Video Article

Social Defeat Stress Paradigm: A Mouse Model of Depression

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Abstract

Major depressive disorder (MDD) afflicts 2-5% of people worldwide, affecting approximately 14.8 million American adults and ranking number one in global mental health burdens[1-3]. Despite the social and economic cost of MDD, there is still a lack in understanding the underlying mechanisms of such disorders, resulting in limited consistent models of depression[4]. Animal models of depression rely on stress exposure to induce a depressive-like phenotype that is quantifiable by correlates of human symptoms, such as social avoidance, anxiety and anhedonia[5]. Importantly, chronic social defeat produces a reliable and long-lasting separation of two phenotypes, susceptible and resilient[6]. Both subtypes have distinct molecular, physiological and behavioral characteristics, many having correlates to human data[7]. Furthermore, only the susceptible subgroup demonstrates depressive-like behaviors, such as a decreased sucrose preference, a correlative of anhedonia which is a symptom of depression in humans [6, 8, 9]. Importantly, the increase in social avoidance behavior and anhedonia observed in susceptible mice is reversed with chronic, but not acute, administration of classical SSRI antidepressants. This is similar to the chronic usage of these drugs required in the clinical treatment of depression in humans [10]. These findings make social defeat a powerful model for investigating the underlying mechanisms of susceptibility and resilience to depression[2, 8, 9, 11]. Understanding both the passive pathogenesis that results in depression and the active neural coping mechanisms that underlie resilience to depression is crucial for revealing treatment targets[12].

An additional valuable paradigm is a sub-threshold social defeat, in which animals undergo 2-3 bouts of defeat in one day. Following this paradigm, mice exhibit no social avoidance or anhedonic behaviors, but have a heightened sensitivity to further stress. This sub-threshold model is very useful for elucidating mechanisms that may contribute to a depression-like phenotype. For instance, combined with optogenetic and molecular manipulations, a sub-threshold defeat can be used to directly link the contribution of specific brain regions and corresponding neurophysiological activity with the induction of social avoidant and anhedonic behavior [13].

Chronic social defeat has revealed molecular, electrophysiological and structural modifications, many of which have been found in human depression. Therefore, both chronic and sub-threshold social defeat are useful animal models to further our understanding of the molecular and cellular mechanisms underlying depression thereby improving the mechanistic scope of therapeutic treatments.

Video Link

The video component of this article can be found at http://www.jove.com/video/4226/

Protocol

1. Three Day CD1 Mouse Territorial Response Screening

1. Prior to screening, individually shoe-cage housed CD1 retired breeder mice are given four days to acclimate to the new facility and cage to ensure a territorial response.
2. During the first day of screening, one naïve C57 screener mouse (7-8 weeks in age) is placed into each home cage of the CD1 retired breeder mouse. Observing five cages at a time and using a stopwatch, record the time it takes for the CD1 mouse to display an aggressive territorial response. The screener mouse is then removed from the CD1 cage after observing aggression.
3. The screening procedure is repeated for the next two days. A screener mouse is placed into each home cage of the CD1 retired breeder mouse and the delay to an aggressive response is recorded.
4. CD1 mice that do not exhibit **consistent** aggressive behavior within 1 min on the third day of screening should not be utilized for the chronic social defeat. Typically, about 50% of CD1 mice qualify for use as experimental aggressors.
5. CD1 mice can be used for multiple rounds of defeat. The aggressors should be re-screened prior to the next round of social defeat experiments.

