

## **Appendix B: Survey questionnaire**

Overleaf is a complete copy of the survey developed by the working group and disseminated between April and July 2020. The aim was to establish current practice in the use of head fixation and fluid restriction, and identify refinements. Further details can be found in Section 2.2.

## Participant Information

### General Information

You are invited to take part in a survey to collect information on rodent high-yield behavioural experiments. This survey is running for a limited period and will close on 10 July 2020 at 4pm BST.

The questions have been developed by an expert working group of the NC3Rs. The aim is to establish current practice in this field and to identify any refinements to improve animal welfare and scientific outcomes. We very much hope you will participate.

Please read through these terms before agreeing to participate by ticking “yes”. You may ask any questions before taking part by contacting the principal researcher (details on next page).

The survey consists of 70 questions on chronic implant surgeries, how animals are monitored post-operatively, motivational tools and behavioural testing protocols. We estimate it will take 30 minutes to complete. We ask that you complete it based on your most commonly used procedures or the ones with which you have most experience. Multiple responses from a research facility are encouraged, but respondents should be the lead person responsible for carrying out the research or the person chiefly involved in the care of the animals involved.

The results will be reviewed by the NC3Rs working group and used to help identify opportunities to refine this area of work. The group’s recommendations will be published in a peer-reviewed paper and promoted within the research community. To keep up to date with the progress of the working group, please visit [the NC3Rs website](#).

### Do I have to take part?

Your participation is voluntary, and all questions are optional. You may withdraw at any point during the questionnaire for any reason by closing the browser. However, once you have submitted your answers, they will be anonymous so you will not be able to withdraw them.

## Data Management and Consent

### How will your data be used?

All data collected in this survey will be anonymous, treated in strict confidence and held securely by the NC3Rs. The NC3Rs data management plan is available upon request ([enquiries@nc3rs.org.uk](mailto:enquiries@nc3rs.org.uk)).

Your data will be stored in a password-protected file and may be used in academic publications. Your IP address will not be stored. Research data will be stored for a minimum of three years after publication or public release.

### Who will have access to your data?

The NC3Rs is the data controller with respect to your responses and these will be processed for the purpose of the research outlined above. Research is a task that we perform in the public interest.

The principal researcher is Dr Chris Barkus, NC3Rs, who is attached to the Department of Biomedical Services at the University of Oxford. Responsible members of the University of Oxford and funders may be given access to data for monitoring and/or audit of the study to ensure we are complying with guidelines, or as otherwise required by law. This project has been reviewed by, and received ethics clearance through, the University of Oxford Central University Research Ethics Committee (ethics approval reference R68817/RE001).

### What if there is a problem?

If you have a concern about any aspect of this project, please contact [chris.barkus@nc3rs.org.uk](mailto:chris.barkus@nc3rs.org.uk). Your concern will be acknowledged within 10 working days. If you remain unhappy or wish to make a formal complaint, please contact Dr Vicky Robinson, Chief Executive, NC3Rs ([enquiries@nc3rs.org.uk](mailto:enquiries@nc3rs.org.uk)). Address: The NC3Rs, Gibbs Building, 215 Euston Road, London, NW1 2BE, or the Chair of the Oxford Medical Sciences Inter-Divisional Research Ethics Committee ([ethics@medsci.ox.ac.uk](mailto:ethics@medsci.ox.ac.uk)); Address: Research Services, University of Oxford, Wellington Square, Oxford OX1 2JD.

Please note that you may only participate in this survey if you are 18 years of age or over.

If you have read the information above and agree to participate with the understanding that the data (including any personal data) you submit will be processed accordingly, please check the relevant box below to get started. Thank you in advance for your participation.

\* 1. Have you read and understood this information, can confirm that you are over 18, and consent to participate in this study?

Yes

No

## General Questions

2. Please select which area best describes your current research.

3. Please select the option that best describes your primary role

4. Please select the country you work in.

5. Please select the species of animal you most commonly use in your research or support as part of your animal welfare role.

- Rat
- Mouse
- None of the above

## PART A. Chronic implant surgery

6. Do you carry out surgery to implant cranial devices in rodents or are you responsible for their post-operative care, for example as a vet or part of the animal care staff?

