

Refined Methods for Handling, Habituation and Training

If you work with animals in research, you will encounter various situations where handling is required. This may occur during routine husbandry, such as health checks or cage changes, or during interventions that are part of the procedures you conduct. Handling can cause stress to the animals, particularly if they are not used to it or if it is forced upon them. When animals experience stress, several physiological functions and systems are affected, which in turn may influence your results and the variability observed in your data.

To enhance animal welfare and ensure the quality of research outcomes, it is important to handle animals calmly and gently and, where appropriate, to train them in preparation for the procedures you intend to carry out. Regardless of your level of experience in handling and training laboratory animals, it is essential to pause and reflect on how you will approach the task. Several factors should be considered, such as the species involved, the animal's previous experiences, and the type procedures included in your study.

Below is information intended to support you in planning how to handle and train your animals. It is primarily intended for those working with research animals kept by humans, in research facilities or otherwise. However, parts of the information may also be relevant to those conducting research involving wild animals.

According to Swedish legislation on laboratory animals (3 ch. 7 § Statens jordbruksverks föreskrifter och allmänna råd (2025:28) om försöksdjursverksamhet, saknr. L 151), the designated laboratory animal veterinarian must develop habituation and training programmes tailored to the animals and the experiments conducted at the facility. You are encouraged to consult your veterinarian regarding the content of this guidance in relation to the studies you plan to undertake.

If you already have an ethical approval and discover a way to refine your procedures without negatively affecting animal welfare, contact your Animal Welfare Body to get an assessment of whether they can help you amend your ethical approval.¹

¹ 5 ch. 6 § SJVFS 2025:28.

Acclimatisation

Being transported from a breeder or relocated within the facility can be stressful for animals. Therefore, you should allow sufficient time for arrival and unpacking and handle the animals calmly and gently to minimise stress.

Before animals are included in procedures, they must be given the opportunity to acclimatise to the new environment, recover from the stress caused by the change, and physiologically adapt to the new conditions. You can find more information about the acclimatisation of mice, rats and zebrafish in guidance we have previously published.

[Acclimatisation of mice prior to experiments | Swedish 3Rs Center \(jordbruksverket.se\)](#)

[Acclimatisation of rats prior to experiments | Swedish 3Rs Center \(jordbruksverket.se\)](#)

[Acclimatisation of zebrafish prior to experiments | Swedish 3Rs Center \(jordbruksverket.se\)](#)

Habituation

During the acclimatisation period and prior to the procedures, you may work with habituation. The purpose of habituation is to create positive associations and minimise unpleasant experiences so that animals gradually become accustomed to handling. At the same time, you and your animals become familiar with one another.

To build positive associations with handling, you can use rewards such as treats or access to an enriched environment like a play area or activity box. The reward should be provided immediately after the handling to help the animal form the desired association. If you use food rewards, make sure the amount is appropriate for the species you are working with.

When working with habituation, it is important to proceed in small steps and introduce handling gradually. Advancing too quickly may cause stress, potentially leading to passivity and learned helplessness. This means that the animals cease to resist your handling, but it is not at all the same as having learned to associate handling with something positive and therefore accepting it.

To avoid progressing too rapidly, you may consult established habituation protocols for guidance. At the same time, you should closely monitor each animal's behavioural responses and adjust the pace accordingly, allowing additional time for individuals who need it.

Often, only short habituation sessions over a few days are needed to achieve a positive effect, and habituation can begin as soon as the animals arrives at the facility.

Once your animals are well habituated to your handling, you may proceed to habituate them to other interventions needed for conducting your procedures with minimal stress to the animals. This may involve receiving a treat in a specific location while you take a blood sample from the tail, accepting to remain still in your lap for an infusion, or being briefly restrained with a particular grip while you administer an injection. As before, you work with rewards and small steps so that the animals gradually accept the required procedures without experiencing stress.

Further information on habituation and examples of protocols for habituating mice and rats

[Habituation | The 3Hs Initiative \(3hs-initiative.co.uk\)](https://3hs-initiative.co.uk/habituation)

[Handling and Habituation | The 3Hs Initiative \(3hs-initiative.co.uk\)](https://3hs-initiative.co.uk/handling-and-habituation)

[Vetbed Habituation | The 3Hs Initiative \(3hs-initiative.co.uk\)](https://3hs-initiative.co.uk/vetbed-habituation)

Handling of Laboratory Animals

The methodology you use when handling animals is of great importance. Keep in mind that you should:

- Handle animals calmly and gently.
- Avoid sudden and rapid movements.
- Avoid loud noises.
- Avoid restraint.

