

Refinement of handling and dosing methods for rats and mice

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Based on a Poster displayed at the IAT Congress 22

Modified handling

Mice

Handling mice by gripping the base of the tail has been shown to negatively impact animal welfare by increasing anxiety and aversion to handling.¹ We have also observed that aggression between mice is targeted at the tail and hypothesised that this may be an ethological reason why mice are particularly averse to this interaction.

Whilst alternative methods of handling (e.g. use of tunnels and cupping) are encouraged, they have not been universally adopted as many believe that gripping the tail is still necessary for restraint.

We aimed to refine the dosing procedure by finding a handling method that allows the same level of control over the animal without gripping the base of the tail.

- Mice were restrained for intraperitoneal dosing using either a conventional method using the tail or a novel method illustrated in Figure 1.

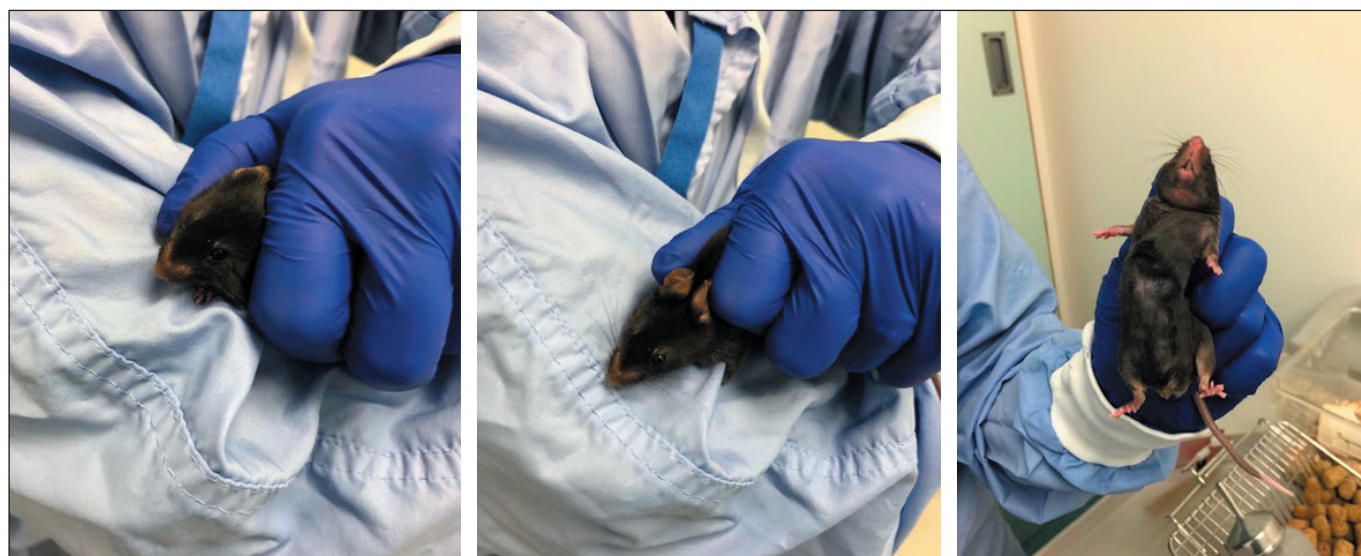


Figure 1. New method of mouse handling.

1. Remove mouse from cage using a cupping method and place on forearm.
2. Place hand over mouse allowing it to push its head out between thumb and forefinger.
3. From this position you can scruff the mouse as you normally would.
4. At no point is the base of the animal's tail held.

- Using simple assessment method overt signs of stress were recorded.
- Researchers with different levels of experience were trained in the new method to assess how readily it could be used.

