McGill

STANDARD OPERATING PROCEDURE #416

SOCIAL DEFEAT MODEL - MICE

1. PURPOSE

This Standard Operating Procedure (SOP) describes the social defeat model in mice.

2. RESPONSIBILITY

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

3. MATERIALS

3.1. Social defeat cage: larger cage containing no nesting material and fitted with transparent, perforated partition

3.2. Tool to stop aggressive bouts, e.g., ruler, rod, or other blunt-end tool

3.3. Timer/stopwatch

3.4. Balance

4. CONSIDERATIONS

4.1. The social defeat model uses social conflict between mice to generate emotional and psychological stress.

4.2. A major obstacle in the assessment and clinical treatment of affective disorders is the limited availability of validated preclinical animal models accurately reflecting the symptoms of human patients. The social defeat stress has been shown to have excellent etiological, predictive, discriminative, and face validity for modelizing social stress and its consequences, such as depression.

4.3. Group-housed animals are constantly affected by social stress and conflict in regular housing conditions. The physical contact is necessary for the expression of natural antagonistic social behaviors in mice. However, the goal is to induce social stress not injuries. Injuries are not necessary to induce stress, and efforts are made to avoid them. The incidence of injuries reaching humane endpoints is, in general, low.

4.4. Applying a range in the number of aggressive bouts and/or duration of exposure to the aggressor (resident) can be necessary to account for the experimenter effect, the aggressiveness of the resident mouse, the susceptibility to stress of the animal model, and research objectives. In any case, the goal is to use the minimum number of aggressive bouts and the shortest duration of exposure necessary to achieve the experimental objectives.

4.5. Experimenters should only work with a maximum of two cages of mice at a time to allow for continuous monitoring of the animals during the phases of screening and induction of social stress.

5. PROCEDURES

5.1. **First phase: screening, selection, and acclimation of the aggressors**

5.1.1. Aggressor mice (retired breeder, preferably > 4 months) are screened for their aggressive potential by presenting them with a different test mouse (intruder) every day for 3-5 days.

5.1.2. Standard acclimation as described in SOP 531 applies.

5.1.2.1. Animals arriving directly from a vendor must be acclimated for a minimum of 72 hours before starting experimental procedures.

5.1.2.2. Animals already present in the animal facility should be acclimated to the social defeat cage or to single-housing for a minimum of 24 hours.

5.1.3. The test mouse (intruder) is placed in the cage of the aggressor (resident) for 3-5 minutes. Since the test mice used in the screening process are not required to develop the depression-like phenotype and can be exposed to multiple aggressors, the total number of defeat interactions should be limited to the shortest duration.

5.1.3.1. The aggressive bouts are stopped after a maximum of 3-5 seconds with a tool (e.g., ruler, rod, other blunt-end tool), not the hands, to prevent injuries to the animals and the experimenter.

5.1.4. To ensure consistent behavior and social stress induction, the selection criteria for the aggressor are:

5.1.4.1. Latency to first attack is less than 1 minute.

5.1.4.2. More than 5 aggressive bouts per screening session over 2 consecutive days.

5.1.5. Overly aggressive animals are excluded, i.e., aggressor that attacks continuously or that consistently inflicts severe wounds.

5.1.6. The test mice (intruder) are examined by the experimenter after each trial for signs of injuries and their body weight is measured daily as described in 5.4. The test mouse will be presented to different aggressors every day for 3-5 days.

5.1.7. The test mice used in this first phase are euthanized at the end of the screening phase.

5.2. **Second phase: induction of the social stress**

5.2.1. The selected aggressor is acclimated to one side of the social defeat cage for a minimum of 24 hours.

5.2.2. Social defeat is initiated when a rodent is introduced into the home cage compartment of an older, aggressive, dominant aggressor.

5.2.2.1. In the social defeat cage with water bottle, and food temporarily removed, the test mouse (intruder) is physically exposed to an aggressor (resident) for a maximum of 10 minutes.

5.2.2.2. The minimal duration required for reaching the scientific objectives shall be justified in the Animal Use Protocol (AUP) and approved by the Facility Animal Care Committee (FACC).

