Custom-made Devices Used for Rodent Restraint and Gaseous Anesthesia

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Authors’ contributions

This work was carried out in collaboration between both authors. Author JS designed the devices with the assistance of author JJ. The devices were used, tested and adjusted by both authors until a satisfactory outcome was achieved. Both authors read and approved the final manuscript.

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ABSTRACT

To obtain scientifically reproducible results in animal studies is challenging because inappropriate handling of animals can negatively impact the final results. This is of significance when new / graduate students are attempting animal studies for the first time. The fear expressed by some students in handling rodents makes experimental research problematic. During the course of a graduate student experience, we developed three (3) devices which were hand made and customized for their intended use in various studies. We prepared a restrainer for tail snips/ IV cannulation, a device for intraperitoneal injections and a chamber for temporary anesthesia. These restrainers consisted of recycled plastic bottles of various sizes and helped to improve the ease of handling laboratory animals. We found that these devices were useful in carrying out experimental research in rodents whilst maintaining minimal risk to the handler. The devices created an environment where students with limited experience in animal handling can perform simple procedures easily without additional assistance. The fact that the risk of zoonotic infections is greatly reduced and the fear of bites and scratches is minimized, students can utilize these devices to aid in...
the conduct of their laboratory experiments with rodents. The devices provided a simple and effective method for blood collection, intraperitoneal and subcutaneous injections. Although good animal handling techniques is advocated, the use of these devices can reduce the risk of scratched and bites and allow novice scientists to perform experimental procedures with relative ease.

Keywords: Isoflurane; animal welfare; anesthesia; animal restraint.

1. INTRODUCTION

Laboratory animal science is a multidisciplinary branch of science contributing to the humane use of animals in biomedical research and the collection of informative, unbiased and reproducible data from animal experiments [1]. The use of animals in pharmacological testing remains an important step in the eventual application of new remedies to humans. It is a pre-requisite that the safety and efficacy of a medicament in these species be well established before extrapolation and testing can begin in humans.

Good handling and restraining techniques reduce stress and the risk of physical injury to the animal. Apart from the welfare of the animal, of great importance is the safety of the individual carrying out the procedure, as scratches, bites and infections are risks to the handler. Young scientists involved in animal research may have difficulty in handling laboratory animals. Though there was access to conventional restraining devices, we found that the devices did not always meet the needs of the experiment being conducted. In the course of our animal experimentation, we developed three devices which were easy to prepare using recyclable material. These could be easily custom made for the animal’s size and ensures safety to the handler as well as ease of manipulating animals.

Despite creating these devices, it was of great importance to ensure the animal’s welfare and the safety of the handler and that the procedure should be executed in the shortest possible time. The devices prepared reduced the time of restraint or confinement. During the experiments, animals must be assessed regularly by a veterinarian or other qualified person not otherwise involved in the project. If any negative impact on an animal is detected, the animal must be removed from the restraint or the method of restraint must be modified to minimize the impact [2].

It has been documented that even very brief handling (30 seconds) can act as a stressor with concomitant rise in plasma glucocorticoid levels [3]. It has also been shown that restraint causes significant changes in the “Disturbance index” of the animal and that the index value increase was proportional to the duration of restraint [4]. It is therefore imperative to address the principles of the 3Rs (Replacement, Reduction and Refinement) in animal research and the impact of restraining devices. The use of our devices adds to the refinement aspect of the 3R approach. Refinement methods refer to all procedures from housing to the experiments conducted on them.

2. IMPORTANCE OF RESTRainers

The use of manual restraining techniques has been well documented [5], the use of restraining devices is commonly used in animal experimentation. This is because the most common problem leading to an accidental injury to both animal and handler is incorrect or inadequate restraint. Often it is a lack of firmness which allows the animal too much freedom of movement, which can have negative implications for animal and handler. Restrainers is useful for three reasons: they reduce the stress with which the animals would be burdened, reduce the chance of physical injury to the animal as well as the risk of zoonotic infection to handler.

For hand restraint to be successful there must be a grip that is firm enough to secure the animal and prevent movement, as well as to ensure that the animal does not sustain an injury during the procedure. Some procedures such as injections can inflict sharp pain or discomfort to which the animal would react. The grip must be firm enough to counteract such swift action of the rat and to prevent physical injury. Evidence suggests that handling mice by a familiar tunnel that is present in their home cage can minimize anxiety compared with standard tail handling [6].

3. CONVENTIONAL RESTRAINING DEVICES

Restraining devices are useful to prevent unpleasant interaction between handler and animal, and also to make administration technique easier where the use of both hands is required. There are simple methods of restrain
such as the use of muslin to immobilize the animal but extra care must be taken to avoid bites and scratches. DecapiCones have the advantage that the thin plastic allows for injections through the material. The disadvantage is that the material does not allow adequate ventilation and animals can become overheated. This is of significance when the animal has to undergo a lengthy [7].

Other useful devices employ acrylic/rigid plastic restrainer which allows the animal inside, before sealing the opening to prevent escape. A major disadvantage is that rats of different sizes cannot be used in the same restrainer. Larger rats were not able to enter, whilst smaller rats were able to turn around in the device. Also, there is the risk of physically trapping the tail as the back-plate is lowered and locked into position [7]. Some of the rigid plastic devices available have numerous holes or grooves to permit the administration of injections. If the animal is able to move slightly, there is always the risk of improper administration and further physical restraint may simply act as a stress inducer in the animal. It has been noted that these devices are inadequate for some procedures and researchers have been actively seeking new devices for specific uses. Since head fixation devices in rats often become unstable within several months, researchers have developed a novel device [8] called the non-invasive “neck collar system” for restraining the head and body movements of rats. Researchers using repetitive transcranial magnetic stimulation have developed a device to restrain rodents which allowed them to investigate the potential cellular and molecular mechanisms for the therapeutic effects of repetitive transcranial magnetic stimulation [9].