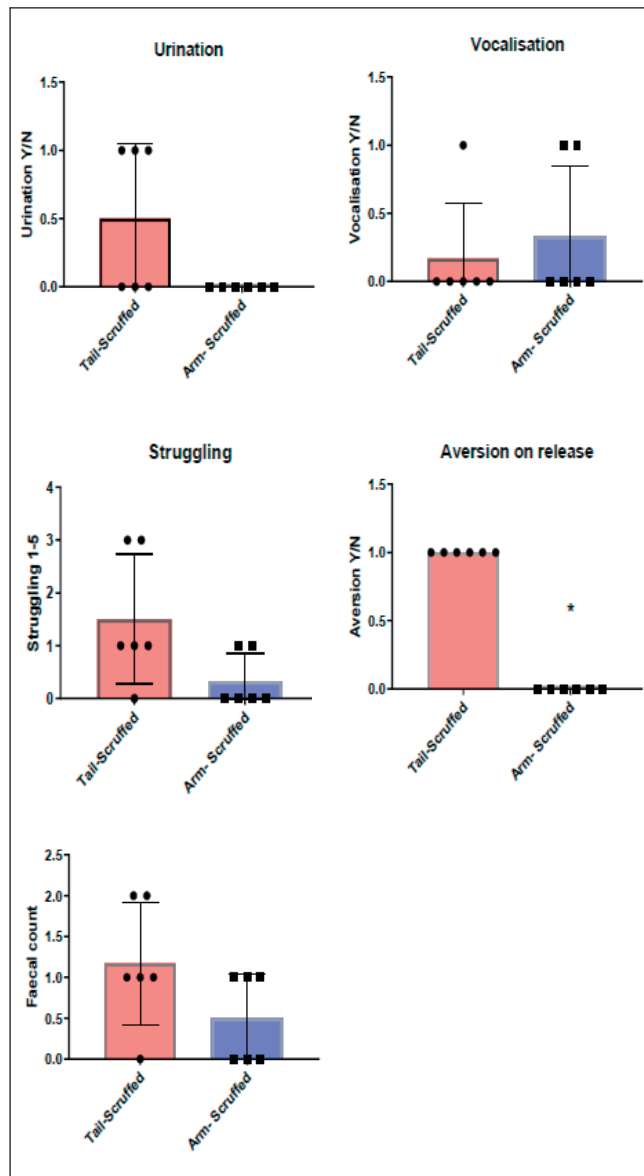


Figure 2: Preliminary studies quantifying overt signs of stress in mice suggest this modified method is associated with better welfare. Aversion on release from restraint was significantly reduced and all other measures except vocalisation were lower for animals in the modified method group (n=6 per group). *p<0.05 Mann WhitneyU-test

Restraint free oral dosing

Rats and Mice

Many scientific procedures involving animals require the oral administration of test substances. Conventional

methods of oral dosing require the animal to be restrained and the insertion of an oesophageal cannula. Restraint of the animal causes stress and the insertion of a cannula carries a risk of injury. Possible adverse events include tracheal dosing and oesophageal trauma (Procedureswithcare.org.uk,2022).² Voluntary ingestion of drugs in palatable solutions enables restraint-free oral dosing of rats and mice. This refinement not only reduces the stress caused to the animal during the dosing procedure but also eliminates the potential risks associated with oral gavage dosing. This method also allows oral dosing to be delegated to non Personal Licence holders (PIL) to increase the resilience of dosing programmes in the case of increased staff absences.

Method

A few days before you intend to start the dosing schedule, expose the animals to the palatable substance that you will be using in their home cage. An example would be 0.3mL 50% strawberry milk shake and 50% water. They may be reluctant to approach the syringe so, if necessary, the palatable substance may be placed on a surface in the cage and left for the animals to investigate in their own time. By the next day, the animals will know that the substance is safe and tasty and will be quicker to approach and drink from the syringe. Once they are happy drinking from the syringe you can start your dosing study.

To make up drugs, you can follow your normal formulation protocol but substitute your usual vehicle for the diluted palatable substance. For example, we would dissolve the drug in water (50% of total vehicle volume) and then add strawberry milk shake (50% of total vehicle volume) once it has dissolved.

