To appear in: Mouse Models for Drug Discovery

Eds: G. Proetzel and M. Wiles, Humana Press, 2009

Mouse Models for Studying Depression-like States and Antidepressant Drugs

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Abstract

Depression is a common psychiatric disorder, with diverse symptoms and high comorbidity with other brain dysfunctions. Due to this complexity, little is known about the neural and genetic mechanisms involved in depression pathogenesis. In a large proportion of patients, current antidepressant treatments are often ineffective and/or have undesirable side effects, fueling the search for more effective drugs. Animal models mimicking various symptoms of depression are indispensible in studying the biological mechanisms of this disease. Here, we summarize several popular methods for assessing depression-like symptoms in mice, and their utility in screening antidepressant drugs.

Keywords: depression, animal models, antidepressant drug screening, despair, anhedonia, chronic stress

1. Introduction

The underlying pathophysiology of depression remains unclear despite the seriousness and prevalence of this disorder (1). Clinical symptoms of depression manifest at psychological, physiological, and behavioral levels, and include changes in appetite and sleeping patterns, sad or irritable mood, psychomotor agitation, fatigue, anhedonia, poor concentration, feelings of guilt, and recurrent thoughts of suicide or death (2-5). While the introduction of monoamine-based antidepressants has promoted various neurotransmitter system-based models of depression (1), little is known about their mechanisms of therapeutic action. Additionally, a sizeable number of depressed patients are nonresponsive to initial treatments, collectively emphasizing the need for newer and more effective drugs (1, 2, 5, 6).

Animal models are widely used to study the neurobiological mechanisms of depression (7-9). Ideal animal depression models must be reasonably analogous to the human condition, be able to be monitored objectively, be reversed by the same treatment modalities as humans, and be reproducible between laboratories (3, 5, 10). Although selected depression symptoms may be irreproducible in animals (e.g., thoughts of suicide), a number of models exhibit considerable construct validity when targeting other clinical endophenotypes of depression (4, 5, 11). Antidepressant treatment has been shown to affect the behavioral responses in these models (see further), indicating that certain depression paradigms are pharmacologically sensitive, and therefore, can be used in the testing of antidepressant drugs in mice.

A clear distinction must be made between animal models of depression and animal tests (or screens) of antidepressant drugs. Both types of animal paradigms, equally important for further progress in biological psychiatry and drug discovery, will be discussed here in detail. Finally, automated versions of some of these tests are currently available (12, 13), enabling

consistent behavioral measurement, standardization of experimental protocols, and increased throughput and testing.

2. Materials and Methods

2.1 Animals

1. Various inbred, selectively bred, and genetically modified (mutant or transgenic (14)) mice (see more details in the chapter on mouse models of anxiety)

2.2 Housing

1. If mice are obtained from a commercial vendor or another laboratory, allow at least 1 week acclimation from shipping stress. In most cases, a much longer time will be required. Food and water should be freely available, unless the intake is being controlled for experimental purposes.
2. Utilize plastic, solid-floored cages with sufficient space. The mouse holding room should be kept at approximately 21°C, on a 12/12 hr light cycle. As mice are nocturnal, the light cycle may be inverted if spontaneous activity measures are needed (15).
3. All experimental procedures (including handling, housing, husbandry, and drug treatment) must be conducted in accordance with national and institutional guidelines for the care and use of laboratory animals.

2.3 Drugs

1. All experimental protocols described here are compatible with testing various antidepressants, administered with a vehicle (e.g., saline).
2. Common routes of injection include *systemic* (intraperitoneal (i.p.), intramuscular (i.m.), intravenous (i.v.), per oral (p.o.), subcutaneous (s.c.)) and *local* (intracerebral (i.c.) or intracerebroventricular (i.c.v)). Route of administration, dose, and pre-treatment time vary depending on strain sensitivity and the drug being used.

2.4 Observations and General Procedures

1. Observers must refrain from making noise or movement, as their presence may alter animal behavior. Assess intra- and inter-rater reliability for consistency. See details in the chapter on animal models of anxiety.
2. Allow at least 1-h acclimation of mice after their transfer from the animal holding room to the experimental room.
3. After each testing session, clean the equipment (e.g., with a 30% ethanol solution) to eliminate olfactory cues.

2.5 Data Analysis

1. Behavioral data may be analyzed with the Mann-Whitney U-test for comparing two groups (parametric Student's t-test may be used only if data are normally distributed), or analysis of variance (ANOVA) for multiple groups, followed by an appropriate post hoc test. Some experiments may require one-way ANOVA with repeated measures, or n-way ANOVA.