4. OUR DEVICES

We therefore tried to acquire devices which are easy to make, would allow for a wide variety of sizes to be easily procured and which were environmentally friendly. We were able to create 3 devices- one for animal restraint which was used for performing tail snips and IV cannulation, another for intraperitoneal (IP) injections and the last device was developed to induce anesthesia in the animals for a short period using isoflurane.

The first device for animal restraint used two plastic bottles. This device utilized two open ended plastic bottles which were able to slide into each other (Fig. 1). At one end there were holes for the animal to breathe (first bottle) and at the other end, there was a hole that allowed free access to the tail (second bottle). Once inside, the bottles were adjusted to ensure the animal movements were restricted. The animal was therefore completely encased and was unable to escape or even turn around in the device, whilst allowing sufficient breathing space. We also used a test tube rack and sealed off the sides to create a holding bay for the restrainer. The restrainer was placed into this holding bay, which allowed a hands-free operation of the device and could easily be handled by one person. We found that once the animal was placed in this device, tasks were successfully performed whilst there were no noticeable signs of discomfort to the animal.

The second device was an intraperitoneal injection chamber, which follows the concept of the previous device, although it is much simpler in design. It uses a single bottle with a small rectangle measuring 6 cm X 4 cm (IP access area) is cut out. (Fig. 2).

The animal is allowed to enter the first chamber (with the IP access area facing downwards. At this point, the device is turned upside down such that the animal now lies on its back. The cut off area gives good access to the intraperitoneal injection site where injections can be administered easily whilst limiting the animal’s movement.

It is important to note the use of a soft collapsible clear plastic bottle which served two important: movement inside the device could be easily monitored and in the event that the animal tried to turn, pressing gently on the bottle was sufficient to restrict the animal’s advances.

Our third gadget uses a round bottom flask which is connected to a restrainer device by the use of plastic tubing (an IV line could be used). To the restrainer (our first device), the top half of a soda bottle is attached to create a sealed chamber.

Isoflurane is placed in the round bottom flask and a 100W light bulb in placed directly below the flask. The heat from the bulb causes isoflurane to evaporate (48.5°C) and travel along the plastic tubing to the device which is holding the animal. The heat rises through convection, which means evaporation of isoflurane could be stopped at anytime simply be removing the light bulb from its position below the round bottom flask. (Fig. 3).
Fig. 1. Picture of animal restrainer- tail snips and IV cannulation can be easily performed using this device

Fig. 2. Picture of plastic bottle being used for IP administration

The animal is held in position and tail reflex is checked at regular intervals, once the animal is sufficiently sedated -which took approximately 30 seconds, simple procedures can be performed (such as IP, IV injection or IV cannula insertion). Once the animal was returned to its cage, activity level returned to normal within fifteen seconds. It should be noted that this device was used only in difficult situations. The device used the simple concept of evaporation and the amount of isoflurane being released cannot be controlled. Also the implications of multiple exposure to isoflurane was not explored during the experiments.
5. DISCUSSION

Laboratory animals are susceptible to stress by restraint [10], however, as long as the same experimenter handles the animal, habituation to handling can be achieved, [11,12] whilst habituation to restraint seems to be more difficult to accomplish [10,13]. It has been shown however, that the stress level can be reduced as rats subjected to repeated restraint can become habituated to the process which was no longer perceived as a stressful situation by the animal [14].

During the course of the study, animals were subjected to the experimental techniques during the acclimatization period as this would allow greater control of variables during the actual testing phase. This was achieved by having the animal handled daily by the same person and allowing them to enter into a manually prepared restraining chamber. During the actual experiment, the exact procedure was repeated, but this time, isoflurane was released into the chamber. It was found that after 30 seconds of isoflurane administration, the animals showed no response to tail pinch. At this point, an intraperitoneal injection was administered, the animal removed from the device and allowed to recover in their cages. In all instances once placed in their cages, the animal overcame the effects of anesthesia in approximately 15 seconds. Thus the animal was perhaps unaware of the painful stimuli.

For our experiments, we chose the agent isoflurane for two (2) main reasons. Firstly, it has been shown that rats anesthetized with isoflurane showed lower increases in blood pressure after, and fewer fluctuations in body temperature during sampling, and the post-anesthetic effect of isoflurane, if any, seems to disappear immediately after sampling [15].

The second reason is that in fed rats, Isoflurane exposure caused no changes in plasma corticosterone, glucose or insulin levels. In rats that were fasted, isoflurane had no effect on corticosterone, but decreased plasma insulin levels [16].

6. CONCLUSION AND RECOMMENDATIONS

The devices described are easy to prepare, cheap and were as effective or even exceeded the capacity of conventional devices. A distinct advantage is the fact that it could be easily...
modified to the size of the animal. The handler can safely carry out a procedure without concerns of being bitten/scratched or zoonotic infections. We recommend the use of this device for blood collection via tail snips, intraperitoneal injections as well as intravenous tail access. Our studies involved injection of an infectious agent and its use is therefore recommended whilst administering agents which can be potentially hazardous to the handler. The anesthetic chamber made short procedures very easy and possibly eased the burden of the experimental procedure to the handler by means of restraint. We used this device in animals that could not be restrained effective by any other method. Since isoflurane is used, this device is recommended in Wistar-Kyoto and spontaneously hypertensive rats. Hypertension. 2000;35:126-129.


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sampling methods on laboratory rats under different types of anaesthesia. Laboratory Animals. 2006;40:261-274.


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