During the dosing schedule the exact volume required for the animal is drawn up into the syringe. If the animals are group housed, then you can either separate the required animal for dosing or dose all animals in the cage at the same time using multiple syringes.

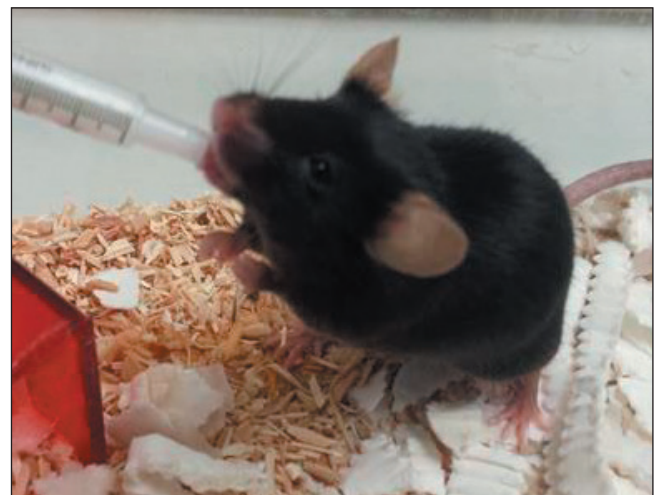


Figure 3. Restraint free dosing of mouse in home cage.

Immediately after the animal has ingested all the required dose from the first syringe it is then presented with a second syringe containing approximately 0.2mL of vehicle solution (e.g. 50% strawberry milk shake and 50% water). At a second timepoint (usually late afternoon) all animals are presented with another syringe of approximately 0.5mL of vehicle solution.



Figure 4. Typical palatable substances for restraint-free oral dosing include strawberry milkshake, peanut butter and condensed milk.



Figure 5. Restraint-free dosing of rat.

Troubleshooting

There are several common issues that prevent or discourage successful oral dosing using voluntary ingestion; our protocol mitigates against these in most cases.

Neophobia

Rats and mice are cautious when presented with new foods and may not drink a novel solution from a syringe when it is first presented. The palatable solution must be introduced about a week before any dosing is planned. The animals are given access to the solution in their home cage and once they discover that the solution is tasty; they will quickly adapt to drinking the solution directly from the syringe.

Conditioned aversion

Drugs that have aversive effects may cause the animals to associate that effect with the palatable solution which could lead to them refusing to ingest the solution.

Some drugs also have a bitter aftertaste that may also discourage future voluntary ingestion.

To prevent this, the animals are given a small amount of the solution without any drug or vehicle immediately after the treatment solution. The dilution of the palatable solution can also be adjusted to mask any unpleasant taste. The animals are also given a second dose of the plain solution at the end of the day. This way they are less likely to associate any effects of the drugs with the palatable solution.

Dosing Accurately

When drugs are mixed in to or placed on palatable substances and presented to the animals it can be difficult, in some cases, to determine the exact amount of drug that the animal has ingested. Our oral drugs are made up to an exact concentration and as animals are drinking from a syringe, the exact amount required for each animal can be drawn up and administered to the animal.

Time and Resources

There is an understandable resistance to switching to dosing methods that might take additional time as this can add to already heavy workloads or even require extra staffing. Once the animals are happy drinking from the syringes, this method can in fact save time as one person can easily dose two animals at the same time. An additional benefit is that, as there is no danger of causing harm to the animal, this dosing technique can be delegated to less experienced or unlicensed members of the team. This can increase flexibility in workloads and resilience to staff absence.