2. Ten Day Chronic Social Defeat (Fig. 1A)

1. Upon arrival to the animal facility, C57 test mice should first be allowed to habituate for 1 week. This reduces the contribution of shipping stress and novel environment on the behavioral paradigm.
2. Clear rectangular hamster cages with a paired steal wire top, two water bottles and a perforated plexiglass divider must be set up with **wood-chip bedding** for each of the 10 C57 test mice that will undergo defeat and for those that are control mice. Each plexiglass divider must fit precisely within the cage and exactly fit with the cage top, to avoid the CD1 aggressor from passing around the barrier. Without the cage divider placed in the proper configuration, the CD1 aggressor can escape to the other side of the cage and the C57 test mice will experience continuous aggressive conditions.
3. Next, the previously screened aggressor mice must be placed in the clear hamster cage on one side of the divider for 24 hr. This period allows for the CD1 aggressor mouse to establish a territorial response.
4. On the first day of defeat one C57 (8-10 weeks of age) mouse is placed into each hamster cage on the side of the CD1 mouse for 5 min or less. Mice are continuously observed and should be separated promptly if judgment is made that the C57BL subject might suffer a serious injury if a severe and continuous aggressive interaction is not stopped. In our experience, with careful observation and prompt interaction, serious injuries occur in less than <5% of tested subjects. In such instances, injured mice should be immediately removed from the study.
5. After the aggressive interaction, the C57 mouse is moved to the opposite side of the plexiglass in the same hamster cage. This allows for continual stressful sensory cues.
6. The control mice must be handled in the same way. Transfer each control mouse from hamster cage to another hamster cage. Control mice only receive sensory cues from another C57 and have no interaction with the CD1.
7. On the second day of defeat, each C57 mouse is moved to a different hamster cage to interact with a new CD1 aggressor for 5 min or less. The C57 mouse is then placed across the barrier from its immediate previous aggressor receiving sensory cues until the following day.
8. This procedure is repeated once daily for ten days directly proceeding the lights being turned off. Each day the C57 mouse encounters a novel aggressor for an aggressive interaction. A cohort of ten animals is the minimum number of defeats recommended for one round of social defeat. This ensures that each test mouse interacts with a novel aggressor each day.
9. On the tenth day of social defeat, after the final aggressive interaction, all the C57 test mice, including the paired C57 control mice are individually housed.

3. Sub-threshold Social Defeat Paradigm

To induce an alternative sub-threshold social avoidant phenotype, a one day micro-defeat paradigm can be utilized (**Fig. 1B**).

1. Previously screened aggressor CD1 mice must be placed in a shoe-cage for 24 hr to develop a sufficient territorial response.
2. On the second day, the C57 test mouse is placed on the same side of the divider as the CD1 mouse and allowed to interact for 2 min. Again, possible injury is avoided, and injured mice are removed from the study.
3. The C57 test mouse is then transferred to the other side of the barrier for 15 min of stressful sensory cues from the CD1 mouse from the other side of the Plexiglass barrier.
4. Following the 15 min of sensory cues, the C57 mouse is moved, once again, to the other side of the divider with a different CD1 for another 2 min. Depending on the genetic strain and age, this procedure can be repeated 2-4 times to reach a sub-threshold social avoidance.
5. The C57 mice are then housed individually in standard mouse shoe-cages with *ad libitum* access to food, water and wood-chip bedding.

4. Social Interaction Test

1. On the eleventh day following chronic social defeat or 24 hr after the sub-threshold social defeat, move the individually housed C57 test mice and a CD1 mouse not previously used during defeat, to the behavioral test area 1 hr prior to testing. This allows for the mice to acclimate to the red light conditions.
2. The open field arena is divided into an interaction zone and two opposing corner zones. Utilizing the Ethovision tracking software program, the relative amount of time each mouse spends in each zone is measured (**Fig. 2A**). Ethovision also can provide measurements of distance travelled, entrances to the different zones, and travel velocity which can also be used for further comparison.
3. Place a metal mesh-plastic target box (**Fig. 2B**) in the interaction zone.
4. Place the first individually housed test animal in the corner of the open field arena opposite the empty metal mesh-plastic target box for 2.5 min (no target). The distance travelled and time spent in each zone is recorded by video-tracking.
5. Remove the C57 mouse from the arena and place it back into the home-cage. Wipe the arena clean with virkon cleaning solution to remove odor cues and feces.
6. Place a novel CD1 mouse in the metal mesh-plastic target box as a social target in the interaction zone. It is important the social target CD1 mouse has not previously interacted with any of the C57 test mice. The metal mesh should be large enough to allow for visual and odor cues, and should have a plastic upper portion to avoid escape-climbing behavior of the CD1 mouse.
7. Place the C57 mouse back in to the arena, at the same corner opposite the target box containing the CD1 mouse (with target). Another 2.5 min of independent video-tracking is used to measure the amount of time spent in each zone.
8. Return the C57 mouse to its home cage.
9. Return the CD1 social target mouse to its home cage.
10. Between each interaction trial it is essential to wipe the arena and mesh cage with antiseptic Virkon solution to remove odor cues, urine and feces.
11. This procedure is repeated for each mouse that underwent social defeat and each control mouse. The same social target CD1 mouse should be used for both groups..