Handling animals in the least stressful manner possible requires patience and training. It is essential to give the animals time and to adapt the handling and training methods to the species, as well as to individual animals. Certain species, strains or individuals may require more time to become accustomed to different procedures. This applies not only to smaller animals such as mice and rats – larger animals such as rabbits, pigs, chickens, sheep and cattle also need to become familiar with the people who will handle them and be treated in a calm and gentle manner.

Regarding mice, the Swedish National Committee for the Protection of Animals Used for Scientific Purposes has issued a statement advising that mice should not be caught, lifted or moved by the tail. It is preferable to handle mice using cupped hands, a tube, or another suitable aid.

Previously, rats have also been handled by the tail, but this can cause injury as rats are significantly heavier than mice. Today, most people lift rats using a grip over the shoulders or under the abdomen. Rats may also be handled using a tube or another type of aid.

When handling fish, you should, as far as possible, avoid removing them from the water. For all aquatic animals, regardless of whether they breathe air or not, care must be taken not to disturb the mucus layer on their skin.

General principles for handling and restraint

[Handling and restraint: General principles | NC3Rs \(nc3rs.org.uk\)](https://nc3rs.org.uk/handling-restraint-general-principles)

[Handling | The 3Hs Initiative \(3hs-initiative.co.uk\)](https://3hs-initiative.co.uk/handling)

More information on handling of mice with cupped hands or a tube

[Mouse handling | NC3Rs \(nc3rs.org.uk\)](https://nc3rs.org.uk/mouse-handling)

Statement from the Swedish National Committee: Don't lift mice by the tail!

[Don't lift mice by the tail | Swedish 3Rs Center \(jordbruksverket.se\)](https://jordbruksverket.se/dont-lift-mice-by-the-tail)

Restraint and Immobilisation

Certain procedures may require you to restrain a part of the animal's body or immobilise the entire animal to prevent movement. For small animals, manual restraint using hands and possibly arms is often sufficient, but there is also equipment designed to immobilise animals, known as restrainers. These are typically shaped like tubes in which the animal is enclosed. For larger animals, restraint or immobilisation often involves limiting the space available for movement by using a halter or pressing the animal against a gate.

Methods of restraint, and especially immobilisation, often cause significant stress to animals. Animal experiments must be designed to cause the least possible suffering.² Therefore, always consider whether you can perform your procedures in an alternative way or whether you can train the animals to avoid the need for immobilisation.

If you use less stressful methods for daily handling, animals may be more willing to accept short periods of restraint. Through training, you may also teach animals to accept restraint or to cooperate with certain procedures so that restraint is not needed.

² 7 ch. 5 § SJVFS 2025:28.

Training of Laboratory Animals

Training laboratory animals to participate in studies offers significant advantages. It makes handling easier and reduces stress for the animals, thereby contributing to improved animal welfare and research quality.

There are many ways to train animals. Generally, the most effective method is training through positive reinforcement, which involves rewarding the behaviour you wish to encourage. Rewards may include food, treats, petting, or access to a toy. Adapt the reward to suit the specific context of your research.

By rewarding small steps, you shape the animal's behaviour and can, for example, train it to remain still without restraint for an injection or blood sample without becoming stressed. For larger animals, the same principles apply, although you may need to train the animal to enter a specific box or to hold a body part against a target in order to receive an injection or provide a blood sample.

Positive reinforcement gives animals the choice to participate in training. It also increases their sense of control over their environment. If animals have a good relationship with humans and predominantly experience positive interactions, brief periods of restraint and discomfort associated with procedures do not result in lasting negative effects.

When training or planning training for your laboratory animals, you should use well-defined standard operating procedures. This helps ensure high-quality training and consistency among staff performing the training. These procedures can be adapted to suit a specific study or a specific species at a facility.

To train animals safely and successfully, you need knowledge, but you also need to train yourself – do not hesitate to seek support from colleagues with more experience.

Keep in mind that:

- When animals are gently handled and/or trained in preparation for a specific procedure, their stress and discomfort are reduced, which contributes to both improved animal welfare and better research quality.
- Animals that are trained in the procedures included in the study and accustomed to being handled by humans are easier to manage, which also reduces stress for you or the staff performing the procedures.
- Training does not need to be extensive or time-consuming; short training sessions over a few days are often sufficient, and training can begin as soon as the animals arrive at the facility.

Videos and Further Reading on Handling and Training of Animals for Research

There are many excellent examples of training laboratory animals. One of the most notable Swedish examples comes from RISE Research Institutes of Sweden, where mice and rats have been trained to voluntarily participate in various types of procedures.