Modified handling

Rats

Not all substances can be administered orally and most alternative dosing methods require the rat to be restrained. Handling and restraint can be a major source of stress

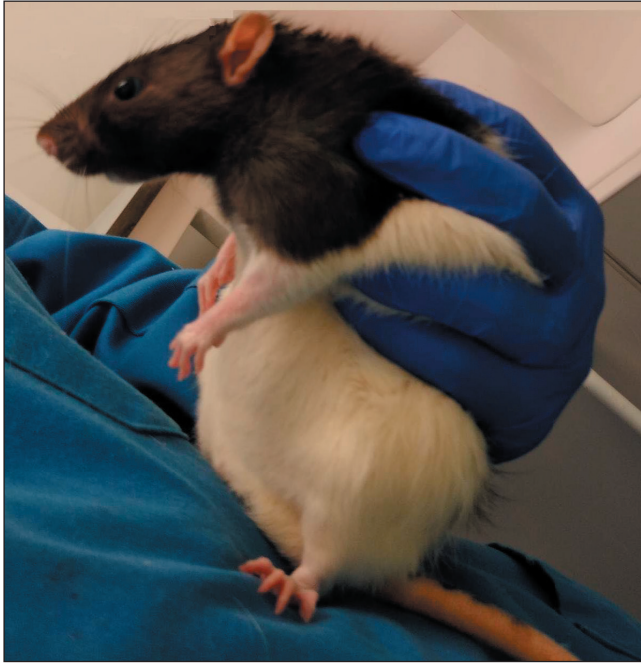


Figure 6. Modified handling for rat I.P. dosing.

which is detrimental to the animal's welfare. It can also make future dosing more difficult and increase the variability of subjects. If the stress caused to the animal during dosing can be lessened, then this could

have a significant impact on the welfare of the animals and the reliability of the experiments.

All members of our laboratory are taught to restrain and dose rats without having to use the standard scruffing or two-person restraint (Procedures with Care) method. For intraperitoneal (I.P.) injections, the rat is held around the shoulders and gently pushed down against the handler's chest, stomach or side. In this position the stomach is relaxed making I.P. injections less aversive and the rat is being controlled without it becoming agitated. Animals handled using this method have been shown to have a more positive affective state, decreased signs of aversion and lower stress hormone levels.²

Results for (a) struggling, (b) vocalisation and (c) faecal counts during dosing in Lister Hooded (LH) 400–550 g, $n=8$ per group, young Wistar, 280–320 g, $n=6$ per group, Stock Wistar 400–5,00 g, $n=5$ per group, Stud Wistar 550–700 g, $n=5$ per group and Sprague Dawley rats 290–320 g, $n=4$ per group, All, $n=24$ per group. Data shown as mean \pm s.e.m. Plasma analysis of (d) corticosterone and (e) amphetamine for conventional ($n=6$) and modified methods ($n=5$; insufficient blood to process was collected from one animal. Data shown as mean \pm s.e.m.. (f) affective bias induced by intraperitoneal dosing by the conventional versus the