5.2.2.3. Latency and number of aggressive bouts are recorded whenever possible.

5.2.2.4. The aggressive bouts are stopped after a maximum of 3-5 seconds with a tool (e.g., ruler, rod, other blunt-end tool), not the hands, to prevent injuries to the animals and the experimenter.

5.2.3. The intruder is then housed for 24 hours in the social defeat cage with the aggressor on the other side of the transparent and perforated partition.

5.2.4. The intruder mouse is exposed to a different aggressor every day for a total of up to 10 defeat sessions (repeating steps 5.2.2 and 5.2.3).

5.2.5. The intruder mice are examined by the experimenter after each trial for signs of injuries, and are treated accordingly, and their body weight is measured daily as described in 5.4.

5.3. **Third phase: behavioral testing of the experimental mice.**

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

5.4. **Monitoring of the socially defeated animals**

5.4.1. Notify the Veterinary Care staff prior to starting a social defeat experiment.

5.4.2. Obtain a pre-trial bodyweight of the test mice (intruders).

5.4.3. Observe test mice (intruders) after each social defeat session.

5.4.3.1. Pay particular attention to the back, genitals, abdomen, and forelimbs. Examine the body to detect lesions that might not be visible. A cotton-tipped swab can be used to detect the presence of fresh blood on the body.

5.4.3.2. Avoid excessive manipulation or restraint of the mice.

5.4.3.3. Measure body weight daily.

5.4.3.4. Log bodyweight and observations. Ensure logs are readily available to the Veterinary Care staff for regular monitoring.

5.4.3.5. Notify the Veterinary Care staff when an animal has a wound.

5.5. **Humane intervention points**

5.5.1. Before starting a social defeat experiment, acceptable treatments should be determined in consultation with the researcher and veterinarian and described in the AUP.

5.5.2. Animals with severe injuries or >20% body weight loss from pre-trial baseline should be euthanized immediately.

5.5.3. For animals with wounds totaling > 1cm:

5.5.3.1. Animals in good overall general condition should be removed from the social defeat experiment and provided with general analgesics and topical treatment to the skin wounds as determined appropriate by the Veterinary Care staff.

5.5.3.2. Animals should not be returned to the social defeat experiment.

5.5.4. For animals with wounds totaling < 1cm:

5.5.4.1. Provide topical treatment to the skin wounds as described in the AUP.

5.5.4.2. Limit the duration of the social defeat sessions and the number of attacks for injured mice until wounds heal.

5.5.5. Animals with 15% body weight loss from pre-trial baseline:

5.5.5.1. Limit the duration of the social defeat sessions and the number of attacks.

5.5.5.2. Provide moistened food in the bottom of the cage.

5.5.5.3. If weight loss persists but does not decrease beyond 20% from the pre-trial baseline, animals should be removed from the social defeat experiment.

5.5.6. If wounding consistently reaches humane intervention points, reduce the duration of defeat sessions, the number and duration of attacks, or remove the problematic aggressor from the study. The veterinarian must be consulted.

6. REFERENCES

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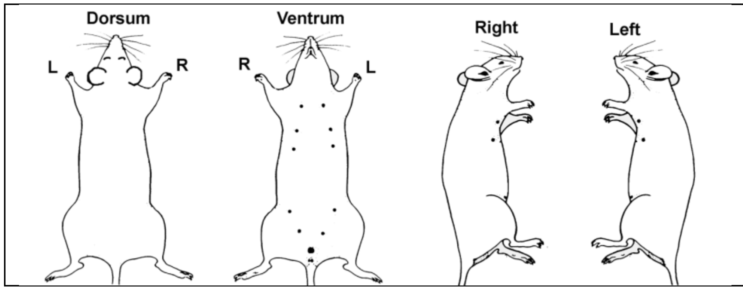
SOP REVISION HISTORY

