

Recommendations on group housing of male mice

A compilation of experiences from Swedish laboratory animal facilities and scientific literature

The Swedish 3Rs Center is the executive body of the *Swedish National Committee for the Protection of Animals used for Scientific Purposes*. Our mission is to compile and disseminate information on methods that can replace, reduce and refine animal experiments (the 3Rs). In cases where animals are used, efforts can be made to increase the animal welfare and improve the quality of research. One way in which we disseminate knowledge is to publish recommendations and share best practices. Our recommendations aim to help businesses and individual employees improve their work. They are not mandatory but can be used as guidance.

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Summary

Aggression in group housed male mice is a common problem in laboratory animal facilities. This causes animal stress and risk for injuries, which ultimately leads to a negative impact on research results. The *Swedish National Committee for the Protection of Animals used for Scientific Purposes* has identified this problem as an important field of activity for the Swedish 3Rs Center.

During 2018-2020, we have worked with external experts to collect a broad knowledge base on the matter, which mainly consists of accumulated experience of staff at Swedish laboratory animal facilities. Based on the collected data we have compiled recommendations aimed at preventing aggression in group housed male mice and thus improve animal welfare, the working environment and, consequently, the quality of research.

The recommendations covers the practicalities of animal housing and the overall work method - procedures, cooperation, and communication. As many of these aspects have not been sufficiently studied in scientific literature, staff experiences are a crucial source of knowledge.

Background

The mouse is one of the most commonly used species in research worldwide. The same is true in Sweden, where we made 202 579 uses of mice in trials in 2019 (Swedish Board of Agriculture, not published). As the mouse is a social species, it shall be housed in groups [1]. In the wild, mice live in groups with a dominant male, several females, and their young [2]. Housing mice this way in a laboratory animal facility is difficult and the mice are therefore housed in same-sex groups or individually if there is a valid reason for doing so. Housing male mice in groups is a major challenge as aggression often occurs, leading to injury, stress, and sometimes even death among the animals. It is a major problem which, in the long run, also has a negative impact on research results [3].

Sweden's National Committee shall advise animal welfare bodies on the purchase, housing, and care of animals [1]. The National Committee is the steering group of the Swedish 3Rs Center, which in turn is the Committee's executive body. The Committee has identified aggression in group housed male mice as an important field of activity for the centre. In 2017, the Committee started a project to compile recommendations on male mice in groups, thus contributing to improved animal welfare and working environment, and ultimately better research results.

The Swedish 3Rs Center was commissioned to identify factors that affect and can prevent aggression in group housed male mice. This was performed together with experts such as researchers, animal technicians, veterinarians and ethologist. The identified factors have been used as a basis for concluding concrete recommendations, to be used by staff working with mice, staff planning the experiments, and those who review applications for research projects involving mice.

The knowledge base resides largely on gathered experiences of animal technicians, scientists and veterinarians in Swedish laboratory animal facilities, but also on a systematic review of scientific literature and a study coordinated by the *British National Centre for the Replacement Refinement & Reduction of Animals in Research* (NC3Rs).

The recommendations shall be a changeable document. It will be updated and revised as new information becomes available, in published literature and from dialogue with staff at facilities.

In this report, we present and discuss the compiled recommendations, but also the methods by which we have gathered knowledge and the documentation that forms the basis for each individual recommendation.

Project group

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Recommendations on group housing of male mice

Aggression in group housed male mice is a common problem in laboratory animal facilities. We have therefore compiled recommendations aimed at preventing and managing aggression and thus improving both animal welfare and the working environment. Consequently, this also contributes to better research.

Five recommendations on how to care for the animals:

1. House the mice in groups that go well together

If possible, it is preferable to house mice in groups with siblings. Mixed groups shall be formed as early as possible, preferably before sexual maturity. Avoid regrouping mice that are used to each other and thrive together. Develop procedures for how and when you assemble groups in the facility. If mice are transported to the facility, keep a dialogue with the breeder for example about housing siblings together during transport.

