

**Murine Neurobehavioral Laboratory
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 on the same shelf of the rack whenever possible. Different shelves on a rack have different lighting levels, which may affect behavior. Avoid placing mice for behavioral testing on top of the rack.

**Number of Subjects.** Group sizes of 20 mice per genotype are preferred, to control for inherent variability and minimize the problem of detecting large differences between groups that are not statistically significant.

**Strain.** Most of our 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 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, only mice from a single line should be provided to the Core. 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, indicate which mice are from which line and include this information. In this case the line of mouse will be included as a blocking factor in statistical analyses, and greater group sizes may be required in order to detect a phenotype.

**Littermate Controls.** Mice used for behavioral testing should be littermates, i.e., derived from heterozygous crossings. 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. 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, small groups of equal numbers of each genotype may be tested separately over a long period of time. However, this approach results in greater variability and may require much 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. For long-term experiments tail tattoo may be required, and the equipment and training for this can be provided by the Core.

**Preparation of Subjects for Behavioral Tests.** The day before testing begins, all mice should be weighed. A scale is 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 in the first 6 hours of the light cycle.