DATE	NEW VERSION
2021.06.14	4.3 Group-housed animals are constantly affected by social stress and conflict in regular housing conditions. The physical contact is necessary for the expression of natural antagonistic social behaviors in ~~male~~ mice. However, the goal is to induce social stress not injuries. Injuries are not necessary to induce stress, and efforts are made to avoid them. The incidence of injuries reaching humane endpoints is in general low. The incidence of injuries reaching humane endpoints is in general low ~~(<5 %)~~.
2021.06.14	4.5. ~~Inexperienced~~ Experimenters should only work with one cage of mice at a time to allow for continuous monitoring of the animals during the phases of screening and induction of social stress. ~~With experience and the proper behavioral tracking software, it is possible to measure social defeat in up to four arenas concurrently~~.
2021.06.14	5.1.1. CD-1 male mice (retired breeder, preferably > 4 months) are screened for their aggressive potential by presenting them with a different test mouse (intruder) ~~that is representative of the experimental cohort,~~ every day for 3-5 days.
2021.06.14	5.1.3. The test mouse (intruder) is placed in the cage of the CD-1 aggressor (resident) for 3-5 minutes or 5-10 attacks, whichever comes first. **Since the test mouse used in the screening process can be exposed to multiple aggressors, the total number of defeat interactions should be limited to the shortest duration and least number of attacks.**
2021.06.14	5.1.3.2. The aggressive bouts are stopped after **a maximum of** 3~~–5~~ seconds with a tool (e.g., ruler, pen, rod), not the hands, to prevent injuries to the animals and the experimenter.
2021.06.14	5.1.4.2. More than 5 aggressive bouts **per screening session** over 2 consecutive days.
2021.06.14	5.2.2. Social defeat is initiated when a ~~male~~ rodent is introduced into the home cage of an older, aggressive, dominant male.
2021.06.14	5.2.2.1. In ~~a large~~ the **Social Defeat** cage **with the grill, water bottle, and food temporarily removed**, the test mouse (intruder) is physically exposed to a CD-1 aggressor (resident) for **a maximum of** 5~~–10~~ minutes or ~~5–~~10 attacks, whichever comes first.
2021.06.14	5.2.2.3. The aggressive bouts are stopped after **a maximum of** 3~~–5~~ seconds with a tool (e.g., ruler, pen, rod), not the hands, to prevent injuries to the animals and the experimenter.
2021.06.14	5.2.4 The intruder mouse is exposed to a different CD-1 aggressor every day for **a total of** up to 10 ~~days~~ **defeat sessions** (repeating steps 5.2.2 and 5.2.3).
2021.06.14	5.2.5 The intruder mice are examined by the experimenter after each trial for signs of injuries**, and are treated accordingly,** and their body weight is measured daily as described in 5.4.
2021.06.14	5.4.3.1. Pay particular attention to the back, genitals, abdomen, and forelimbs. ~~Palpate~~ **Carefully examine** the body to detect lesions that might not be visible. **A cotton-tipped swab can be used to detect the presence of fresh blood on the body. Avoid excessive manipulation or restraint of the mice.**
2021.06.14	5.4.3.4 Notify the Veterinary Care staff when an animal has a ~~serious~~ wound.
2021.06.14	5.4.5. For animals with wounds < 1cm: 5.4.5.5. Provide treatment as determined in the AUP or in consultation with the veterinarian **and limit the duration of the social defeat sessions and the number of attacks.** 5.4.5.6. Euthanize.
2021.06.14	5.5.6 If wounding consistently reaches humane intervention points, reduce the duration of defeat sessions, the number and duration of attacks, or remove the problematic aggressor CD-1 from the study. **Veterinary Care staff should be consulted.**
2022.01.07	3.1. Social defeat cage: larger cage **containing no nesting material and fitted** with transparent, perforated partition
2022.01.07	~~4.2. Validity of the social defeat model~~
