THE SWEDISH 3Rs CENTER

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Acclimatisation of mice prior to experiments

A support material from the Swedish 3Rs Center

Photo: Sören Andersson/2See AB

Contents

Background

This document is part of a series of acclimatisation documents that the Swedish 3Rs Center has produced on behalf of the Swedish National Committee for the Protection of Animals Used for Scientific Purposes. The series consists of three parts with a focus on mice, rats and zebrafish respectively. This part is intended for everyone who works with mice as laboratory animals and can be used as a support when writing acclimatisation routines of the animal facility, which is a requirement according to the Directive (2010/63/EU) of the European Parliament and of the Council on the protection of animals used for scientific purposes, as well as chapter 16, 7 § of the Swedish research animal legislation (Statens jordbruksverks föreskrifter och allmänna råd (2019:9) om försöksdjur). The material is based on scientific literature and the Swedish animal welfare legislation. In addition, the Swedish 3Rs Center, in collaboration with veterinary students at the Swedish University of Agricultural Sciences, have collected experiences from people and organisations who work with mice, rats and zebrafish as laboratory animals in Sweden. The data collection was carried out through a questionnaire in order to get an overview of how acclimatisation is carried out in Swedish research animal organisations. At the end of the material, the references that have been used are listed. Please use the list of references to get deeper and more detailed information about the subjects we address. You are also welcome to contact the Swedish 3Rs Center if you have any further questions.

Introduction

Acclimatisation is a term that can be used in a variety of contexts. In this document, we use the term to describe the process that the body goes through to adapt to changes that are often linked to the environment. During an acclimatisation period, the animal is given an opportunity to recover from the stress that a change entails, and physiologically adapt to the new conditions. Acclimatisation is of importance both for the welfare of the animals and for more reliable research results. The length of the acclimatisation process depends on several different factors, such as the cause of stress, new environmental conditions and the animal's individual circumstances.

According to the Directive (2010/63/EU) of the European Parliament and of the Council on the protection of animals used for scientific purposes, Annex III point 3.1.a, introduction of new animals shall be a part of the health strategy that every facility must have. In point 3.7 there is also a requirement that facilities must have habituation programs for the animals. Furthermore, the Commission Recommendation of 18 June 2007 on guidelines for the accommodation and care of animals used for experimental and other scientific purposes (2007/526/EC), general section, 4.4 states that a period of acclimatisation is needed to allow animals to recover from transport stress, to become accustomed to a new environment and to get used to new husbandry and care practices. According to the Commission's recommendation, a period of acclimatisation is necessary even if the animals are perceived to be in good health. The amount of time required depends on what the animals have been subjected to. For example, a longer acclimatisation period may be required after long international transports that disrupt the animals' circadian rhythm, compared to after shorter transports within the country.

In chapter 16, 7 § in the Swedish Research Animal Regulations (Statens jordbruksverks föreskrifter och allmänna råd (2019:9) om försöksdjur) it is clear that there must be a written plan for assessing the physical and psychological well-being of the experimental animals. The plan must also include habituation and training programs adapted to the experimental animals and animal experiments in question. This plan must be produced by a laboratory animal veterinarian or, where applicable, by an expert. If an ethologist is associated with the facility, the plan must be developed in consultation with this ethologist. Thus, there are legal requirements for experimental animals to be acclimatised, but it is not clear how the acclimatisation is to be carried out. Therefore, the approaches vary between different organisations in Sweden.

What is stress?

It is known that stress in laboratory animals can affect research results negatively. In a stressed animal, several different physiological functions and systems are affected, which leads to an increased variation in the results. For example, the

levels of adrenaline, norepinephrine, cortisol and corticosterone in the blood becomes elevated. These hormones are secreted to some extent in an unstressed state, but production increases in stressful situations in order to prepare the body to flee or fight. Stress over a longer period of time also affects the animal's ability to maintain the body's balance, which negatively affects the immune system and increases the risk that the animal will suffer an illness.

Stress is a difficult concept to define. It usually means the body's response to an external stimulus, called a stressor. The response can be both physiological and behavioural. Stressors can consist of a variety of things and what constitutes a stressor can differ between individuals. Examples of stressors are social hierarchy, an injury, exhaustion, or difficulty managing the temperature of the environment. Stress can often be linked to a change in the animal's environment that the animal is trying to cope with. Stress does not have to be negative, but prolonged or severe stress can affect the individual negatively, especially if the individual lacks control over the situation.

