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# Acclimatisation of rats prior to experiments

## A support material from the Swedish 3Rs Center


Photo: Emelie Jansson

# 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 rats 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 has shown that rats flown from the US to Korea are acclimatised after seven days based on the levels of corticosterone in the blood. When the researchers instead looked at stress proteins in the heart, brain, kidneys, lungs, liver and muscles, these were still elevated after a week of acclimatisation.

# 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 rats

A total of 38 people completed the survey that included questions about rats. 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 rats was carried out in Sweden.

# When are rats acclimatised in Sweden?

Respondents who answered yes to the question **Are rats acclimatised when first arriving at your facility?** were given the opportunity to describe in free text how long their rats were acclimatised. There were 36 respondents (95 %) who answered yes to the question and described the length of the acclimatisation period. Other respondents did not acclimatise rats 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 responses showed that there was a variation between 4 and 14 days. The majority of respondents acclimatised their rats for 5–7 days, see figure 1.

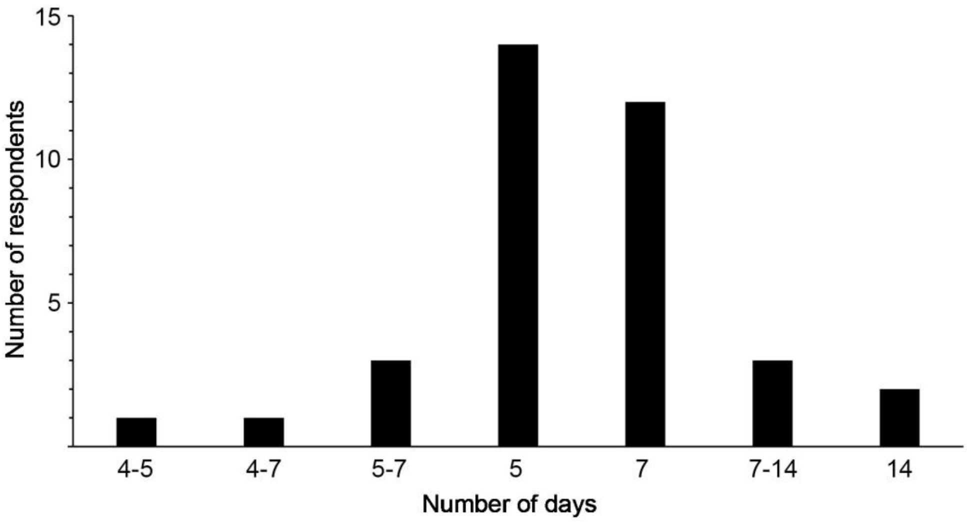


**Figure 1. Number of days stated for rat acclimatisation upon arrival to a new facility. The answers were given in free text and may therefore overlap.**

When the survey respondents were asked, **Are rats acclimatised when they are re-grouped at your facility?** there were only two respondents (5 %) who answered yes and described how it was performed. One of the respondents told us that rats were always acclimatised when regrouped, but that the length of acclimatisation varied. Adult rats that would remain on the same circadian rhythm were acclimatised for seven days. The other respondent indicated that rats were acclimatised for 2–4 days upon regrouping. The remaining respondents did not acclimatise rats when regrouping, did not know if it was performed at their facility, or chose not to answer the question.

To the question **Are rats acclimatised when they are moved to a new location within your facility?** there were three respondents (8 %) who answered yes and indicated that they acclimatised rats for one hour, three days, and five days, respectively. Other respondents did not acclimatise rats when moving them within the facility, did not know if it was done at their facility, or chose not to answer the question.

Eight respondents (21 %) answered yes to the question **Are rats acclimatised when the circadian rhythm is changed at your research facility?** and then described how it was performed. Their responses showed that the time for acclimatisation varied between 5 and 14 days. Other respondents did not acclimatise rats to the altered circadian rhythm, did not know if it was carried out at their facility or chose not to answer the question.

# How are rats acclimatised in Sweden?

The survey respondents were given an opportunity to briefly describe in free text, how they carried out acclimatisation of rats. We received 36 descriptions of how they approached it. The answers showed two general approaches, see figure 2. The majority (56 %) responded that they handled or trained rats during the acclimatisation period. The other group (44 %) described that rats were left alone in the home cage during the acclimatisation period, without further measures being taken.