5. Social Interaction Ratio

1. To determine the interaction ratio, divide the time the C57 spends in the interaction zone with the target CD1 mouse present by the amount of time spent in the interaction zone with the target CD1 absent, and normalize to 100.
2. The interaction ratio is defined as:

Social interaction ratio= (Time spent in Interaction zone target present/Time spent in interaction zone no target present)*100

C57 mice with scores <100 are determined to be susceptible and those with scores ≥ 100 are determined to be unsusceptible or resilient [7]. A split of approximately 2/3 will be susceptible and have an interaction ratio score below 100. Non-stressed control C57 mice typically find social interactions rewarding and will spend significantly longer amounts of time in the interaction zone when it contains a social target, compared to the no target trial.

Additional Measurements: All additional measurements such as sucrose preference should be performed after the social interaction test to avoid interference with social avoidance behavior.

6. Sucrose Preference

1. Following the social interaction test, acclimate the C57 test mice to a two bottle choice for 24 hr with two bottles placed in the home-cage. Acclimation may be done with either two water bottles or with one bottle containing 1% sucrose solution.
2. On day 12, interchange the bottles and sucrose consumption can be measured for either a 6, 12 or 24 hr period. Measurements may be done either by weight or marking the level of liquid change.
3. After each measurement, interchange the bottle locations to avoid a side preference.
4. Sucrose preference is represented as percent of sucrose solution consumed divided by the total volume of water consumed.

7. Representative Results

A typical distribution of susceptible and resilient phenotypes in a cohort of mice is determined by a comparison of the total time spent in the interaction zone during each social interaction test when the target absent or present varies. Approximately 2/3 of a cohort exhibits social avoidance behavior. A social interaction ratio score of 100, in which equal amounts of time is spent in the interaction zone with target as compared to without target, is used as the cutoff for dividing the mice between susceptible or resilient subgoups. The subgroups can also be represented by the relative time spent in the interaction zone and the corner zone with the target absent or present. Susceptible mice spend more time in the corner zone than the interaction zone with the target present. Resilient mice spend more time in the interaction zone than the corner zone with the target present, similar to stress-naïve control mice.

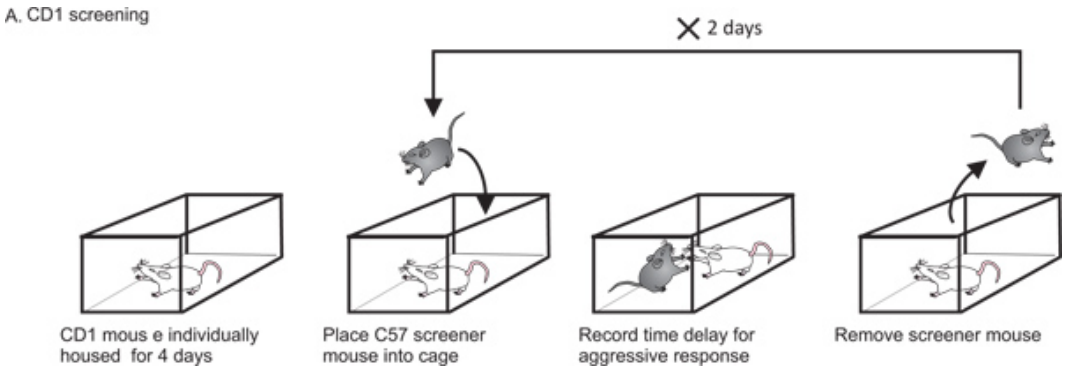

Figure 1. Schematic of (A) chronic social defeat procedure and (B) sub-threshold social defeat procedure.