2. Transfer nesting material when changing cages

Transfer clean and dry nesting materials and other enrichment. Do **not** transfer material soiled by urine. Carry out the cage change in a way that minimizes disturbance and stress for the mice. Avoid too frequent changes and do spot cleaning of dirty cages to spread out the number of changes.

3. Avoid disturbances and handle the mice with care

Avoid disturbances in the animal room by minimizing all forms of interference, noise, light, odours, and unnecessary passage through the room. Also, minimize the number of people handling the mice and make sure they handle and lift the mice with care.

4. Use enrichment adapted to your conditions

Use environmental enrichment that works at your laboratory animal facility, adapted to the cage type, strain, and research area. Avoid enrichment that risks being monopolised and cause competition. Constantly evaluate how the mice are affected by the enrichment you use.

5. Choose a strain with a low level of aggression

Evaluate the relevant strains for your research in terms of aggressiveness and choose the least aggressive one. Aggressiveness varies between different strains and the behaviour differs between both different strains and between genetically modified mice and their wild type.

Two recommendations on procedures and cooperation in the workplace:

6. Establish procedures in your organisation

Create routines for the selection of strains, ordering of animals, grouping, cage change, and rules in the animal room. Describe the measures to be used in the event of aggression. This may include using more environmental enrichment or separating the group by, for example, relocating the aggressive or injured mouse. Regularly follow up on and evaluate the procedures.

7. Promote collaboration and communication

Promote good cooperation and communication between animal facilities, researchers, and breeders. Support dedicated staff and raise the issue of aggression at all stages; when ordering animals, when breeding, when planning experiments, in experimental processes, when changing cages, and when handling animals. Cooperation between all those concerned makes it easier to group house male mice, which results in increased animal welfare and better research.

Knowledge acquisition

The basis for the recommendations is mainly experiences of animal technicians, veterinarians and researchers at Swedish laboratory animal facilities. They were collected through a survey and workshops on site. In total, ten facilities from six universities, two pharmaceutical or biomedical companies, and two government agencies participated. Together, they represented 97% of the total use of mice in trials during 2016 [3].

We also base the recommendations on a systematic review of scientific literature and on a study published by NC3Rs.

Survey

All organisations using laboratory animals must have an animal welfare body. The task of the animal welfare body is, *inter alia*, to advise on animal welfare issues and to promote the use of new methods and approaches to replace, reduce, and refine animal testing [1].

A web-based survey was sent to the animal welfare bodies of the ten facilities and they were encouraged to forward it to researchers, animal technicians, and veterinarians in their organisation. The survey was open from 30 August 2018 until 16 April 2019 and was available in Swedish and English. The questions covered practical aspects of keeping animals as well as issues related to animal housing. Some questions were specifically addressed to researchers.

In total, we received 95 responses from 56 animal technicians, 33 researchers, and 6 veterinarians. A few responses were in English and these were translated into Swedish before we analysed the results [3].

Workshops

An offer to hold local workshops on the housing of male mice was sent out to the ten facilities through their animal welfare bodies. Nine out of ten invited facilities participated in a workshop, and we held a total of ten workshops in seven locations between October 2018 and March 2019.

We conducted all workshops in the same manner. Participants reported problems and measures based on their own experience of group or single housing of male mice. We asked them to write all comments on Post-it Notes, which we then compiled and analysed [3].

Systematic literature review

Karolinska Institutet's University Library conducted a systematic literature search in December 2018. The search generated 840 unique scientific articles. The search criteria we used included articles where male mice were studied and where the concepts of aggression (or similar) and some form of housing (group, single, social) were included.

We then reviewed the articles in two steps:

- First, we only reviewed the summaries of the articles. Articles where the authors had studied male mice in combination with single or group housing qualified for closer examination.
- In the next step, we read the full articles and included only those that dealt with aggression in group housed male mice.