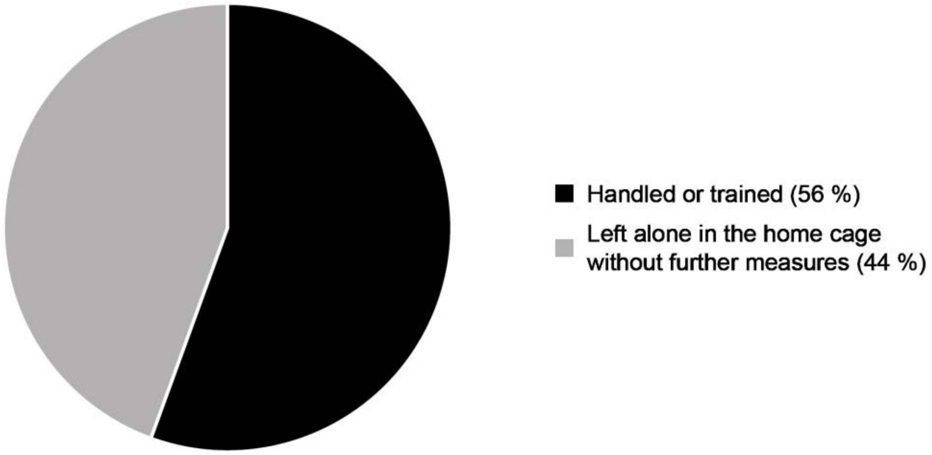


**Figure 2. How rats were acclimatised in Sweden. Expressed in percent of the respondents choosing to describe their approach.**

# What does the research say about acclimatisation of rats?

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 rats are affected by transportation, but there are still gaps in the knowledge regarding 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 sex of the rat. 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.

The scientific literature in combination with the results of the survey we conducted, show that an acclimatisation length of five to seven days is common. Five to seven days may be sufficient for several parameters to stabilize, such as blood pressure, heart rate, body temperature, weight and the level of corticosterone in the blood. However, levels of corticosterone seem to be an uncertain parameter as several studies have shown a large variation occurring between individuals despite them undergoing the same treatment. Additionally, in female rats, levels can vary depending on where they are in the oestrous cycle. Therefore, recommendations for acclimatisation length based on corticosterone levels vary widely, from three days up to three weeks. Other research results indicate that three weeks is not enough time to stabilize the corticosterone levels. Based on blood glucose and corticosterone levels, one study suggests that the length of acclimatisation should be two weeks for males and three weeks for females.

There are several other parameters that researchers have looked at in relation to acclimatisation. Research results have shown that stress proteins in the heart, brain, kidneys, lungs, liver and muscles require more than one week of acclimatisation to return to normal levels. Behaviours and activity levels are other parameters for which there are mixed recommendations in the literature, with some studies suggesting that three days is sufficient and others showing that a minimum of 16 days is required. Heart rate has been shown to exhibit fewer variations and can thus be a better indicator of acclimatisation. In males, the heart rate stabilizes after seven to eight days. Females need more time and may require up to two weeks to return to a normal heart rate. Overall, several studies agree that two weeks of acclimatisation for males and three weeks for females should be standard when rats are transported between facilities.

## Transport within a facility

Rats are also affected by being moved short distances within the same building. In one study, rats were transported using roller carts for five minutes. The rats' body weights, levels of corticosterone in the blood, heart rates, blood pressures and activity levels were examined. The results showed that at least two days of acclimatisation are needed to restore these levels after such transport.

## Changed circadian rhythm

A change in the circadian rhythm, for example before an experiment or as a result of transportation between different continents, affect rats physiologically and behaviourally. The results of one study suggest that male rats should be acclimatised for at least ten days after a reversed circadian rhythm, based on the rats' activity level, blood pressure and heart rate. According to the same study, there is a rule of thumb stating that one day of acclimatisation is needed for each hour shift in time. The basic rule can be used as support for the acclimatisation of males and for the parameters activity level, blood pressure and heart rate. In another study conducted on male rats, researchers examined the same parameters, meaning activity level, blood pressure and heart rate, after the light period was shortened by four hours over six weeks. When the conditions were changed back to 12 hours of light and 12 hours of darkness, it took one to three weeks for the rats to acclimatise. Most studies dealing with the subject have only looked at male rats, but there are a small number of studies that have also included females. One of these states that two weeks of acclimatisation after transport for males and three weeks for females should be standard regardless of whether the circadian rhythm has been affected or not.

## Differences between age, sex and strain

Several studies indicate that female rats are more easily stressed in comparison to male rats. Both physiological parameters and the behaviour of females are more affected by a transport compared to males. It also takes longer for them to acclimatise to a new environment. The oestrus cycle is important when it comes to how females react to stress. Those in heat (oestrus) display a higher level of corticosterone in the blood after stress and they also need more time to recover, compared to males and females before or after heat. Males always recover faster compared to females, regardless of where the females are in the cycle. In one study, males were compared to females after transport. The researchers looked at the rats' body weights, levels of corticosterone in the blood, heart rates, blood pressures and their activity levels. The results showed that eight days of acclimatisation was sufficient for males, while females needed 14 days. This study, along with several others, show that consideration should be given to the sex of rats regarding acclimatisation. Studies also indicate that stress sensitivity is affected by rat strain and age, with inbred strains and young rats, younger than 6 months, being more sensitive to stress. There are also research results suggesting that rats from the same strain can have different responses to stress depending on the breeder they originate from.

# Conclusion

Every researcher should ensure that the rats 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 rats 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 rat
- The sex of the rat
- 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 rats 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 stabilizing. However, for behavioural experiments or other studies of the whole rat, a longer acclimatisation period may be necessary for rats to acclimatise completely.

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