Yes

No

## Information about the implant

7. How many times is the animal typically anaesthetised for your most common surgical procedure?

- 1  
 2  
 3 or more

8. What permanent devices are typically implanted? *Select all that apply.*

- |   |  |
|---|--|
| <input type="checkbox"/> Head fixation device                 | <input type="checkbox"/> Optical fibres          |
| <input type="checkbox"/> Chronic single electrodes            | <input type="checkbox"/> Miniscope               |
| <input type="checkbox"/> Electrode arrays                     | <input type="checkbox"/> Cranial window          |
| <input type="checkbox"/> Ground and/or reference electrode(s) | <input type="checkbox"/> Intercranial cannula(e) |
| <input type="checkbox"/> Ground screw(s)                      | <input type="checkbox"/> Lenses / prisms         |
| <input type="checkbox"/> EMG electrode placement              |  |
| <input type="checkbox"/> Other (please specify)               |  |

9. Is this combined with other types of surgical intervention in the same surgery? *Select all that apply.*

- |   |  |
|---|--|
| <input type="checkbox"/> Yes, viral delivery of genetic material                    | <input type="checkbox"/> Yes, mini-pump with intra-cranial cannula(e) implantation |
| <input type="checkbox"/> Yes, lesion of a particular brain area                     | <input type="checkbox"/> No  |
| <input type="checkbox"/> Yes, mini-pump implantation for osmotic diffusion delivery |  |
| <input type="checkbox"/> Other (please specify)                                     |  |

10. In your experience, how important are the following to the welfare of the animals over the course of the experiment?

	Not at all important	Slightly important	Moderately important	Very important	Extremely important
Weight of the implant	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Position of the implant	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Dimensions of the implant	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Depth of placement	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

11. What is used to secure your device? *Select all that apply.*

Skull screws (please give further details below)

Bone cement

Dental adhesive (e.g. superbond)

Other (please specify). Please also add further details such as type of screw used (material, self-tapping or not, etc) and drill type (handheld, frame-mounted etc) used.

## Peri-operative care

12. Which of the following form part of your standard surgical drug regimen?

	Pre-emptive (shortly before surgery)	During surgery (continuous or discrete)	Post-operative (within 12 hours of end of surgery)
Opioids, for example buprenorphine	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Sustained-release opioids, for example buprenorphine SR	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
NSAIDs, for example meloxicam	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Steroids, for example prednisolone	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other anti-inflammatories	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Local anaesthetic, for example lidocaine	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Inhalation anaesthesia, for example isoflurane	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Injectable anaesthesia, for example ketamine/medetomidine	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Fluids, for example saline or gluco-saline	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Oral/injected antibiotics (if given routinely for most surgeries)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Other (please specify)

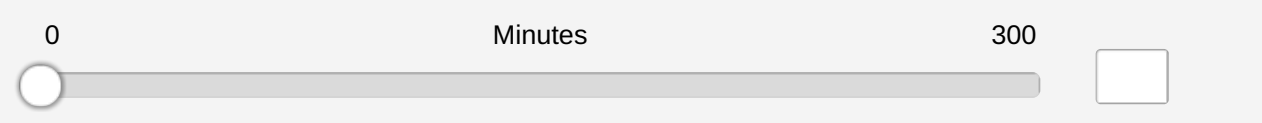
13. What steps are taken to ensure aseptic conditions? *Select all that apply.*

- |   |   |
|---|---|
| <input type="checkbox"/> Sterile equipment  | <input type="checkbox"/> A trained assistant  |
| <input type="checkbox"/> Sterile foil or similar for handling equipment that cannot be fully sterilised (e.g. hand drill) | <input type="checkbox"/> A trained anaesthetist   |
| <input type="checkbox"/> Sterile instruments  | <input type="checkbox"/> Separate rooms/air compartments for preparation of the animal and the surgery itself                 |
| <input type="checkbox"/> Separate sterile instruments for each animal   | <input type="checkbox"/> Surgeon is scrubbing up with skin disinfectant, for example with a chlorhexidine-containing solution |
| <input type="checkbox"/> Sterile consumables (e.g. gloves, drapes, swabs etc)   | <input type="checkbox"/> Mask   |
| <input type="checkbox"/> Sterile drapes/sterile tray/disposable sterile surface for instruments                           |   |