Below are links to further information, recorded lectures and other videos related to handling and training. Keep in mind that you do not need to limit your search for information to training specifically for laboratory animals. There is a wealth of knowledge on training companion animals using positive reinforcement, which may also be relevant to your work.

We have also included a selection of scientific articles addressing the effects of refined handling, habituation and training.

Positive reinforcement training for laboratory animals

[Training animals | NC3Rs \(nc3rs.org.uk\)](https://nc3rs.org.uk)

Examples of protocols, standard operation procedures and guidelines

[Protocols, SOPs and Guidance Documents | The 3Hs Initiative \(3hs-initiative.co.uk\)](https://3hs-initiative.co.uk)

Handling och training of mice and rats

[Positive reinforcement | The 3Hs Initiative \(3hs-initiative.co.uk\)](https://3hs-initiative.co.uk)

[Housing, handling and training mice | RISE Research Institutes of Sweden \(youtube.com\)](https://www.youtube.com/watch?v=...)

[Housing, handling and training rats | RISE Research Institutes of Sweden \(youtube.com\)](https://www.youtube.com/watch?v=...)

[Recorded webinar: Handling and training of mice and rats for low stress procedures at RISE Research Institutes of Sweden | NC3Rs \(nc3rs.org.uk\)](https://nc3rs.org.uk)

Training of pigs

[Target training pigs within an isolation unit – a pilot study | The Pirbright Institute \(pirbright.ac.uk\)](https://pirbright.ac.uk)

Guidelines for training of animals at zoos and aquaria

[Animal Training Guidelines | EAZA \(eaza.net\)](https://eaza.net)

[Recorded webinar: Animal Training Guidelines | EAZA \(youtube.com\)](https://www.youtube.com/watch?v=...)

Examples of scientific articles addressing the effects of refined handling, habituation or training

Dickmann, J., Gonzalez-Uarquin, F., Reichel, S., Pichl, D., Radyushkin, K., Baumgart, J., Baumgart, N. (2022). Clicker Training Mice for Improved Compliance in the Catwalk Test. *Animals*. 12(24):3545.
<https://doi.org/10.3390/ani12243545>

Ghosal, S., Nunley, A., Mahbod, P., Lewis, AG., Smith, EP., Tong, J., D'Alessio, DA., Herman, JP. (2015). Mouse Handling Limits the Impact of Stress on Metabolic Endpoints. *Physiology & Behavior*. 150: 31-37.
<https://doi.org/10.1016/j.physbeh.2015.06.021>

Gouveia, K., Hurst, J. (2017). Optimising Reliability of Mouse Performance in Behavioural Testing: The Major Role of Non-Aversive Handling. *Scientific Reports*. 7: 44999. <https://doi.org/10.1038/srep44999>

Gouveia, K., Hurst, J. (2019). Improving the practicality of using non-aversive handling methods to reduce background stress and anxiety in laboratory mice. *Scientific Reports*. 9: 20305. <https://doi.org/10.1038/s41598-019-56860-7>

Hurst, J., West, R. (2010). Taming anxiety in laboratory mice. *Nature Methods*. 7: 825–826. <https://doi.org/10.1038/nmeth.1500>

Jønholt, L., Bundgaard, C.J., Carlsen, M., Sørensen, D.B. (2021). A Case Study on the Behavioural Effect of Positive Reinforcement Training in a Novel Task Participation Test in Göttingen Mini Pigs. *Animals*, 11(6): 1610.
<https://doi.org/10.3390/ani11061610>

Leidinger, C., Herrmann, F., Thöne-Reineke, C., Baumgart, N., Baumgart, J. (2017). Introducing Clicker Training as a Cognitive Enrichment for Laboratory Mice. *Journal of Visualized Experiments*. 121: 55415.
<https://doi.org/10.3791/55415>

Neely, C., Lane, C., Torres, J., Flinn, J., (2018). The Effect of Gentle Handling on Depressive-Like Behavior in Adult Male Mice: Considerations for Human and Rodent Interactions in the Laboratory. *Behavioural Neurology*. 2018: 2976014. <https://doi.org/10.1155/2018/2976014>

Pichl, D., Dechert, P., Börner, A., Gonzalez-Uarquin, F., Reichel, S., Baumgart, N., Baumgart, J. (2025). Enhancing Gerbil Welfare Through Positive Reinforcement Training in Research Environments. *Journal of Visualized Experiments*, 222: e68657. <https://doi.org/10.3791/68657>

[Mouse handling: Research papers | NC3Rs \(nc3rs.org.uk\)](https://nc3rs.org.uk/mouse-handling-research-papers)