2022.01.07	4.4. Applying a range in the number of aggressive bouts and**/or** duration of exposure to the aggressor (resident) can be necessary to account for the experimenter effect, the aggressiveness of the resident mouse, the susceptibility to stress of the animal model, and research objectives. In any case, the goal is to use the minimum number of aggressive bouts and the shortest duration of exposure necessary to achieve the experimental objectives.
2022.01.07	4.5. Experimenters should only work with ~~one cage~~ **a maximum of two cages** of mice at a time to allow for continuous monitoring of the animals during the phases of screening and induction of social stress.
2022.01.07	~~5.1.2. After a standard acclimation period upon arrival (see SOP 531), the CD-1 mouse is acclimated to the social defeat cage for a minimum of 3 days.~~ **5.1.2. Standard acclimation as described in SOP 531 applies.** **5.1.2.1. Animals arriving directly from a vendor must be acclimated for a minimum of 72 hours before starting experimental procedures.** **5.1.2.2. Animals already present in the animal facility should be acclimated to the social defeat cage for a minimum of 24 hours.**
	5.1.3. The test mouse (intruder) is placed in the cage of the CD-1 aggressor (resident) for 3-5 minutes ~~or 5-10 attacks, whichever comes first~~. Since the test mice used in the screening process **are not required to develop the depression-like phenotype and** can be exposed to multiple aggressors, the total number of defeat interactions should be limited to the shortest duration. ~~and least number of attacks.~~
2022.01.07	~~5.1.3.1. The aggressive bout is defined as a rapid motion towards the other mouse with biting, and adoption of a submissive or defensive posture, or fleeing by the intruder mouse.~~
2022.01.07	5.1.3.3. The aggressive bouts are stopped after a maximum of 3-**5** seconds with a tool (e.g., ruler, pen, rod), not the hands, to prevent injuries to the animals and the experimenter.
2022.01.07	5.1.5. Overly aggressive CD-1 males are excluded~~:~~ **i.e., males that attack continuously or that consistently inflict severe wounds.** ~~5.1.5.1. Males that attack suddenly and continuously or that consistently inflict severe wounds.5.1.5.1. Males that attack suddenly and continuously or that consistently inflict severe wounds.~~
2022.01.07	5.2.1. The selected CD-1 aggressor is acclimated to the social defeat cage for a minimum of ~~3 days~~ **24 hours**.
2022.01.07	5.2.2.1. In the social defeat cage with ~~the grill,~~ water bottle, and food temporarily removed, the test mouse (intruder) is physically exposed to a CD-1 aggressor (resident) for a maximum of ~~5~~ **10** minutes ~~or 10 attacks, whichever comes first~~. The minimal duration **required** ~~and number of attacks~~ for reaching the scientific objectives shall be justified in the Animal Use Protocol (AUP) and approved by the Facility Animal Care Committee (FACC). Latency and number of aggressive bouts are recorded **whenever possible**.
2022.01.07	~~5.2.2.2. The aggressive bout is defined as a rapid motion towards the other mouse with biting, and adoption of a submissive or defensive posture, or fleeing by the intruder mouse.~~
2022.01.07	5.2.2.2. The aggressive bouts are stopped after a maximum of 3-**5** seconds with a tool (e.g., ruler, pen, rod), not the hands, to prevent injuries to the animals and the experimenter.
2022.01.07	~~5.4.4. Immediately euthanize animals with wounds > 1cm, swollen shoulders/elbows, severely injured genitals, or >20% body weight loss from pre-trial baseline.~~ **5.4.4. For animals with wounds > 1cm:** **5.4.4.5. Animals in good overall general condition should be removed from the social defeat experiment and provided with general analgesics and topical treatment to the skin wounds as determined appropriate by the Veterinary Care staff.** **5.4.4.6. Animals with severe injuries or >20% body weight loss from pre-trial baseline should be euthanized immediately.**