14. What is the average length of surgery?

0 Minutes 300



15. Are both the cranium and the dura typically removed?

- Yes
- No (please provide further information e.g. soften the dura chemically, thin the cranium)

16. Is brain tissue typically excised/lesioned to ease your implant?

- Always
- Usually
- Sometimes
- Rarely
- Never

## Post-operative care

17. In addition to the drugs detailed above, which of the following additional steps form a part of post-operative care **immediately after surgery** (i.e. for the first day or several days post-surgery)? *Select all that apply.*

	Never	Rarely	Sometimes	Usually	Always
Provide additional warmth	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Provide extra nesting material	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Additional palatable foods	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Assessment of body weight/condition	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Easily accessible source of fluids (e.g. gel packs)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Assessment of fluid intake	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Assessment of locomotor activity	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Grimace scale or other pain assessment	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Advice from animal care or veterinary staff	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

Other (please specify). Please also use this text box for any further information from the above options.

18. In addition to regular health checks, how frequently are "enhanced" post-operative health checks performed?

- More than once a day                       Weekly
- Daily     We do not perform any checks other than usual husbandary checks
- Several times a week
- Other (please specify)

19. For how long do these checks continue?

1 day

2 - 4 days

5 - 7 days

More than 7 days

N/A

Until defined criteria are met/Other (please specify)

20. Which of the following do you provide as part of more long-term monitoring of implanted animals (i.e. for the days and weeks after surgery)? (Select all that apply)

	Never	Rarely	Sometimes	Usually	Always
Provide additional warmth	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Provide extra nesting material	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Additional palatable foods	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Assessment of body weight/condition	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Easily accessible source of fluids (e.g. gel packs)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Assessment of fluid intake	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Assessment of locomotor activity	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Grimace scale or other pain assessment	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Advice from animal care or veterinary staff	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Administration of analgesia	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Administration of steroids	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Provide saline/glucosaline via injection	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Routine administration of antibiotics (i.e. to every animal regardless of any evidence of infection)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Administration of anti-inflammatory agent to prevent brain swelling	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Administration of steroids	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

Other (please specify)

21. Overall, how important do you think the following are for the long-term routine welfare of animals that have undergone surgery i.e. which make the biggest impact on their recovery and welfare throughout the rest of the study?

	Not at all important	Slightly important	Moderately important	Very important	Extremely important
Provide additional warmth	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Provide extra nesting material	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Additional palatable foods	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Assessment of body weight/condition	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Easily accessible source of fluids (e.g. gel packs)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Assessment of fluid intake	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Assessment of locomotor activity	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Grimace scale or other pain assessment	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Administration of analgesia	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Advice from animal care or veterinary staff	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Administration of steroids	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Provide saline/glucosaline via injection	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Routine administration of antibiotics (i.e. to every animal regardless of any evidence of infection)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Administration of anti-inflammatory agent to prevent brain swelling	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Administration of steroids	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

Other (please specify)

22. What period of time are the animals typically given to recover from surgery before food/fluid restriction is started/resumed?

- |                                  |   |
|----------------------------------|---|
| <input type="radio"/> 0 days     | <input type="radio"/> 7 - 9 days                              |
| <input type="radio"/> 1 day      | <input type="radio"/> 10 - 14 days                            |
| <input type="radio"/> 2 - 3 days | <input type="radio"/> More than 14 days                       |
| <input type="radio"/> 4 - 6 days | <input type="radio"/> We do not use food or fluid restriction |

23. What period of time are the animals typically given to recover from surgery before the first behavioural test?

- |                                  |  |
|----------------------------------|--|
| <input type="radio"/> 0 days     | <input type="radio"/> 7 - 9 days   |
| <input type="radio"/> 1 day      | <input type="radio"/> 10 - 14 days   |
| <input type="radio"/> 2 - 3 days | <input type="radio"/> More than 14 days                                    |
| <input type="radio"/> 4 - 6 days | <input type="radio"/> We do not perform behavioural tests on these animals |

24. How are animals typically housed during an experiment?

	Singly-housed	Pair-housed	Group-housed
Before surgery (i.e. non-instrumented animals)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Immediately after surgery (i.e. instrumented animals)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Following recovery from surgery (i.e. instrumented animals)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