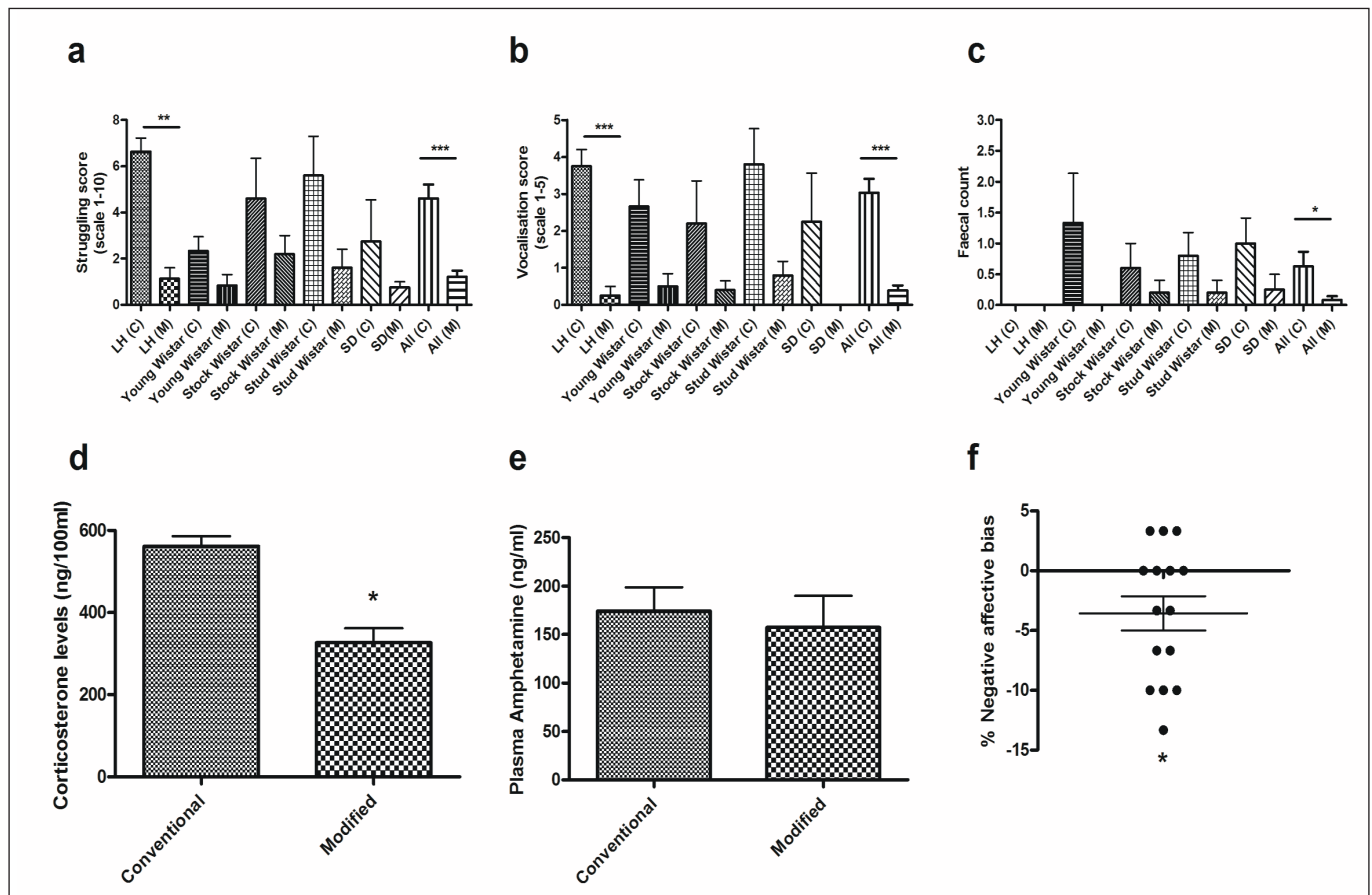


Figure 7. Effect of IP dosing using the conventional scruff method (C) versus the modified method (M) on behavioural, physiological and psychological measures of stress.

modified method as assessed in the ABT. Each data point represents s.e.m. (f) Affective bias induced by intraperitoneal dosing by the conventional method versus the modified method as assessed in the ABT. Each data point represents an individual rat. Error bar s.e.m, n=15 rats, *p<0.01, ***p<0.01 (Stuart & Robinson (2015)).²

References

- ¹ **Hurst J.L., West R.S.** (2010). Taming anxiety in laboratory mice. *Nat Methods*. 2010 Oct;7(10):825-6. doi: 10.1038/nmeth.1500. Epub 2010 Sep 12. PMID: 20835246.
- ² **Procedureswithcare.org.uk.** (2022). *Procedures With Care – Administration of Substances*. [online] Available at: <http://www.procedureswithcare.org.uk/administration-of-substances/> [Accessed 07 February 2022].
- ³ **Stuart, S. and Robinson, E.S.J.** (2015). Reducing the stress of drug administration: Implications for the 3Rs. *Scientific reports*. 5.14288.101038/srep14288.



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