DATE	NEW VERSION
2022.01.07	5.4.5.1. Provide **topical** treatment **to the skin wounds** as determined in the AUP or in consultation with **the researcher** and veterinarian. ~~and limit the duration of the social defeat sessions and the number of attacks.~~ **5.4.5.2. Consider limiting the duration of the social defeat sessions and the number of attacks for injured mice.** ~~5.4.5.3. Euthanize.~~
2022.07.19	**5.5.4. Animals with 15% body weight loss from pre-trial baseline:** **5.5.4.1. Consider limiting the duration of the social defeat sessions and the number of attacks.** **5.5.4.2. Provide moistened food in the bottom of the cage.** **5.5.4.3. If weight loss persists but does not decrease beyond 20% from the pre-trial baseline, animals should be removed from the social defeat experiment.**
22.09.01	**5.5.1. Before starting a social defeat experiment, acceptable treatments should be determined in consultation with the researcher and veterinarian and described in the AUP.**
2022.11.01	**3.3. Timer/stopwatch** **3.4. Balance**
2022.11.01	5.1.2.2. Animals already present in the animal facility should be acclimated to the social defeat cage **or to single-housing** for a minimum of 24 hours.
2022.11.01	5.2.1. The selected CD-1 aggressor is acclimated to **one side of** the social defeat cage for a minimum of 24 hours.
2022.11.01	5.2.2. Social defeat is initiated when a rodent is introduced into the home cage **compartment** of an older, aggressive, dominant male.
2023.08.28	3.2. Tool to stop aggressive bouts, e.g., ruler, ~~pen,~~ rod, **or other blunt-end tool**
2023.08.28	**5.5.3.2. Animals should not be returned to the social defeat experiment.**
2023.09.04	5.5.4.2. ~~Consider limiting~~ **Limit** the duration of the social defeat sessions and the number of attacks for injured mice **until wounds heal**.
2023.09.04	5.5.5.1. ~~Consider limiting~~ **Limit** the duration of the social defeat sessions and the number of attacks.
2023.09.11	5.1.1. ~~CD-1 male~~ **Aggressor** mice (retired breeder, preferably > 4 months) are screened for their aggressive potential by presenting them with a different test mouse (intruder) every day for 3-5 days.
2023.09.11	5.1.3. The test mouse (intruder) is placed in the cage of the ~~CD-1~~ aggressor (resident) for 3-5 minutes. Since the test mice used in the screening process are not required to develop the depression-like phenotype and can be exposed to multiple aggressors, the total number of defeat interactions should be limited to the shortest duration.
2023.09.11	5.1.3.1. The aggressive bouts are stopped after a maximum of 3-5 seconds with a tool (e.g., ruler, ~~pen,~~ rod, **other blunt-end tool**), not the hands, to prevent injuries to the animals and the experimenter.
2023.09.11	5.1.4. To ensure consistent behavior and social stress induction, the selection criteria for the ~~CD-1 males~~ **aggressor** are:
2023.09.11	5.1.5. Overly aggressive ~~CD-1 males~~ **animals** are excluded, i.e., ~~males~~ **aggressor** that attacks continuously or that consistently inflicts severe wounds.
2023.09.11	5.2.1. The selected ~~CD-1~~ **aggressor** is acclimated to one side of the social defeat cage for a minimum of 24 hours.
2023.09.11	5.2.2. Social defeat is initiated when a rodent is introduced into the home cage compartment of an older, aggressive, dominant ~~male~~ **aggressor**.
2023.09.11	5.2.2.1. In the social defeat cage with water bottle, and food temporarily removed, the test mouse (intruder) is physically exposed to ~~a CD-1~~ **an aggressor** (resident) for a maximum of 10 minutes. The minimal duration required for reaching the scientific objectives shall be justified in the Animal Use Protocol (AUP) and approved by the Facility Animal Care Committee (FACC). Latency and number of aggressive bouts are recorded whenever possible.
2023.09.11	5.2.2.2. The aggressive bouts are stopped after a maximum of 3-5 seconds with a tool (e.g., ruler, ~~pen,~~ rod, **other blunt-end tool**), not the hands, to prevent injuries to the animals and the experimenter.
2023.09.11	5.2.4. The intruder mouse is exposed to a different ~~CD-1~~ aggressor every day for a total of up to 10 defeat sessions (repeating steps 5.2.2 and 5.2.3).
2023.09.11	5.5.3. For animals with wounds **totaling** > 1cm:
2023.09.11	5.5.4. For animals with wounds **totaling** < 1cm:
2023.09.11	5.5.6. If wounding consistently reaches humane intervention points, reduce the duration of defeat sessions, the number and duration of attacks, or remove the problematic ~~CD-1~~ aggressor from the study. The veterinarian ~~should~~ **must** be consulted.

Social Defeat Daily Observations Log



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BW:

COMMENTS:

DATE	**BW**	**WOUNDS**	**OBSERVATIONS** Include location, size, and depth of wounds, treatments administered, general condition, etc.
		☐ Yes ☐ No ☐ Vet Care contacted	
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