## Adverse effects

25. How do you monitor for expected adverse effects and record these signs?

- We have a scoresheet used for all post-operative animals.
- We have a bespoke scoresheet specifically for these types of experiments.
- We keep detailed notes of each animal in a lab book.
- We keep detailed notes of each animal on their cage card/in our colony record system.
- We have no specific system but record concerns as they arise.
- We monitor but do not keep detailed records.
- Other (please specify)

26. How often do you see each of these adverse effects at any point post-surgery?

	Never	Rarely	Sometimes	Usually	Always
Scabbing/wounding around the head implant	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Secondary infections	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Wound rupturing/loss of stitches	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Loss/repair needed of head implant	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Bleeding	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Loss of appetite	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Piloerection	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Shivering	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Reluctance to move	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Lack of alertness	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Lack of grooming	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Hunched posture	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Vocalisation	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Inflammation	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Excessive weight loss (for example >20% over a few days)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Facial indicators of pain	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

27. Are there any other details of your surgical and peri-operative care procedure which you feel impact on the welfare of the animals? What could be done to acheive further improvements?



## PART B. Fluid and food restriction

28. In your experience, what are the most important considerations when choosing how to motivate rodents in your studies, for example choosing between using food or fluid control?

	Not a consideration at all	Least important	Not very important	Important	Most important
Used by others in the field	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Established lab practice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Better for scientific outcomes of the study (e.g. lower number of trials to criterion)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
The alternative (food/fluid) has not worked in the past	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
The experimental set-up practically requires it/technical consideration	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Animal welfare considerations	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Regulatory advice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

Please note here if you do not use either food or fluid control routinely.

29. Are the animals that you use or care for often under fluid control?

- Yes  
 No

## Fluid restriction protocol

30. How is fluid restriction initially introduced?

- We gradually reduce access to water to the level used during testing (i.e. starting with a large volume and decrease from there).
- We restrict to the level used during testing from the start (i.e. the volume used remains more-or-less constant throughout the procedure).
- We restrict at a level lower than is typically necessary and gradually increase this as weight and/or performance stabilises (i.e. starting with a low volume and increase from there).
- Other (please specify)

31. How do you determine that your animals are at an appropriate level of fluid restriction? *Select all that apply.*

- Task performance
- Number of trials completed
- Time engaged with task
- Other (please specify)
- We give a fixed amount of food/fluid
- We work to a fixed percentage of baseline weight

32. Is there a daily minimum amount of water that **MUST** be provided to each animal? If so, please specify this value with units.

Rats

Mice

33. What are the most important considerations for using this value?

	Not a consideration at all	Least important	Not very important	Important	Most important
Veterinary advice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Values given in the literature	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Experience of collaborators	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Guidance from local ethical review process (e.g. from your IACUC or AWERB)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Regulatory advice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Established lab practice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

Please list any guidelines used in making this decision.

34. Do animals receive fluid on testing days outside of the behavioural training? (i.e. a "top-up")

- Yes, immediately after testing
- Yes, some time after testing
- Yes, at a fixed time of day
- No, animals only receive the fluid they gain in testing
- Other (please specify)

35. Do animals receive a different amount of fluid on non-testing days?

- Yes - 12 hours or longer/constant access
- Yes - 6 – 12 hours access
- Yes - 2 – 6 hours access
- Yes - 1 hour access
- Yes - less than 1 hour access
- Yes - until they have consumed a fixed amount of liquid
- No - animals receive the same amount of fluid access on testing and non-testing days
- N/A - we test seven days a week

36. How often do you see the following signs of dehydration?

	Never	Rarely	Sometimes	Usually	Always
Reduced skin turgor (e.g. skin tent test)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sunken eyes	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Marked variation in general behaviour (e.g. change in locomotor activity, increased/decreased activity)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Change in faecal pellet consistency	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Dry, 'tacky' oral mucous membrane	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Reduced capillary refill time	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Hunched posture	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Abnormal gait	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Deviation from growth curve	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Rapid, undesirable weight loss	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Deterioration in body condition	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

Other (please provide further information if you do not monitor dehydration)

37. How often do you monitor for signs of dehydration?

- More than once a day  Weekly  
 Daily  Never  
 Several times a week  
 Other (please specify)