Of the 840 articles we reviewed, about 80 matched our criteria. They are all published during the period 1970-2018 (The Swedish 3Rs Center, not published) and the information in these articles form part of the basis for the recommendations. Scientific articles published later than 2018 are thus not included in our review.

We plan to publish the systematic summary in 2021, and all articles relevant to the recommendations are listed in Appendix A of this report.

British recommendations on housing male mice in groups

After our systematic literature search had been completed, a relevant article was published by NC3Rs. The study aimed to develop recommendations for group housing of male mice and we have therefore included this study in our knowledge base.

In the study, 143 animal technicians in 44 laboratory animal facilities collected data from a total of 137,580 mice. British facilities participated to the greatest extent (35 out of 44 facilities) [4]. The study therefore mainly reflects UK conditions. Further knowledge acquisition based on other countries could potentially contribute to other important factors in the matter.

Knowledge base for each recommendation

The following is a report of the knowledge base for each recommendation.

House the mice in groups that go well together

In the study published by NC3Rs, two recommendations focus on achieving compatible groups. The study advocates for groups to be made up of siblings and that this approach can also be used to make randomized groups by marking the mice while they are still in the same group as their siblings. The study also mentions communication with breeders ahead of transportation as a crucial factor in reducing aggression in male mice [4].

In the survey we conducted in Swedish facilities, 35 out of 95 respondents stated that they had tested forming groups of male mice from different litters before sexual maturity and that this measure had a significant positive effect in terms of reduced aggression. During our workshops, a small number of participants mentioned the importance of not regrouping stable groups [3].

In the systematic literature review, we identified a few studies that have compared aggression in groups of siblings with groups of mice unfamiliar with one another. The results are not conclusive, and as group formation and measurement of aggression have been conducted in different ways and at different ages, it is difficult to make a comparison. Despite this, none of the five studies reported increased aggression in groups of siblings, compared with groups of mice unfamiliar with one another (The Swedish 3Rs Center, not published).

Considering this, we recommend that groups are made up of siblings. If this is not possible, and unfamiliar mice are grouped together, they shall be grouped before reaching sexual maturity.

Transfer nesting material when changing cages

The British study shows that aggression decreases in mice where nesting material is transferred when changing cages, why they recommend transferring dry and clean nesting material upon cage change. They also highlight that spot cleaning of the cage rather than frequent cage changes reduces aggression [4].

Our investigations also show that the transfer of nesting material and other enrichment reduces aggression. During our workshops, this was highlighted as the second most common measure in case of problems with aggression. The survey also discussed this measure and showed a moderate decrease in aggression [3]. We wish to point out that workshop participants and survey respondents stated that they are moving both nesting material and other enrichment. This is something they feel has a positive effect on aggression and we have therefore also included the transfer of other enrichment in the recommendation.

In the literature, opinions differ somewhat as to whether the transfer of material from the old cage increases or decreases aggression. Gray and Hurst (1995) found that aggression decreased in a particular CFLP strain when the new cages were completely clean compared with partially changed cages [5]. An investigation by Van Loo *et al.* (2000) showed that the transfer of bedding material increased aggression, while the transfer of clean nesting material tended to reduce aggression [6].

It is important that nesting material and other enrichment are clean when transferred. It must not be soiled with urine as this may have the opposite effect on aggression [6].

Based on this, we want to highlight the importance of disturbing the mice as little as possible during cage change. We would also like to point out that the transfer of clean and dry nesting materials and other enrichment can contribute to reduced aggression in group housed male mice.

Avoid disturbances and handle the mice with care

When the workshop participants were asked to name measures to facilitate group housing, it was mainly about avoiding disturbances in the animal room, such as minimising the number of people handling the animals, not allowing experiments in the animal room, and keeping a calm environment with as little disturbing light, sound and passage through the room as possible [3]. These are all comprehensive environmental conditions for the mice, which have rarely been studied by researchers or others (The Swedish 3Rs Center, not published).

