
1. PURPOSE

This Standard Operating Procedure (SOP) describes the social defeat model in mice, its methodology and possible refinement alternatives.

2. RESPONSIBILITY

Principal investigator (PI) and their research staff, veterinary care staff.

3. MATERIALS

3.1. Larger cages with dividers

4. CONSIDERATIONS

4.1. Generalities

- 4.1.1. The purpose of this model is to study social stress. When submitting an animal use protocol, it is critical that the researcher explain, in lay terms, the relevance of this model in the experimental context. For instance:
 - 4.1.1.1. Social stress is one of the most common and pervasive sources of stress in animals and humans. This model is generally used to study what makes some individuals resistant and others susceptible to adverse effects of social stress. Rodent models of social defeat have been shown to be relevant methods to study behavioral and physiological effects of stress.
- 4.1.2. The goal is to induce stress not injuries. Injuries are not necessary to induce stress, and efforts are made to avoid them. The incidence of injuries reaching humane endpoints (see section 5.4.6) is in general low (estimated 5-10%). Aggression is a common behaviour observed in general in animal facilities, particularly in sexually mature males housed together.
- 4.1.3. Only work with one cage of mice at a time to allow for continuous monitoring of the animals during the induction of social stress.

5. PROCEDURES

5.1. First phase: screening/selection of the aggressors

- 5.1.1. CD-1 male mice are screened for their aggressive potential by presenting them with a different test mouse every day for 3-5 days.
- 5.1.2. The latency and number of attacks are used to score the degree of aggressivity.
- 5.1.3. The CD-1 mice with low scores are not selected (because they will not induce social stress). The ones with the highest scores are also not selected due to the risk of causing injuries.

5.2. Second phase: induction of the social stress

- 5.2.1. A large cage is divided with a transparent and perforated panel.
- 5.2.2. A different cohort of test mice is used.
- 5.2.3. One test mouse is physically exposed to a CD-1 aggressor for a period of up to 5 minutes. Latency and number of attacks are recorded.
- 5.2.4. The test mouse is then housed in the same cage as the aggressor but on the other side of the partition.
- 5.2.5. The next day, step 5.2.3 and 5.2.4 are repeated with a different CD-1 aggressor for a total of up to 10 days.

5.3. Third phase: behavioral testing of the test mice.

- 5.3.1. Various behavioral tests are performed depending on the research objectives (e.g., anxiety testing, Morris water maze, etc.).

5.4. Refinements:

- 5.4.1. Most of the refinements identified below are already applied by investigators at McGill or in the scientific literature. Other refinements would need to be validated. All refinements might not be applicable to specific research projects depending on the degree of social defeat required to obtain significant results.
- 5.4.2. Screening/selection of CD-1 aggressors:
 - 5.4.2.1. Retired breeders are used most commonly; age can vary significantly (4-10 months; not specified by the supplier). Although the use of 2-month old CD-1 males with a successful social stress induction with no observable injuries has been previously reported. Retired breeders are likely to be more aggressive but that might not be necessary to induce the stress. The age variation in retired breeders could introduce a significant variable and could increase the number of aggressors to be screened.
 - 5.4.2.1.1. Obtain CD-1 males based on a more specific age range (e.g., retired breeder 4-5 months old).
 - 5.4.2.1.2. Consider using sexually mature CD-1 but not breeders (e.g., 2-3 months old)
- 5.4.3. The screening could be shortened by reducing the number of exposure days and exposure time:
 - 5.4.3.1. Select CD-1 aggressor that attacked consistently within a latency of 2 minutes but eliminating the individuals with highest aggressivity scores (number of attacks and severity), or causing injuries.
 - 5.4.3.2. Submissive behaviours could be predictor of social defeat even if the attacks score is low.
 - 5.4.3.3. Behaviours such as upright defensive posture, side defensive posture, full submissive posture, and fleeing, could be scored to select aggressor even with a low attack score.
- 5.4.4. Induction of the social stress:
 - 5.4.4.1. The duration of the exposure and the number of days of exposure varies. The lower range could be used:
 - 5.4.4.1. Place the experimental mouse in the aggressor's cage compartment for 5 minutes. Then house the experimental mouse in the adjacent compartment for 55 minutes before returning to its home cage (individually housed).
 - 5.4.4.1.1. Expose the experimental mice for 4 days.
- 5.4.5. The duration of the exposure could be shortened based on the number and severity of the attacks:
 - 5.4.5.1. For example, the exposure could be ended after 10 regular attacks or 5 severe attacks (see below definition of an attack).
 - 5.4.5.1. The attack could be defined and graded to be used as scoring and intervention points:
 - 5.4.5.1.1. Define an attack as a lunge followed by a bite.
 - 5.4.5.1.2. A severe attack could be defined as lasting more than 3 seconds and/or causing injuries (see injury endpoints below).
 - 5.4.5.1.3. An attack could be stopped by the observer after 3 seconds.
 - 5.4.5.1.4. Submissive behaviors could be predictors of social defeat in absence of attacks.
- 5.4.6. Monitoring of the socially defeated animals and humane endpoints:
 - 5.4.6.1. Observe animal after each social defeat session. Palpate the rump to detect lesions.
 - 5.4.6.1. Provide systemic and/or local analgesia for animals with wounds > 1cm or swollen shoulders/elbows, or euthanize. Provide treatment to the skin wounds as determined by the veterinarian.
 - 5.4.6.2. Euthanize animals with severely injured penis.
- 5.4.7. As there is a normal incidence of aggression, experimental mice could have lesions even before being selected for a social defeat study:
 - 5.4.7.1. Whenever possible, request littermates and house mice individually at receiving instead of mixing animals from different cages.

SOP REVISION HISTORY

DATE	PREVIOUS VERSION	NEW VERSION
2017.12.22	4.1.3 (NO TEXT)	4.1.3 Only work with one cage of mice at a time to allow for continuous monitoring of the animals during the induction of social stress.