

Preparation of Subjects for Behavioral Experiments

It is important to keep in mind that everything that happens to a mouse, from prenatal events up to the present moment, will affect its behavior. This includes the number of pups per litter, the genotype and parity status of the dam, whether the subject was situated between males and/or females in the uterus, maternal stress, early postnatal handling, adolescent handling, adult handling, the number of adults per cage during the pre-weaning period, the presence or absence of an adult male in the cage during the pre-weaning period, cage-changing frequency, time since the last cage change, type of bedding, perfume/cologne/breath of the experimenter, behavioral habits and other idiosyncrasies of the experimenter, time of day, time of week, time of month, time of year, genotype, gender, background strain, and age of the mouse, number of mice per cage, distribution of genotype and gender of the mice within a cage, relative position in the intra-cage dominance hierarchy, behavioral history, drug history, dietary history, and any significant noises that occur before or during the behavioral test session, to name a just a few. Each of these can contribute to the variability of the data; thus as many as possible should be controlled as tightly as possible.

Animal Care. Housing, mating, weaning, and all other aspects of animal care are the responsibility of the User and Principal Investigator. Mice must be taken back to the vivarium at the end of the user's allotted time. Mice may not be housed in the Core overnight except with prior approval from IACUC and approval on a case-by-case basis by the Core Director.

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 or LACU vet. 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 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 20 mice per genotype are typically preferred, to control for inherent variability and minimize the problem of detecting large differences between groups that are not statistically significant.

Strain. Behavioral abilities vary dramatically by the strain of the mouse or rat. Many mutant mice are created using a hybrid of the C57BL/6J and a 129 or D2 substrain. Ideally, hybrid mice should be back-crossed to one strain or the other for 7-10 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. Larger group sizes will be needed to handle the additional variability. Keep in mind that with mixed genetic backgrounds it is not always possible to definitively attribute a behavioral phenotype to the mutant gene. Also note that some strains carry allelic variant that can render them useless for certain behavioral tests. For example, some commonly-used strains such as C3H/HeJ, SJL/J, and FVB/NJ all carry the *rd1* mutation of the *Pde6b* gene, making them blind by weaning age. SJL/J also carries the *im* mutation of the *Dysf* gene, causing a spontaneous myopathy and essentially immobile mice. Hybrids of these strains, e.g., B6SJLF1/J and B6C3F1/J are commonly used to generate transgenic mice and will be heterozygous for the parental strains' mutant alleles. The vendor's website should have list of any characteristics that the strains have that might be of concern for behavioral testing. It's also important to keep in mind that many strains have age-related hearing loss, with considerable differences in the ages and spectra across strains. For example, the C57BL/6J strain has hearing loss for frequencies above 8 kHz by 6 months of age, which is worse by 12 months. The DBA/2J and many of its BxD hybrids have impaired hearing for sounds greater than 16 kHz by weaning. At 6 weeks of age they're impaired for frequencies above 8 kHz and practically deaf for frequencies above 16 kHz.

Littermate Controls. Mice used for behavioral testing should be littermates, i.e., derived from heterozygous crossings, even if only the wild-type and homozygous mutant will ultimately be used. Thus, 20-30 heterozygous matings may be required in order to achieve the number of mice required for behavioral testing.

Gender. Groups should be evenly divided between male and female when possible. If interactions between genotype and gender are detected, larger group sizes may be required.

Age. Unless the experimental protocol dictates otherwise, behavioral testing should begin when mice are 8-10 weeks old. 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, cohorts containing equal numbers of each genotype may be tested separately over a 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, tail tattoo, or subcutaneous radio-frequency (RFID) tag. Every subject must have a unique identification number.

Preparation of Subjects. The day before testing begins, all mice should be weighed. 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 in the first 6 hours of the light cycle. Mice should be transported to the Core at least one hour before behavioral testing begins, and allowed to rest undisturbed. For tests that are particularly sensitive to stress, the mice should be transported to the Core several hours before the testing sessions. It's a good practice to move your mice to the testing area first thing in the morning if possible, to avoid the noise and uncontrolled environment (people) in the vivarium (see below).

Cleaning cages. The LACU staff change out the cages once per week, and sometimes twice if they're found to be dirty. This is a noisy, stressful period in the mouse's life, which continues for hours and is accompanied by intense fighting among males of many strains when placed in a clean cage. The behavior of mice will be different after a cage change than on other days. Thus it is always important to know which day of the week the cages are changed. If possible these days can be avoided altogether, but sometimes a particular task must be repeated every day for many consecutive days. In this case it is wise to remove the mice from the vivarium before the cage changing time, and let them rest in the core or some other location, undisturbed, until it is time for behavioral testing. Notify LACU staff that you will be providing clean cages so they can skip yours, and take clean cages and fresh water and food with you to the Core. Then when a particular mouse is finished behavioral testing for the day put it in a clean cage rather than back in the dirty cage. You can put a little dirty bedding from the old cage into the clean cage to reduce fighting among males, but make sure the dirty bedding is from the same mouse's cage.

Good practices

The overall experimental design must be planned well in advance.

This is not only good science, it's essential for co-ordinating 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 ten 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 the Core 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.

In general, at least two tests measuring a single construct are recommended.

If spatial memory is of interest, at least two measures of spatial memory 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 Director if there is any question about the order of testing.