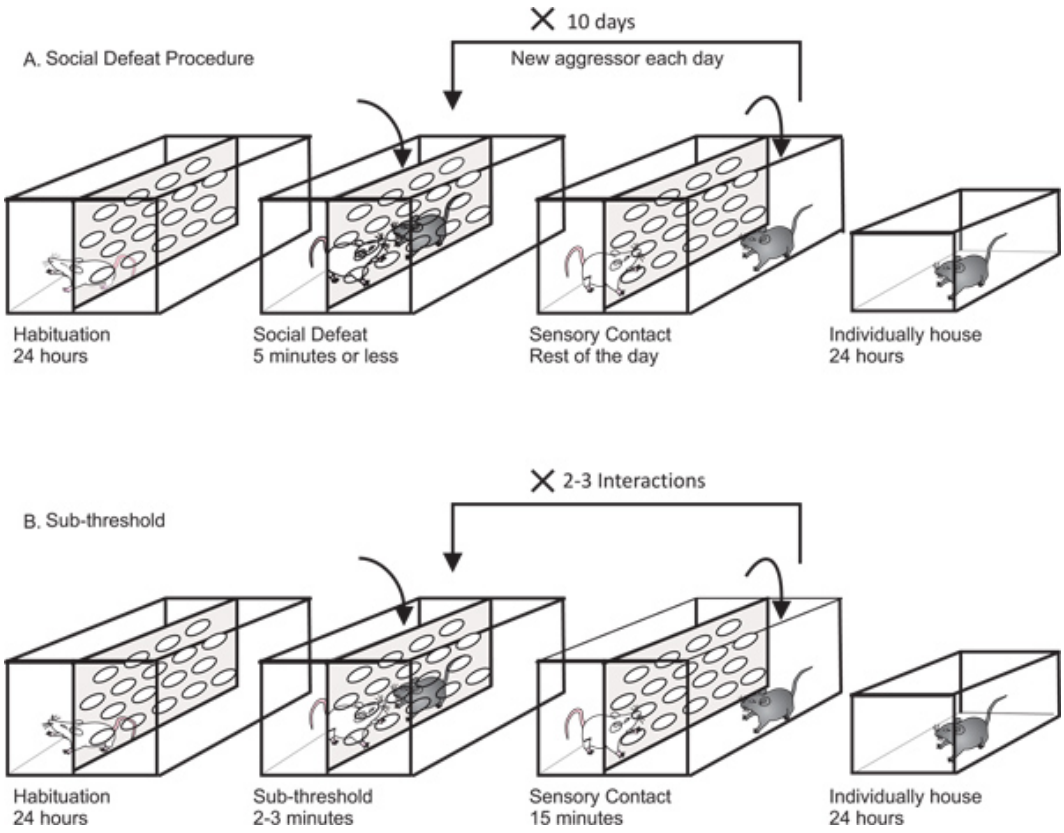

Figure 2. Social interaction test. (A) Representative diagram of the open field arena and the interaction and corner zone designation. (B) Schematic of metal-mesh-plastic target box. (C) Schematic of social interaction test.

