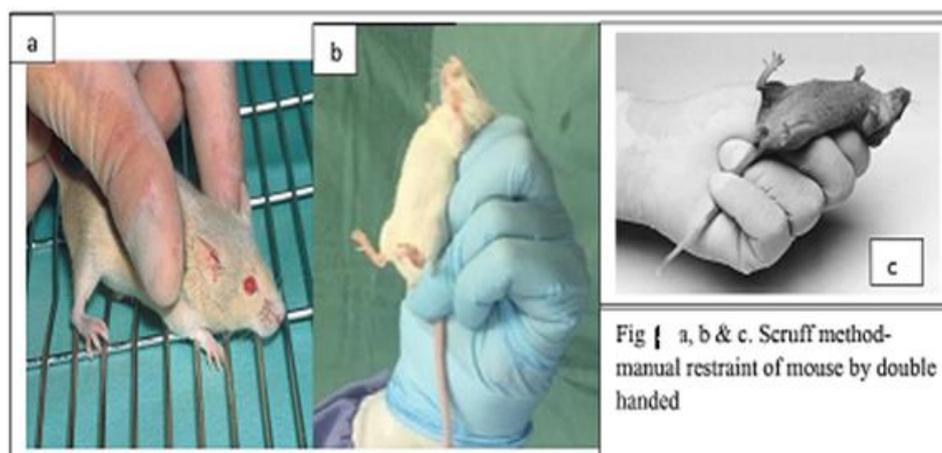


Figure 1: Represents the scruff method for mouse.

One-handed restraint technique (refer fig. 2)

- Mouse can be held by the tail with the help of thumb and index finger and kept on the stainless-steel grill.
- The tail can be held between the ring finger and little finger and restrained against the palm

- with the help of middle finger, at the same time slowly released the thumb and forefinger.
- Following this, the index finger and thumb can be used to hold the scruff of the neck from its back. This procedure enables the scruff to be held firmly over the shoulder.

- d. The animal can be lifted by grasping the loose skin at the neck.
- e. The animal can be stabilized with the help of a middle finger on the back by restraining the dorsal skin on the base of the thumb and further by fixing the tail.

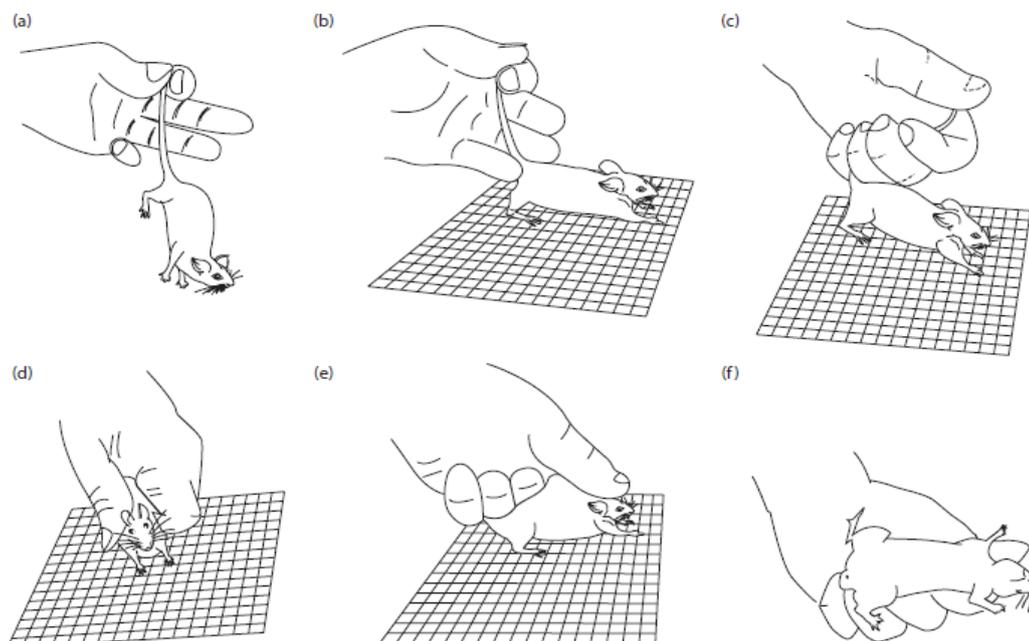


Figure 2: Represents one-handed restraint method for mouse.