38. When do these checks take place? *Select all that apply.*

- At the start of each testing day  Shortly after fluid access  
 Shortly before behavioural testing  At the end of each testing day  
 Shortly after behavioural testing  Around lights on in the animal house  
 Shortly before fluid access  Around lights off in the animal house  
 Other (please specify)

39. What is the limit for intervention, for example increased monitoring or free access to water?

- Animals fall below 85% of reference weight  
 Animals fall below 80% of reference weight  
 Animals fall below 75% of reference weight  
 Animals fall below another proportion of reference weight/other measure (please specify)

40. What is the limit for removing the animal from the study? (e.g euthanasia)

- Animals fall below 85% of reference weight acutely  Animals fall below 75% of reference weight acutely  
 Animals remain below 85% of reference weight for an extended period of time (please specify)  Animals remain below 75% of reference weight for an extended period of time (please specify)  
 Animals fall below 80% of reference weight acutely  Animals fall below another proportion of reference weight (please specify)  
 Animals remain below 80% of reference weight for an extended period of time (please specify)  We do not remove animals from the study based on this measure (please specify)

Please specify the length of time considered if appropriate or if another measure is used.

41. Do you use a fixed value for the reference weight or adjust this throughout the study?

- We use a fixed value based on one measurement of free feeding weight.  We adjust based on data from within our lab/unit.  
 We use a fixed value based on several measurements of free feeding weight.  We adjust based on control mice from the same cohort.  
 We adjust based on publicly available growth curves (e.g. those available through JAX).  We adjust by occasionally taking a new reference weight.

42. Are there other details of your fluid restriction protocol that you feel are important in maximising the welfare of the animals? Are there particular unanswered questions in this area that you would like see addressed?

## Food control

43. Are the animals that you use or care for often under food restriction?

Yes

No

## Food control protocol

44. How is food restriction initially introduced?

- We gradually reduce the food given to the level used during testing (i.e. starting with a large amount and decrease from there).
- We restrict to the level used during testing from the start (i.e. the amount given remains more-or-less constant throughout the procedure).
- We restrict at a level lower than is typically necessary and gradually increase this as weight and/or performance stabilises (i.e. starting with a small amount and increase from there).
- Other (please specify)

45. How do you determine that your animals are at an appropriate level of food restriction? *Select all that apply.*

- Task performance
- Number of trials completed
- Time engaged with task
- Other (please specify)
- We give a fixed amount of food
- We work to a fixed percentage of baseline weight

46. Do you typically provide a fixed amount of food or is it adjusted? What is your primary guide if you adjust?

- Adjusted to maintain good body weight
- Adjusted to maintain task performance
- Fixed (please specify)

47. Is there a daily minimum amount of food that MUST be provided to each animal? If so, please specify this value with units.

Rats

Mice

48. What are the most important considerations for using this value?

	Not a consideration at all	Least important	Not very important	Important	Most important
Veterinary advice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Values given in the literature	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Experience of collaborators	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Guidance from local ethical review process (e.g. from your IACUC or AWERB)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Regulatory advice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Established lab practice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

Please list any guidelines used in making this decision.

49. On non-testing days is a larger amount of food given?

- Yes - a variable amount based on the animal's body weight
- Yes - a variable amount based on the animal's task performance
- Yes - a fixed amount less than ad lib but greater than the typical amount on a test day
- Yes - a fixed amount approximately equal to ad lib intake
- No
- N/A - animals are used in tests seven days a week.

50. How often are the animals under food control weighed?

- More than once a day
- Daily
- Several times a week
- Weekly
- Other (please specify)



51. When do these weight checks occur? *Select all that apply.*

- At the start of each testing day
- Shortly before behavioural testing
- Shortly after behavioural testing
- Shortly before feeding
- Shortly after feeding
- At the end of each testing day
- Around lights on in the animal house
- Around lights off in the animal house
- Other (please specify)

52. What is the limit for intervention, for example increased monitoring or free access to food?

- Animals fall below 85% of reference weight
- Animals fall below 80% of reference weight
- Animals fall below 75% of reference weight
- Animals fall below another proportion of reference weight/other measurement (please specify)

53. What is the limit for removing the animal from the study? (e.g euthanasia)

- Animals fall below 85% of reference weight acutely
- Animals remain below 85% of reference weight for an extended period of time (please specify)
- Animals fall below 80% of reference weight acutely
- Animals remain below 80% of reference weight for an extended period of time (please specify)
- Animals fall below 75% of reference weight acutely
- Animals remain below 75% of reference weight for an extended period of time (please specify)
- Animals fall below another proportion of reference weight (please specify)
- We do not remove animals from the study based on this measure (please specify)

Please specify the length of time considered if appropriate or if another measure is used.