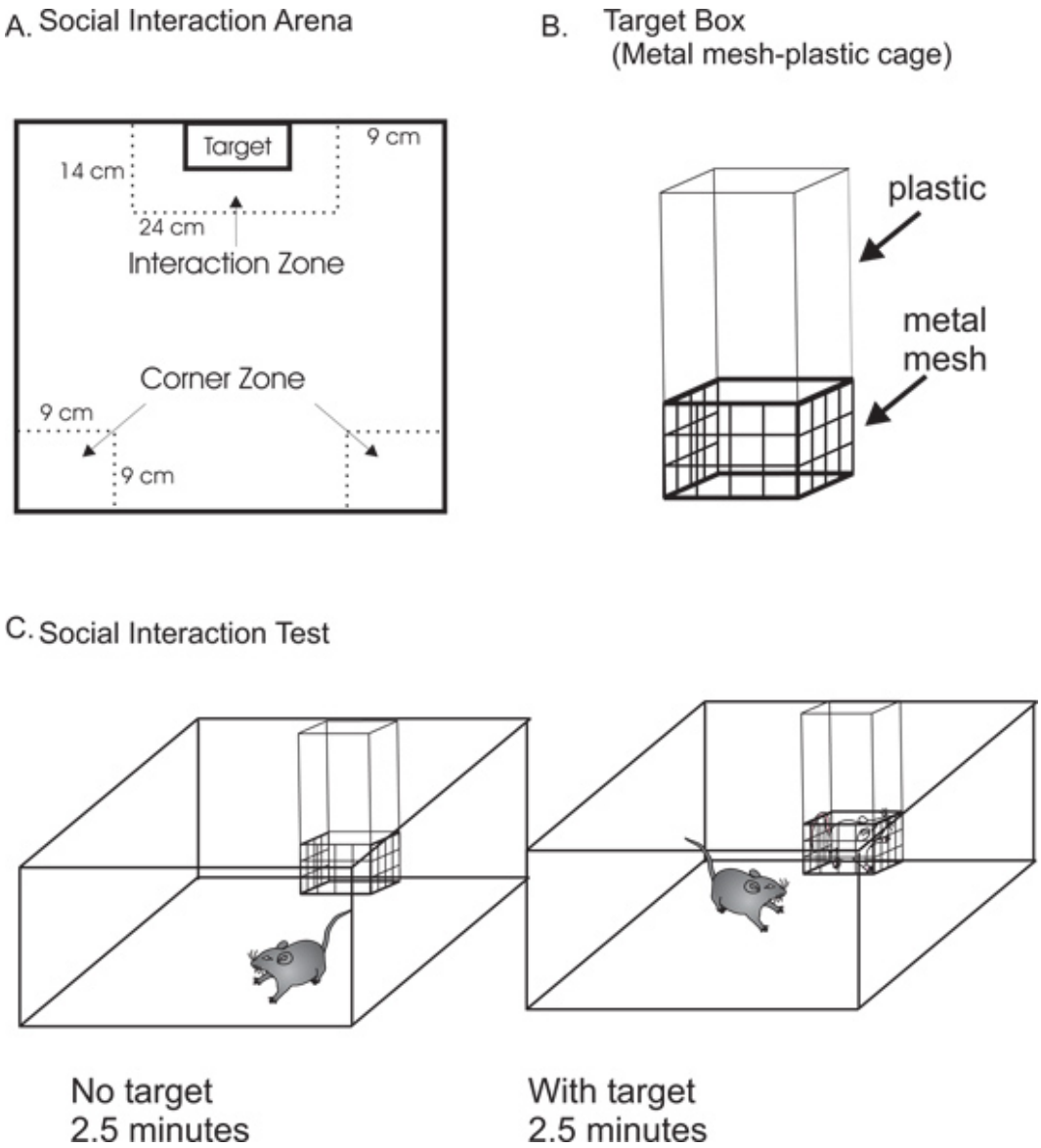

Figure 3. Typical results of the social defeat paradigm, represented by time spent in the interaction and corner zones with target and social interaction ratio. (A) Chronic social defeat: Susceptible mice spend significantly less time in the interaction zone (n=40, P< 0.001) and significantly more time in the corner zone (n=40, P<0.001) with the target present. (B) Social interaction ratio can also be used as a measure of social avoidance by representing the relative time spent in the interaction zone without and with a social target. Susceptible mice have a significantly lower social interaction ratio (n=40, P<0.001 value). (C) Sub-threshold social defeat: Mice that undergo sub-threshold social defeat or control conditions have no significant difference in time spent in the interaction zone or corner zone with the target present (n=20, P=0.71). (D) There is no significant difference in the interaction ratio between mice that undergo control or sub-threshold social defeat conditions (n=20) (Error bars represent ± SEM **P<0.001).