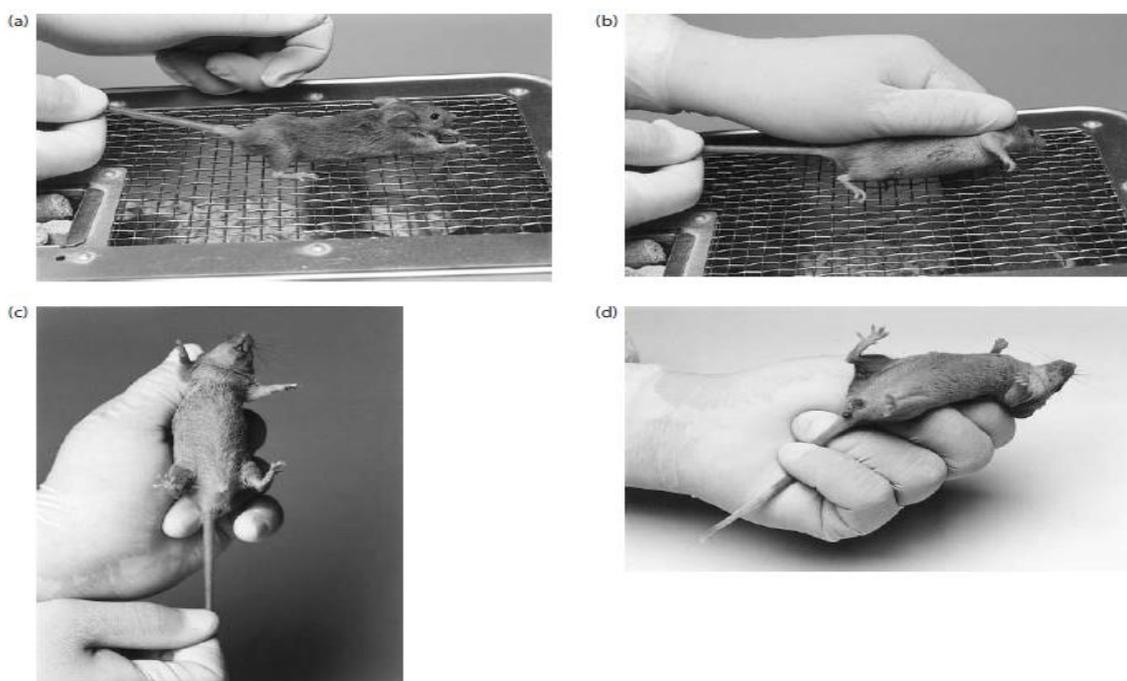


Figure 3: Represents two-handed restraint method for mouse

Two-handed restraint technique (refer fig. 3)

- a. Like one handed method, mouse can be lifted and kept on the stainless-steel grill or rough surface.
- b. Then the mouse tail can be pulled back, and at the same time pulling made the mouse hold the surface firmly with four paws and move forward.

- c. Then by the other hand an index finger and thumb can be used to hold the scruff of the mouse neck. The loose skin of the neck can be restrained and lifted the animal.
- d. Animals can stabilize with the help of the middle finger on the back by restraining the dorsal skin on the base of the thumb.
- e. With the help of a first-hand tail can be transferred to another hand and fixed through the ring finger between the little finger and palm.