2.6 Sucrose consumption test

Materials:

1. Home cage
2. Two drinking bottles with water and sucrose

A core symptom of depression is anhedonia – a decreased interest in pleasurable activities (2). There are several commonly used tests to assess hedonic deficits in mice. The sucrose consumption test examines anhedonia in a relatively short period of time without the need for expensive equipment or extensive training of the test animals. In this model, a mouse is given free choice between regular tap water and a sucrose solution to drink. Usually, mice show a clear preference for the sweetened water, while depressed animals demonstrate markedly less

interest. A pure chance would result in animals drinking equally (50%) from each bottle, and a preference for sucrose of less than 65% is considered to be an indication of hedonic deficit (16). Since various antidepressant drugs reverse the anhedonia-like reduction in preference for sucrose (e.g., (17-19)), this test is widely used in the screening of antidepressant drugs.

Procedure: (16, 20, 21)

1. For a set period of time (e.g., 1, 3, or 7 days), allow mice access to two freely available water bottles – one containing tap water and the other containing a solution of up to 35% sucrose. To preclude side preference in drinking, switch the positions of the bottles halfway through the procedure.
2. Measure the volumes of sucrose solution and water consumed. Calculate the preference for the sucrose solution as a percentage of total liquid consumed, and total sucrose intake in mg per g body weight. In addition, a commercially available lick-counter may be used. Calculate the number of licks at each bottle per 100 mg of body weight, and the preference for sucrose as a percentage of total licks.

Notes:

1. The use of a 4-10% sucrose solution will usually generate good results for most mouse strains. However, some strains (especially mutant mice) may have abnormally reduced taste sensitivity, which would make assessment of their hedonic responses in this test difficult. Always check taste sensitivity prior to performing a sucrose consumption test by using a standard taste sensitivity test (see specific mouse phenotyping literature for details). Consider using a different strain if the problem persists. Alternatively, higher concentrations of sucrose (e.g., 20-35%) may be required.
2. To avoid the confounds of metabolic factors and acute stress, allow food and water ad libitum prior to performing the sucrose consumption test. However, some strains may

have altered water consumption (e.g., polydypsia), and the sucrose consumption test may not always be suitable for such strains.

3. Although this test can also be performed over a short time period (e.g., 2 h), mice consume so little over such a brief time and errors in measurement can result. Consider lengthening the period of the test to at least 24 h (a three-day test will be more appropriate in most cases).
4. Neophobia to the presence of multiple water bottles and to the taste of sucrose may also confound behavioral results in this model. To avoid this problem, acclimate mice by giving them 2 bottles, each with the sucrose solution, for 72 h before the test, or with one water and one sucrose bottle for 1 h/ day for one week. Also, consider lengthening the period of the test to at least 24 h.
5. Depending on the length of time over which this test is conducted, mice may alternate between active and inactive phases, which demonstrate marked differences in the animals' liquid consumption. When switching the positions of the bottles to avoid side preference, be sure to take shifting activity levels into account so that each bottle is in each position for the same amount of each activity phase.
6. Since some mouse strains may develop a metabolic syndrome-like phenotype or have pathologically high reward-related phenotype, their sucrose consumption may be abnormally high. In these cases, alternative methods of depression testing or other mouse strains may be required.

2.7 Coat state assessment

Materials:

1. No special materials are required for this test.

The coat state assessment is a fast and simple qualitative method of assessing mouse depression-like states through observation of the condition of an animal's fur. In rodents, coat state tends to decline with increased depression, similar to depressed patients who frequently exhibit poor hygiene (22-24). Antidepressants have been shown to improve the coat condition of mice while reducing depression-like symptoms (22-24).

Procedure:

1. After removing the animal from the homecage, assess the coat state in each of 8 regions: head, neck, forepaws, dorsal coat, ventral coat, hindlegs, tail, and genital region. A well-kept coat may score a 0 in each region, while each region which is dirty or disheveled may receive a score of 1. The average of the 8 scores for each animal can then be compared among individuals or groups (23, 24).

Notes:

1. Mouse strains may have different sensitivity in this test. For example, C57 mice can be resistant to deleterious effects of chronic stress on the coat state (25). Strain differences may result in differing levels of grooming activity. For example, some inbred strains may be inherently poor (e.g., BALB/c) or excellent (e.g., A/J) groomers, regardless of stress levels. Some mutant mouse strains also display "compulsive" grooming behavior (26) that may mask any alterations in the animal's coat state. Consider a more suitable strain if floor or ceiling effects occur.
2. In socially-housed mice, hetero-grooming may confound self-grooming data. Single-housing mice may eliminate this confound, but this practice should be used with caution, as social isolation stress may induce aberrant behavioral effects.
3. Some mouse strains may display pronounced balding patches due to alopecia (27) or increased barbering behavior (28-30), which will make the coat state data less valid.