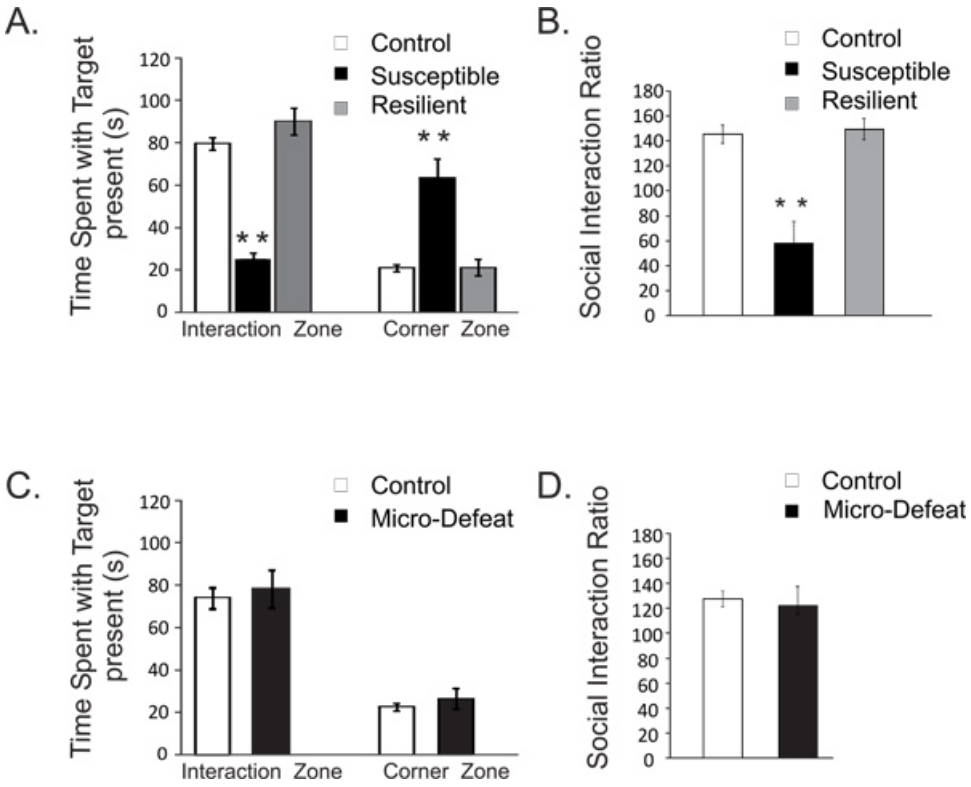

Figure 4.

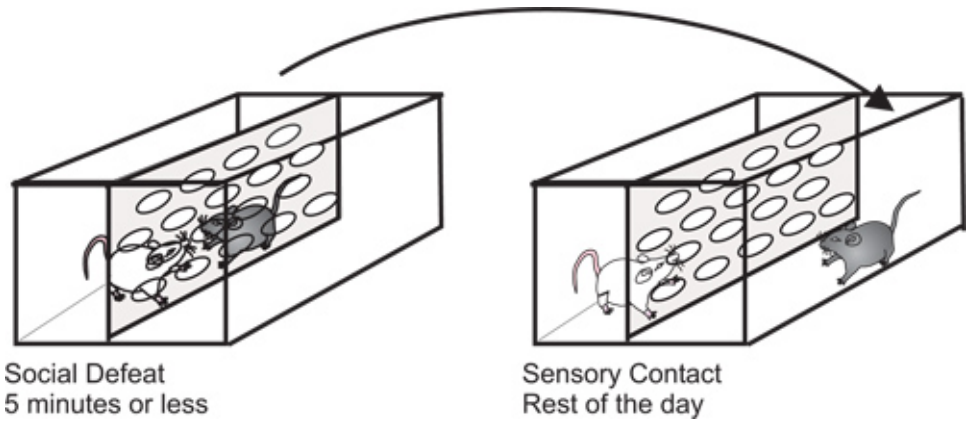

Figure 5.