There are a number of different parameters that can be used to measure stress in an animal, such as the hormones cortisol, corticosterone, adrenaline and norepinephrine, the animal's behaviour, weight loss or changes in feed and water consumption. In order to get a good insight into the reaction, it is necessary to assess several parameters of the animal. For example, making the assessment only from the animal's behaviour has proven to be difficult since a stressed animal both can freeze and become completely still or run around, as well as vocalise or be completely silent. Results in studies dealing with acclimatisation vary depending on which parameter or parameters researchers have chosen to investigate. For example, one study looked both at the mice's levels of corticosterone in the blood and their behaviours as indicators of stress. The results showed that the levels of corticosterone were back to normal within 24 hours when mice were moved between different rooms in the same building, but four days was not enough for the studied behaviours to return to normal.

Survey on acclimatisation in Sweden

During the autumn of 2022, a survey was sent out to the networks of animal welfare bodies, laboratory animal veterinarians and animal technicians with whom the Swedish 3Rs Center was in contact. The survey was also sent to the principal investigators who, during the years 2018–2022, had been granted ethical permission for research on any of the animal species in question. Recipients were able to spread the survey further, since the links were reusable. The survey was available in both Swedish and English. There were three versions of the survey and the recipient was asked to answer the ones corresponding to the animal species they worked with, mice, rats or zebrafish. The questions were the same in all three versions. All responses were anonymous and it is therefore not possible to determine whether several responses came in from the same organisation. A total of 153 people completed the survey, of which 105 people answered regarding mice, 38 people regarding rats and 10 people regarding zebrafish. A large majority of the respondents were researchers, see table 1. The next two largest groups consisted of laboratory animal technicians or other care staff and laboratory animal veterinarians.

Table 1. Distribution of the respondents' professions.

Profession	Number of respondents
Researcher	108
Animal technician or other care staff	15
Laboratory animal veterinarian	11
Laboratory assistant	8
Named Animal Care and Welfare Officer (NACWO)	7
Animal facility manager	2
License holder	1
Other	1
Total	153

Written plan for acclimatisation

According to the National Animal Welfare Report 2022, one of the most common insufficiencies found during physical controls of laboratory animal facilities is linked to the following control point "There are written plans for preventive animal health care, animal welfare-related measures and assessment of the physical and psychological well-being of the laboratory animals". This control point includes the written plan for acclimatisation. Also, the responses from our survey indicate that there may be insufficiencies in the written plan for acclimatisation. According to the survey, the shortcomings can be found in the design and accessibility of the plan, as well as in the application of the plan. A number of respondents were unsure of what the written plan contained and where it could be found. When asked

what types of sources the written plan for acclimatisation was based on, the survey indicated that the experiences of the respondents were the most common source. For those who responded to the survey, legislation and scientific studies were two other important resources. One respondent specifically described that other scientific studies in the same field were consulted to apply a similar approach to acclimatisation in order to increase comparability between studies.

Survey questions about mice

A total of 105 people completed the survey that included questions about mice. The relatively low number of respondents, combined with the fact that it is not clear which organisation the respondents belong to, should be considered when conclusions are drawn. The purpose of the survey was to investigate when and how acclimatisation of mice was carried out in Sweden.

When are mice acclimatised in Sweden?

Respondents who answered yes to the question **Are mice acclimatised when first arriving at your facility?** were given the opportunity to describe in free text how long their mice were acclimatised. There were 87 respondents (83 %) who answered yes to the question, of which 83 respondents also described the length of the acclimatisation period. Other respondents did not acclimatise mice upon arrival at a new facility, did not know if it was performed at their facility, or chose not to answer the question. The survey results show that mice were acclimatised between 3 and 28 days. The majority of respondents (57 %) indicated that they acclimatised their mice for seven days, see figure 1.

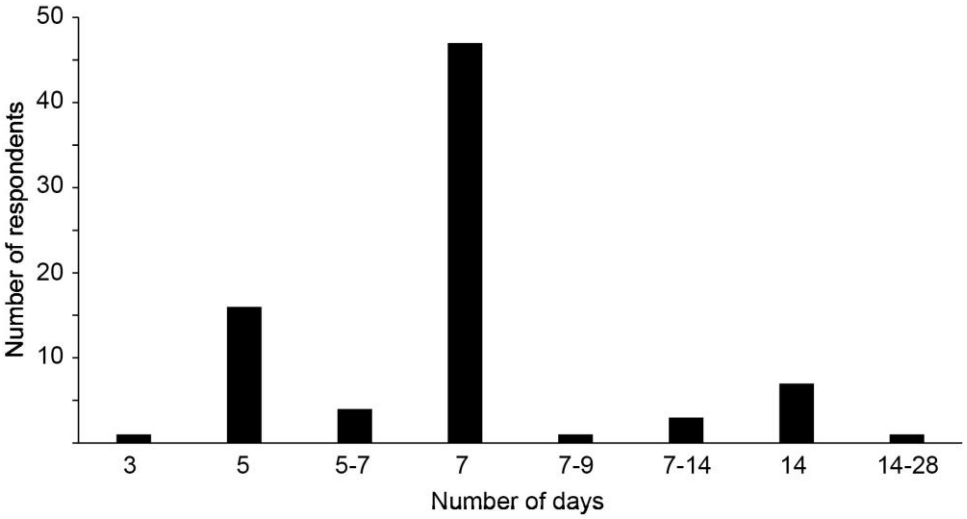

Figure 1. Number of days stated for mouse acclimatisation upon arrival to a new facility. The answers were given in free text and may therefore overlap.