The survey dealt with questions regarding animal handling, including a question about the methods used to lift mice. Just over half of the respondents said that they use both modern methods, such as lifting the mice in tunnels or cupped hands, and more traditional methods, such as lifting the mice with tweezers or by the tail. One-tenth of respondents use only modern methods [3]. Several studies have shown that mice become less stressed by being lifted in tunnels or with cupped hands and these methods can therefore improve animal welfare [7-9]. One study investigated the link between handling and aggression in male mice in groups. Lifting the animals using tunnels was compared with lifting by the tail or with tweezers, and aggression was more frequent in mice where tweezers had been used [10].

Based on this, we recommend minimising all interventions and other disturbances in the animal room. Animal handling and lifting shall be done with care.

Use enrichment adapted to your conditions

European legislation on laboratory animals requires animals to have access to enrichment that increases the possibility of activity and improves the ability of animals to cope with life in a cage [1]. Swedish legislation goes into further detail and states that enrichment shall be available in such quantities that it does not cause competition over the material. For mice in particular, nesting material shall always be available [11].

In our workshops, participants discussed more enrichment as a measure to facilitate group housing. It was the increased amount of enrichment that was mentioned and not specified what kind of enrichment that works. The survey also touched on the subject and it was mainly adding more nesting material that was claimed to have had a significant positive effect in reducing aggression. Houses, tunnels, and an increased amount of enrichment were said to have a weaker positive effect and some responses even indicated increased aggression [3].

In the systematic literature review, we identified just under 30 articles that had tested the effect of enrichment on aggression. However, the studies use different strains, group sizes, and cage sizes, in combination with different types or compositions of enrichment. It is therefore difficult to draw conclusions about the effect of a specific type of enrichment (The Swedish 3Rs Center, not published). Nor did NC3Rs' study identify any clear effect of the forms of enrichment studied [4].

In light of this, we recommend using the enrichment that work best based on each individual facility's conditions.

Choose a strain with a low level of aggression

When our workshop participants were asked to identify the most common reason for aggression, strain differences were at the top. They mentioned specific strains as more or less aggressive, but also described a general experience of differences between strains [3].

Strains were also often cited in the survey as a cause of aggression. Forty percent of respondents described how they had noted that a particular strain was more aggressive. Meanwhile, 46% said they identified strains that were less aggressive and therefore easier to house in groups [3]. Both of these responses are well in line with what NC3Rs reports in their study [4].

In the systematic review, we identified more than 20 articles examining the difference in aggression between different strains. In the experiments, comparisons were made between different strains, between sub strains of the same strain, and comparisons of transgenic mice with its wild type. It is obvious that it is important to consider which strain to choose, including when choosing the sub strain,

as differences in aggression have been noted there, as well (The Swedish 3Rs Center, not published). The results of the survey also show that there may be clear differences within the same strain and between different facilities. For example, some respondents specifically highlight BALB/c and there are as many who consider it aggressive as non-aggressive [3].

The literature study also shows how transgenic mice often have a different aggressive behaviour compared with its wild type. In all experiments we have identified where transgenic mice have been compared with its wild type, the authors describe a difference between the groups (The Swedish 3Rs Center, not published). This indicates that it cannot be assumed that transgenic mice show the same aggressive behaviour as its wild type.

NC3Rs' study, including a large number of mice from several facilities, compared the incidence of aggression between several different mouse strains. The lowest incidence of aggression was seen in 129S, C57BL/6, and BALB/c and the highest incidence in C3H, CBA, and CD1 [4]. Survey, workshops, and other literature do not give a clear picture of which strains are the least or most aggressive in group housing. Therefore, we cannot suggest strains that are easier or more difficult to house in groups.