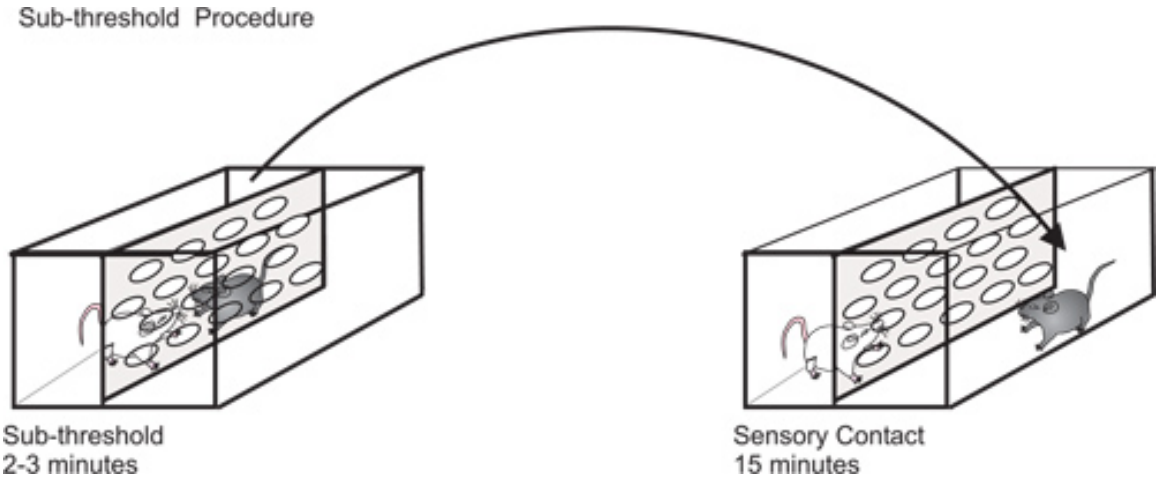

Figure 6.

Discussion

Chronic social defeat may be suitable for a large range of genetic backgrounds of male mice. The ten day paradigm has been shown to establish stable, long-lasting behavioral, cellular and molecular changes. Although variations in the duration of this paradigm may be possible, they have not been fully validated. It is important to note that the age and genetic background of the mice used will result in varying social avoidant phenotypes and that the 8-week age and C57BL/6J line have resulted in the consistent behavioral results using this paradigm. Additionally, success of the social defeat, as measured by the following avoidance behavior, is sensitive to a series of subtle factors listed within the protocol and highlighted here: The relatively larger size of the defeat cage such as large shoe-cage is optimal to ensure successful chronic social defeat and to avoid a learned helplessness phenotype. The wood chip bedding listed in the equipment provides necessary traction for the aggressive bout of defeat. Performing the defeat 1 hr before the lights are turned off, the same time of day for ten days will result in a more consistent level of interactions.

The social interaction test is performed to measure the relative social avoidance or amount of time the C57 mouse spends in the target or corner zone in an open field under two different conditions, either with or without a social target present. The social avoidance behavior is sensitive to loud noises and disturbances which can induce freezing behavior during testing; therefore reasonable measures should be taken to reduce excess noise. Ideally, during testing of social avoidance, a novel behavior space that is separate from the housing area should be used to avoid the influence of environmental cues and excess noise. It is also crucial to thoroughly clean the social interaction arena to avoid stressful olfactory cues between each 2.5 min trial.

Both the chronic social defeat and sub-threshold social defeat paradigm are highly adaptable. While only the susceptible subgroup exhibit social avoidance behavior, all the C57 mice that undergo social defeat exhibit an anxiety phenotype [7]. Therefore, this model may also be beneficial in understanding the different and overlapping molecular and structural changes that occur in both depression and anxiety phenotypes [14]. Further, work has also incorporated the use of optogenetic stimulation during social defeat, sub-threshold social defeat and social interaction [13, 15].

An experimental advantage of the chronic social defeat is that the molecular and behavioral differences between the resilient and depressive-like mice have been shown to last at least three months. Importantly, this model has been further utilized to understand the underlying epigenetic, molecular and physiological phenotypes of depression and resilience to depression [7, 12, 16, 17]. Used with extreme care to avoid injury to the test subjects, this is one of the best pre-clinical models to assist in the development and testing of novel therapeutics for the treatment of depression. However, it is very important that during these procedures, injuries should be very carefully avoided and mice should be used with extreme care and excluded from the study if injury occurs.

Disclosures

No conflicts of interest declared.

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