54. Do you use a fixed value for the reference weight or adjust this throughout the study?

- We use a fixed value based on one measurement of free feeding weight.
- We use a fixed value based on several measurements of free feeding weight.
- We adjust based on publicly available growth curves (e.g. those available through JAX).
- We adjust based on data from within our lab/unit.
- We adjust based on control mice from the same cohort.
- We adjust by occasionally taking a new reference weight.

55. Are any measures other than body weight assessed?

Body condition

Home cage activity

Other (please specify)

56. Are there any other details of your food restriction protocol that you feel are important in maximising the welfare of the animals? Could anything further be done to improve welfare further?

## PART C. Behavioural testing

57. Which of the following are routinely paired with the behavioural testing of your animals?

- Head fixation
- Tethered device
- Home cage testing
- Testing without restraint e.g. wireless recording or no recording device

58. Are the animals habituated to the behavioural procedure and the tethering/restraint method together or separately?

- Together, we acclimatise animals to being tethered/restrained whilst they also make basic responses.
  - We acclimatise animals to being tethered/restrained first before adding any elements of the task, i.e. we have some sessions in which they are restrained but the task is not run.
  - Animals receive some behavioural training before being tethered/restrained for the first time (or before surgery).
  - Other (please specify)
- N/A - animals immediately enter testing using the full task and tethering/restraint method.
  - N/A - We only perform wireless recordings or no recording so do not restrict the animals' movement in any way.

59. On average, how many habituation/acclimatisation sessions in total do animals normally receive (i.e. before formal testing begins)?

- 0
- 1
- 2
- 3
- 4+

60. Do the animals have a habituation/acclimatisation period at the start of each formal testing session?

- No
- Yes (please specify length of time and other details)



64. Are animals typically tested in the light or dark period of the animal's circadian cycle? Please also specify if you use reverse light or not.

- Light
- Dark
- It varies as part of our research (i.e. scientific reasons)
- It varies due to testing schedule (i.e. for non-scientific reasons)

Please provide further information (e.g. please specify if a reverse light cycle is used)

65. What approximate percentage of the initial cohort of animals typically **fail** to complete behavioural testing?

0 % 100

66. How frequently are the following a cause for an animal having to be removed from the study?

	Never	Rarely	Sometimes	Usually	Always
Animals persistently do not engage in the task	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Animals frequently show signs of distress during the task	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Removed due to ill health/implant complications	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

## Choice of reward

67. What type of reward is typically used to reinforce behavioural performance? *Tick all that apply.*

- Water
- Sucrose solution
- Saccharine solution
- Milkshake
- Soya milk
- Fruit juice
- Food pellet similar to standard lab chow.
- Sucrose pellet
- Flavoured food/sucrose pellet
- Electrical stimulation of, for example, the dopamine system
- Optical stimulation of, for example, the dopamine system
- A sensory cue (e.g. visual and/or auditory cue(s))

Please provide further information and specify any other rewards not listed above.

68. Has this choice of reward altered your food/fluid restriction regime?

- Yes, using this reward means less food/fluid restriction
- Yes, using this reward means more food/fluid restriction
- No
- N/A - We have only ever used this reward

69. Has this choice of reward altered behavioural performance in your animals?

- Yes - using this reward means animals complete a greater number of trials than previously or compared to other groups.
- Yes - using this reward means animals have greater behavioural performance than previously or compared to other groups.
- No - we have not seen any change/they respond as expected.
- We have only ever used this reward but would consider changing if it could improve behavioural performance or response rates.
- We have only ever used this reward and would not consider changing this.
- N/A - we do not use food/fluid restriction.

70. Are any of the following aversive training methods used? *Select all that apply.*

- Air puff
- White noise
- Time out
- Shock
- Other (please specify)

71. Finally, is there any other key information about your behavioural protocol that is important in ensuring the welfare of the animals? Can you identify any parts of the procedure where further improvements are possible?

Thank you for participating in this survey.