When the survey respondents were asked **Are mice acclimatised when they are re-grouped at your facility?** there were 20 respondents (19 %) who answered yes, of which 17 respondents also described how it was performed. The remaining respondents did not acclimatise mice when regrouping, did not know if it was performed at their facility, or chose not to answer the question. The majority of respondents (30 %) reported that they adjusted the length of acclimatisation according to which trial the mice were going to be included in, which sometimes meant that they did not acclimatise the mice at all. Otherwise, the answers indicated that the length of acclimatisation varied between 2 and 21 days.

To the question **Are mice acclimatised when they are moved to a new location within your facility?** there were 37 respondents (35 %) who answered yes, of which 34 respondents also described how it was performed. The other respondents did not acclimatise mice when moving them within the facility, did not know if it was done at their facility, or chose not to answer the question. The majority of

respondents (35 %) acclimatised their mice for a week when moving them within the facility. Seven respondents (19 %) wrote that it varied whether, and for how long, they acclimatised the mice depending on the experiment. One respondent (3%) commented that they acclimatised the mice for a week if the climate, such as the temperature, changed, otherwise they did not acclimatise them at all. Other responders acclimatised mice for 1–28 days.

For many of those who responded to the survey, it was not relevant to acclimatise mice to a changed circadian rhythm, as this was not stated to occur at their facility. There were 17 respondents (16 %) who answered yes to the question **Are mice acclimatised when the circadian rhythm is changed at your research facility?** The answers showed that the time for acclimatisation varied between 1 and 14 days. The other respondents did not acclimatise mice to altered circadian rhythm, did not know if it was carried out at their facility or chose not to answer the question.

How are mice acclimatised in Sweden?

The survey respondents were given an opportunity to briefly, in free text, describe how they carried out acclimatisation of mice. We received 84 descriptions of how they approached it. The answers showed five overall approaches, see figure 2. The majority (76 %) said that the mice were left alone in the home cage during the acclimatisation period, without further measures being taken. The second largest group (19 %) described that they handled or trained the mice during the acclimatisation period. Two respondents (2 %) described that the acclimatisation took place in a reception room or a quarantine. One respondent (1 %) indicated that they adapted the acclimatisation approach to the type of experiment the mice would be included in. Another respondent (1 %) described that the mice were monitored more frequently during the acclimatisation period.

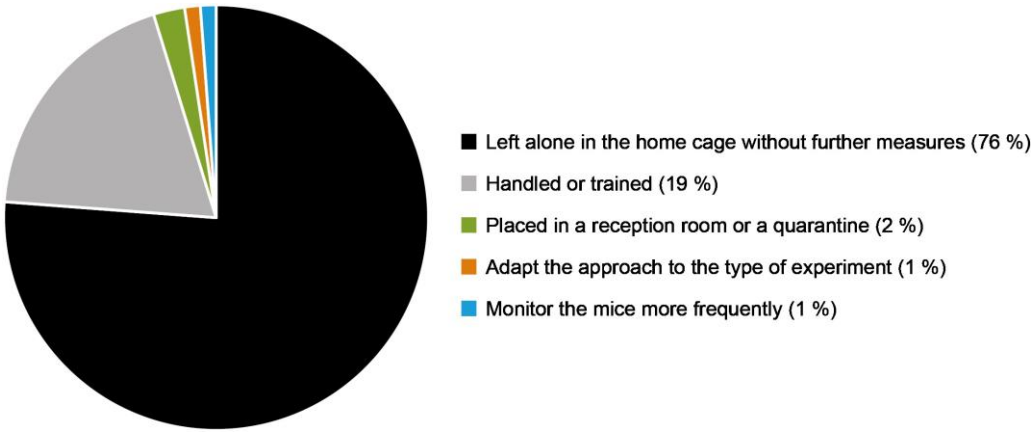

Figure 2. How mice were acclimatised in Sweden. Expressed in percent of the respondents that chose to describe their approach.

What does the research say about acclimatisation of mice?

When reviewing the scientific literature, four areas of acclimatisation were identified, which we chose to focus on; transport between facilities, transport within a facility, changed circadian rhythm and differences between age, sex and strain. These areas are explored in the headings below.