- a. In this method, dominant hand can be used to hold near the base of the tail and kept on the rough surface. Then, the tail of the rat can be pulled back on the rough surface.
- b. The non-dominant hand can be used and placed over the shoulder by approaching from the rear back.
- c. The movement of the head can be restricted by placing the index finger on one side of the head and the middle finger on the other side.
- d. The third finger along with little finger and thumb can be used to secure the rat by encircling the body behind the forelegs.
- e. The tail and hindquarters can be stabilized by grasping the base of the tail.
- f. This method is efficient for restraint the larger rats and when access to the head.

Restraint Methods: Rat

Over the shoulder grip restraint method (refer fig. 4)

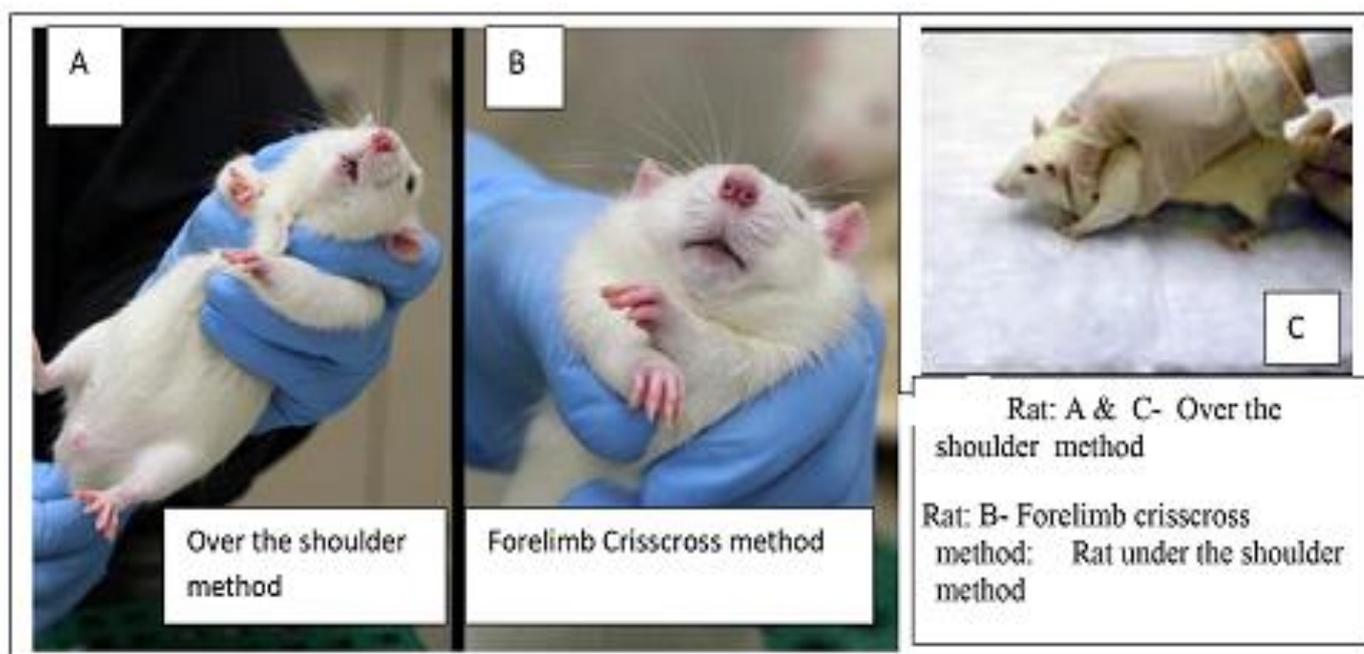


Figure 4: Represents over the shoulder restraint method and crisscross restraint method for rats.

Under the shoulder grip method (refer fig. 4)

- a. Normally, rats can be held near the base of the tail as similar to the above method.
- b. Then use non-dominant hand to hold right under the shoulder blades by approaching from the rear back.
- c. Rat can be grasped around the thorax with the help of the thumb and index finger, and the forearm can be lightly pushed up. This procedure can restrict the movement of the head.
- d. Caution- One should take care that the chest must not be compressed, otherwise it may compromise the respiration.

