

Recommendations for reviewing ethics applications for work involving rodent head fixation and fluid control

This summary presents the main recommendations from <u>a review</u> of the use of head fixation and fluid control in rodents in a format designed to aid in the ethical review of applications to use these approaches. These recommendations are the product of an <u>NC3Rs working group</u> and you can find further material on this topic at the dedicated <u>resource page</u>.

This summary is intended for members of ethical review bodies who may be scientists themselves or staff involved in animal care but are not familiar with these specific methodologies. It is presented as questions that should be explored as part of a discussion on the use of these techniques. Summaries for animal care staff and researchers actively involved in this area are also available on the dedicated resource page.

The recommendations are split into three categories; <u>head fixation surgery</u>, <u>dietary control</u>, and <u>behavioural set-up</u>. Links to sections of the paper and to other useful material are given where relevant for further information.

Introduction

Head fixation in rodents is often used to enable certain recording techniques that would be difficult or result in poor quality data if used with less restraint. In some cases, a constant head position is also important, for example for the precise delivery of stimuli. However, this form of restraint can have a negative impact on the animal's welfare. Refinements are possible to minimise the impact of these approaches without compromising scientific goals.

Head fixation is often combined with the use of fluid control to enable high-yield testing where hundreds, if not thousands, of trials are necessary. Fluid control involves restricting access to water in the homecage environment to motivate behaviour – it is also increasingly used in studies of behaviour in freelymoving animals. It can leave animals dehydrated for prolonged periods if not implemented carefully. Very small fluid rewards can be delivered more reliably than solid food rewards, meaning more rewarded trials can be conducted before animals are satiated. Licking a reward spout is also quicker and more comfortable for the animals in a head fixed set-up than consuming solid food.

- 1. As with any technique, the first step should be assessing whether these techniques are appropriate for the proposed work and whether more refined alternatives could be used in their place. These include using less restraint and/or more rewarding substances without the need for dietary restriction.
- 2. The ability of the group to responsibly conduct the work should also be considered, taking into account resource and training provisions, previous experience and any prior or ongoing welfare concerns with the existing work of the lab
- 3. Regular review of the use of these techniques should also be conducted to ensure any further refinements are implemented and any welfare concerns not originally anticipated discussed further.

If head fixation and/or fluid control are needed, there are still steps that could be taken to refine their use, minimising the level of suffering involved.

Head fixation surgery – ensure good preparation and communication

- 4. <u>Pre-surgical steps</u> are key to a successful outcome. Are procedures in place to ensure that animals are health checked before surgery and that there is adequate time to habituate the animal to the researcher and facility? Preparations for post-operative recovery should also be conducted before surgery is carried out including discussions with animal care staff if they are to be involved in this step and/or the monitoring of postoperative animals. Are staff aware of what monitoring they may be asked to carry out, its frequency and important points of intervention? Are they trained and confident in what should be monitored?
- 5. If rodents are also going to be under caloric control, they should be returned to *ad libitum* access and allow them to regain their full weight before surgery.
- 6. Good aseptic technique should always be observed (further advice on this is available, for example from the <u>Laboratory Animal Science Association</u> and <u>Research Animal Training</u>) and local guidance on combined <u>anaesthetic and analgesic</u> use should be followed. The local veterinarian should be involved in regular reviews of both the surgical procedure and use of anaesthesia and analgesia.
- 7. Have researchers considered the delivery of <u>fluids</u>? Often providing fluids during surgery is unnecessary and may prolong surgery further. Instead, adequate fluids can be delivered pre- and post-surgery.
- 8. Are clear plans in place to <u>monitor</u> the site of surgery and general health of the animal closely in the days following the procedure? How will this be recorded? Will animal care staff help in these assessments? The <u>example health monitoring template</u> give key indicators for recovery, including body weight, integrity of the wound site and general activity level of the animal, as well as a way to clearly record the assessments made.
- 9. Have refinements to housing and husbandry been considered? Group housing should be routine, even following implants, to avoid the negative welfare impacts of single housing. <u>Group housing has</u> not been observed to lead to greater post-surgical complications or implant loss.

Dietary control - balancing motivation and welfare

- 10. Has every opportunity to reduce the level of dietary control been considered? The <u>degree of</u> <u>restriction</u> should be dynamic and adjusted and minimised throughout the study to maintain motivation whilst minimising the welfare impact on individual animals. Clear intervention points should be set to avoid animals becoming dehydrated unnecessarily.
- 11. Younger animals should be allowed to grow during the course of prolonged studies, and many groups successfully incorporate "holiday" breaks from restriction, top-ups of food and water outside of testing and periods of recovery of body weight before and after surgery. Have these been considered and incorporated into the proposed regimen?
- 12. <u>Other factors</u>, for example habituation to any restraint needed, may also limit the amount of restriction required to motivate the desired behaviour. Have these factors also been considered?
- 13. The overall <u>welfare</u> of each animal must be monitored and recorded daily and compared against clearly defined intervention points. Animal care staff, as well as the local ethical review body, should be involved in these discussions with researchers. See the <u>example health monitoring templates</u> for key welfare measures, , including body weight, grooming and general activity level of the animal.
- 14. Responses to dietary control may differ in mutant rodent lines compared to their wildtype counterparts. Are any additional concerns expected from mutant lines? Has the literature been

reviewed to identify any concerns? If novel lines are being used, are extra precautions in place? Have animal care staff been made aware of any additional monitoring required?

Behavioural set-up – improving welfare for better performance

- 15. <u>Habituation to restraint</u> should be practiced before formal testing as this will reduce stress responses to head fixation, improving task engagement and decreasing the likelihood of the loss of headcaps.
- 16. Further steps to reduce stress throughout the task should also be taken, for example allowing for <u>naturalistic behaviours</u> as part of the response and <u>locomotion during testing</u> or using self-initiated head fixation. Can these be incorporated into the proposed experiments?
- 17. Monitoring factors such as pupil size and facial expressions, even when unrelated to the task, provides useful metrics of welfare and engagement. <u>Other measures of welfare</u> also exist that could be incorporated into most study designs for example noting overt signs of distress, the presence of faeces following restrain and using task-specific measures of engagement as an indicator of habitation to the testing set-up. Will these be monitored and recorded? How will this information be acted upon?