Transport between facilities

It is known that mice are affected by transportation, but there are still gaps in knowledge about how they are affected. Because of this, it is also unclear how a period of acclimatisation after transport should be designed. Several studies have determined that acclimatisation after transport is necessary, but many times these studies have considered different types of parameters, which makes it challenging to draw a general conclusion. The length of acclimatisation is also dependent on a number of different factors, such as the length of transport and the strain of the mouse. Long transports can require a longer recovery time. For example, there is a correlation between transport length and weight loss, where animals lose more weight the longer they are transported. It also takes longer for them to return to their normal weight. Furthermore, a long transport can disrupt the circadian rhythm, which several studies have shown to negatively affect research results. See more about this below.

The scientific literature in combination with the results of the survey we conducted show that an acclimatisation length of seven days is common. Seven days have also been shown to be sufficient for certain physiological parameters to normalise, such as levels of the stress hormones adrenaline, noradrenaline corticosterone and cortisol. Other parameters that are affected by transport and usually have time to return to normal levels after one to seven days are increased heart rate, decreased weight, and increased blood sugar. Additional blood parameters, including the percentage of red blood cells in the blood and some parameters of the immune system, usually recover within one to seven days. There are also scientific studies that show other results. In one study, young mice were transported overnight to a new facility. These mice showed signs of stress for at least four weeks and gained weight slower compared to the control group.

Other parameters, such as blood pressure, may require a longer period of acclimatisation in order to return to normal levels. In a study where young mice were transported, they still showed elevated blood pressure after a week of acclimatisation. It took three weeks for the blood pressure to drop, and only after six weeks did the blood pressure match the levels of the control group. Based on the results, the researchers stated that mice should be acclimatised for at least three

weeks when they are to be included in experiments where blood pressure is of interest.

For some immunological parameters, the results indicated that it required four weeks of acclimatisation before the presence of faecal corticosterone and faecal antibodies returned to more normal levels. In addition, the reproductive capacity of the mice can be negatively affected after a transport. It may take several weeks, up to months, before reproductive capacity is normalised.

Transport within a facility

Mice are also affected by being moved short distances within the same building. Studies have shown that after such transport, mice show signs of stress for at least 24 hours. It has also been shown that the immune system is affected where levels of white blood cells decrease when mice are moved within a facility. These levels normalise after a day or two. In one study, the mice's behaviours and levels of corticosterone in the blood were examined after a move between different rooms in the same facility. The results indicated that it took 24 hours for the mice's corticosterone levels to return to normal. However, it turned out that four days was not long enough for the mice's behaviour to return to normal.

Changed circadian rhythm

A change in the circadian rhythm, for example before an experiment or as a result of transport between different continents, affects mice physiologically, behaviourally and cognitively. The studies dealing with the subject suggest that it takes several weeks, up to months, for mice to acclimatise to a new circadian rhythm. When cognitive studies are performed, it is therefore crucial for the results that the mice are acclimatised to the circadian rhythm and that the experiments are performed during the mice's naturally active period. As mice are nocturnal animals, they are active when it is dark. Behavioural testing during what corresponds to dawn and dusk for the mice is not recommended since they exhibit a different activity level during those periods.

Differences between age, sex and strain

Several studies have shown that sensitivity to stress differs between mouse strains. This means that some strains are more negatively affected than others by changes in the environment or a stressful experience, such as transportation. It has also been shown that the learning process and the way the mice show fear can differ between different strains. How the mice are housed and how old they are can also cause variations in stress sensitivity. Finally, there are studies suggesting that females who are housed solitarily and young mice tend to be more sensitive to stress. These animals may therefore require a longer period of acclimatisation.

Conclusion

Every researcher should ensure that the mice they use in research have been acclimatised for a sufficient period of time. It is not only important because it is included in the legislation, but also because stressed mice can experience reduced animal welfare and negatively affect research results. It is not obvious what an adequate period of time is. Since the time needed depends on a variety of factors, a balanced decision must be made in each case. Procedures and routines outside of experiments are difficult to standardize. It could, for example, regard a transport that contains many different elements. These elements during transport can be handling, weighing, new cage mates, handling the transport box, travel by plane, car or boat, constant disturbances, sounds, smells and other impressions. Factors that should be considered prior to such acclimatisation include:

- Length of transportation
- Changes in circadian rhythm
- Stress sensitivity of the strain
- The age of the mouse
- The sex of the mouse
- Physiological parameters of importance for the experiment

Examples of additional changes that may affect research results include:

- New humans
- New routines
- New feed
- Changed temperature
- Changed humidity

Scientific literature should be consulted to provide guidance on how stress affects the strain of mice that is going to be used, and the type of research that is going to be conducted. In some experiments it may be sufficient that the parameters relevant to the experiment return to stable levels while others are still stabilising. However, for behavioural experiments or other studies of the whole mouse, a longer acclimatisation period may be necessary for mice to acclimatise completely.

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