Rat restraint: Scruffing method

- a. This method is used for smaller rats and performed with two hands.
- b. Rat can be lifted by non-dominant hand, holding near the base of the tail, and kept on the rough surface. Rat can be pulled back lightly by the base of the tail on the rough surface.
- c. The other hand can be used to hold the scruff of the neck by approaching slowly from the rear back.
- d. Then put the pressure on the back of the rat and can be restrained by holding the scruff from the base of the skull with the aid of

- thumb and index finger and further by pinning the rat's dorsal skin with the middle and ring fingers against the palm of the hand.
- e. The scruff can be secured well to keep control from bites.
- f. By this procedure, usually, rats vocalize after restraint.

Restrainer device (refer fig. 5)

- a. In this method, the restrainer device made of acrylic is used when the injection to be made in the animal's tail. This procedure exposed the tail without stressing the animal.
- b. Such devices can be commercially available or customized in the laboratory.
- c. The specific size device should be used for restraint procedure, so that the animal must stay in one direction and unable to turn around.
- d. The animal can be restrained in a tube or restrainer, which has a horizontal slot on tube body and vertical slot at base. It involves the following steps: -

- i. The animal can be held by the base of the tail
- ii. Then, the animal can be inserted into the device from the wide opening with an adjustable head by pulling the base of the tail through the horizontal slot. This process can be directed at the animal's head towards an adjustable head and further, the tail can be pulled through the other end or slot of the restrainer body, which enabled the tail end free.
- iii. The animal can be accommodated and held immovable with minimal resistance, and the head holder can be adjusted as per the animal's size. The restrainer allows minimal distress to the animal. The restraint of animals is shown in a picture.
- e. As the restrainer tube is slotted and having a wide opening on one side of the tube, which allows less risk to limbs getting trapped.
- f. The procedure should be conducted at minimum time as the animal may overheat in restraint position.



Figure 5: Represents restraint of mouse by acrylic device.

Applications- Restraint method used exclusively for route of administration/blood withdrawal:

- a. To perform an intraperitoneal injection the animal's ventral surface is exposed. The following methods can be used:
 - i. Scruffing method

- ii. Over the shoulder and crisscross method.
- b. If an injection to be made in subcutaneous space, then the animal's dorsal surface must be exposed. The methods used for this procedure are:
 - i. Scruffing
 - ii. Rigid restrainer

- c. For Intramuscular injections, animal's hind limb has to be restrained. Further, the hind limb restraint procedure can be applied for blood withdrawal from the saphenous vein, femoral vein or cannulated femoral artery, and injection into the footpad. The methods are:
- i. Scruffing of mouse and small rat (under 200 g).
 - ii. Over the shoulder grip or forelimb crisscross method.
 - iii. Rigid restrainer.

Observations

- The IAEC approval is necessary when invasive or painful procedures are involved in experimental study.
- Acclimation and a gentle approach before handling are required to facilitate minimal stress.
- The manual restraint for mouse, one- and two-handed method can be followed as the protocol demanded.
- For rats, restraint by over the shoulder, under the shoulder, or scruffing method is used as per the purpose of the protocol e.g. scruffing method for an i.p. injection.
- Restraint procedure by acrylic device is an effective method when accessing the tail. This is the most commonly used method for intravenous drug administration.

Conclusion

The efficient handling and restraint technique is essential in preclinical research to minimize the distress to the animals. The techniques are comprehensively illustrated for the ease of understanding and in perspective of application.

Physical restraint may include manual handling or device to limit some or all of an animal's normal movement for the purpose of examination, collection of samples, drug administration, therapy, or experimental manipulation. However, the animals are restrained for a brief period in most research applications but still have major impact on the physiology. Hence, the competent personnel by practicing these techniques as well as use of appropriate restraint equipment can mitigate most of the stress and facilitate the better research findings.

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Conflict of Interest

None declared.

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