

FAU Neurobehavior Core Preparation of Subjects

Animal Care. Housing, mating, weaning, and all other aspects of animal care are the responsibility of the Core User. Core personnel may give advice about these issues in an effort to improve the quality of the behavioral data obtained, but animal care is always the responsibility of the user and his/her PI.

Housing Conditions. Mice should be separated by gender as soon as possible after weaning, to minimize fighting. Whenever possible, avoid housing mice from more than one litter in the same cage, also to minimize fighting. Avoid housing mice individually unless required by the experimental protocol. Isolation induces stress in mice, which will affect performance of many behavioral tasks. Because the behavior of individual mice is affected by the number of mice in the home cage, it is preferable to have an equal number of mice in each cage. In addition, it is preferable to keep the cages of mice, to be used in the same procedure, on the same shelf of the rack whenever possible. Different shelves on a rack have different lighting levels, which may affect behavior.

Number of Subjects. Group sizes of greater than 10 mice are typically needed to detect group differences with statistical significance.

Strain. Most of the behavioral tasks have been optimized using mice of the C57BL/6J strain. This strain is good for most behavioral tests and is the most commonly used strain for behavioral testing. However, under some circumstances the use of another strain may be preferable or unavoidable. If a strain other than C57BL/6J is used or if a novel task is requested, investigators may have to work out the parameters of the task with one or more groups of wild-type mice of that particular background strain. Many mutant mice are created using a hybrid of the C57BL/6J and the 129S6/SvEvTac (formerly 129/SvEvTac). Ideally, hybrid mice should be back-crossed to one strain or the other for a minimum of seven generations before being used for behavioral testing. However, this is not always practical for initial publication of a mutant phenotype, and so the use of hybrid mice may be required. Keep in mind that with mixed genetic backgrounds it is not always possible to definitively attribute a behavioral phenotype to the mutant gene.

Line. When mice are derived from more than one line, it is preferable that a single line be chosen to use for behavior. There are sometimes large differences in behavior between different lines of mice harboring identical mutations. If it is necessary to use mice from more than one line, it is critical to keep records and label cages to indicate which mice are from which line.

Littermate Controls. Mice used for behavioral testing should be littermates, and derived from heterozygous crossings if genetically modified. Keep in mind that 20-30 heterozygous matings may be required in order to achieve the number of mice required for behavioral testing.

Sex. If testing both sexes, groups should be evenly divided between male and female. If interactions between genotype and gender are detected, larger group sizes may be required. Test males and females separately.

Age. Unless the experimental protocol dictates otherwise, behavioral testing should begin when mice are 8-10 weeks old, young adults. All mice should be of the same age or close to the same age (i.e., within 2 weeks of each other). In cases in which this is not possible, small groups of equal numbers of each genotype may be tested separately over a longer period of time. However, this approach results in greater variability and may require larger group sizes.

Identification of Subjects. The investigator must provide proper identification of subjects, which may be ear tag or punch, toe clip, or tail tattoo. Every subject must have a unique identification number.

Preparation of Subjects for Behavioral Tests. The day before testing begins, all mice should be weighed. Scales are available in the Core for this purpose. In addition, for most behavioral tests the tails should be marked for identification using a waterproof marking pen, in addition to ear tags or punches. This allows easy and quick identification of mice in the cage without handling or scruffing the mice, which induces a high level of stress immediately before testing. Ideally, all behavioral testing should be performed or commence in the first 6 hours of the light cycle, and at the same time of day for multiple days of testing or for groups tested separately that will be combined for analysis.

Good practices

The overall experimental design must be planned well in advance.

This is not only good science, it's essential for coordinating Core and animal usage. Because of the variability inherent in behavioral experiments, groups of at least 20 mice are recommended. Most experiments have more than two groups of mice, and often must be conducted using separate cohorts of mice over long periods of time. Thus staggered breeding schedules are often needed, breeding more pups than anticipated to ensure at least five mice per group per cohort, and roughly equal numbers of mice from each group represented in each cohort. Importantly, the **Core rooms are often scheduled months in advance**; thus the breeding schedules must be coordinated with the future availability of Core equipment (i.e., when the mice are to reach the desired age of testing). Consult with Core Managing Director regarding long-term experimental planning.

A single experimenter should complete an experiment in its entirety.

This rule is to reduce variability. Importantly, if multiple cohorts of mice are used, the same experimenter must conduct testing on all the mice in all the cohorts. Thus if a summer student is trained to conduct a particular behavioral test but it's anticipated that the final cohort will not finish before the student leaves, someone else should be used to conduct the experiment, if possible.

In general, at least two tests measuring a single construct are recommended, and multiple tests should be performed in a specified order.

If anxiety is of interest, for example, at least two measures of anxiety should be conducted. In addition, all behavioral tests must be conducted in the same order in every mouse, and at the same time of day. Each behavioral test conducted will affect performance on all subsequent tests, often in immeasurable ways. In general, multiple behavioral tests conducted within subjects are ordered from least to most aversive. Consult with the Core Managing Director if there is any question about the order of testing.