It is clear that the choice of strain has a major impact on the risk of aggression between male mice. To avoid this, one should evaluate strains based on aggression and, to the extent possible, choose the least aggressive strain.

Establish procedures in your organisation

This is a general recommendation on how important it is that all facilities have clear routines for dealing with aggression in group housed male mice. Clear procedures that are followed up and evaluated ensure that all relevant parties take the same approach. We deem this will benefit both staff and animals.

Promote collaboration and communication

This recommendation is difficult to substantiate using scientific literature. But cooperation was frequently discussed during our workshops. A better dialogue between researchers and animal technicians was something that many highlighted as an important strategy to facilitate group housing of male mice.

Through a close dialogue between different stakeholders, early warning signs that could contribute to aggression may be identified. Several workshop participants argued that better planning of breeding and euthanization, for example, would improve the work [3].

If aggression occurs after all

Despite preventive measures, aggression can occur after grouping and during ongoing research studies. There may also be other situations where group housing is difficult, for example when breeding. It is then necessary to find other solutions.

In their study, NC3Rs has tested various measures that can be used when aggression occurs. These include keeping the cage under extra supervision, adding more enrichment, and removing the injured or aggressive mouse from the group. The measures were followed up for seven days and, with respect to both enrichment and removal of the injured or aggressive mouse, no further incidents of aggression were seen in more than 90% of cases [4].

In the survey, removing an aggressive or injured mouse from a group was the most common reason for single housing. Approximately one third (35%) also stated that their facility had criteria for when a mouse should be removed from the group to avoid further aggression or injury. The criteria mostly covered visible injuries to mice, but also that there were signs of aggression or fighting. The second most common reason for single housing was that a male had been used for breeding and thus could no longer be housed together with other males [3].

During the workshops, measures that reduce single housing were discussed, but also how animal welfare can be improved when single housing is necessary. The measures relate to recommendations on

promoting cooperation and communication. It involves the planning of animal housing and experiments, communication, cooperation, and exchange of experience between groups of staff such as researchers and animal technicians. Proposed measures to make it easier for mice in single housing included adding more enrichment or alternative forms of companionship, such as a female [3].

Our recommendations on procedures, cooperation, and communication (number 6 and 7) can also help in situations where the recommendations on practical animal housing does not sufficiently reduce problems of aggression.

Conclusions

Aggression between group housed male mice is a recurring problem for laboratory animal facilities. Our hope is that these recommendations will help prevent and manage aggression and thus improve both animal welfare and the working environment. This also contributes to better research.

In the absence of documentation from scientific studies, it is necessary to gather experiences from those dealing with problems with aggressive mice on a daily basis. The material from workshops and surveys is therefore very valuable.

During the collection process, it has become clear that there are gaps in the literature and, in many cases, it is difficult to draw clear conclusions based on scientific evidence. This applies to overall environmental factors for the mice, which are rarely studied systematically. This also applies to enrichment, which is part of several studies but with conflicting results. Another example where more research is needed is the level of aggression in different strains. A better characterisation of strains would lead to a better basis for the choice of strain and awareness that certain strains require specific measures to prevent aggression.

It also turned out that a measure that prevents aggression at one facility does not necessarily have the same positive effect at another facility, and that the level of aggression in strains is perceived differently at different facilities. It is important to document the measures used, based on these recommendations or on other grounds. This applies both to outcome and details on how the mice are housed and is an important part of the work to reduce aggression in male mice and facilitates the exchange of knowledge.

The compiled recommendations are based on the knowledge and experience available today – in scientific literature as well as in Swedish laboratory animal facilities. The aim is to share knowledge and facilitate the prevention of aggression. These recommendations will not solve all problems and research and other forms of knowledge acquisition are needed to move the issue forward. The National Committee and the Swedish 3Rs Centre will treat the recommendations as a changeable document and we will add new knowledge when relevant.

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Appendix A: Relevant references from the ongoing systematic literature review

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