Therefore, this model may not be used in high barbering strains. Likewise, stress *per se* may promote barbering in mice (31), thereby further confounding the coat assessment protocol.

2.8 Forced Swim Test:

Materials:

1. Clean glass cylinder (e.g., height 25 cm, diameter 10-15 cm).
2. Water maintained at 23-25 °C.
3. Towels to dry animals after swimming.
4. Stop watch to calculate the duration of immobility.
5. Optional: video-camera for subsequent video-tracking and data analysis (e.g., (12)).

Although it does not induce experimental depression in mice, the forced swim test (FST) is one of the most commonly utilized ethological models of fast high-throughput antidepressant screening. FST places mice in an inescapable aversive situation and measures their "despair," (learned helplessness) by an increased duration of immobility in the water. Animal FST immobility is markedly reduced by antidepressant drugs. FST has good predictive validity and is widely used in research investigating acute and chronic effects of antidepressant drugs (16, 32-35).

Procedure:

1. Place mice individually into a glass cylinder filled with 10 cm of water for 6 min.
2. As a measure of depression-like behavior, the total duration of immobility and the number of immobility episodes should be recorded. Immobility is defined as the absence of movement, unless they are necessary for the animal to stay afloat (head above water).
3. After testing, dry mouse thoroughly with towels and return to their homecages.

Notes:

1. Consider strain and individual differences in baseline immobility duration.
2. Motor or vestibular deficits may result in poor (abnormal) swimming, including aberrant spinning, turning, and sinking, that may confound FST data. Examine such mice in specific motor or vestibular ability tests. Mice with poor swimming should be excluded from the FST. Also note that some popular inbred mouse strains (e.g., 129 mice) are poor swimmers and develop spastic behaviors in FST situations that complicate their swimming.
3. Some mice exhibiting increased levels of FST immobility may be suffering from fatigue rather than depression *per se*. Evaluate the fatigueability of animals in separate tests. If mice display high fatigueability phenotypes, consider shortening the length of the test.
4. There is a growing number of mouse models with metabolic syndrome-like phenotypes, as well as with altered bone physiology (14). Consequently, mutant animals with such phenotypes may have affected swimming abilities/buoyancy, and therefore may not be adequately compared in the FST with their wild type littermates.

2.9 Tail Suspension Test (TST):

Materials:

1. A shelf or tail suspension apparatus to suspend mice. The apparatuses may be wooden or plastic boxes (e.g., 680 × 365 × 280 mm), painted to contrast with mice.
2. Tape measure to determine the height of suspension.
3. Adhesive tape to secure mice to suspension apparatus.
4. Optional: automated electromechanical strain gauge device, video tracking system

In the tail suspension test (TST), mice initially engage in vigorous escape behaviors, but eventually succumb to immobility. Like the FST, longer durations of TST immobility infer a heightened degree of behavioral despair. As such, TST is a commonly used screening method for

antidepressant properties of drugs, and is highly sensitive to pharmacological manipulations. Antidepressant drugs generally decrease the duration of TST immobility in mice (13, 33-35).

Procedure:

1. Mice may be suspended by the tail on the edge of a shelf or in a special apparatus, at least 35 cm above the floor (from the beginning of the tail).
2. The mice should be secured by adhesive tape approximately 1 cm from the tip of the tail for 6 min.
3. Researchers may choose to manually record data through direct observation or automatically collect data using a strain gauge device to detect movements.
4. Mice are considered immobile only when hanging passively and completely motionless.

Notes:

1. Some mice may fall from the apparatus due to poor fixation by the adhesive tape. Use a cushioned floor for the TST to prevent any damage to the animal and exclude such mice from the experiment.
2. Some strains (e.g., C57 mice) display specific tail climbing behaviors and may not be an appropriate mouse model for this test (36).
3. Some strains with vestibular deficits may show an abnormal “spinning” phenotype in the TST, thereby confounding behavioral data in this model. Consider using other models of depression for testing these mice.
4. Some mutant mice display other specific neurological abnormalities relevant to their TST performance. For example, hind leg clasping behavior, reported in some mice, may result in abnormally high immobility in this test (which can incorrectly be interpreted as low depression). In contrast, spontaneous mild seizures in some mutant mice may lead to reduced TST immobility, again confounding depression-related data.

2.10 Chronic Mild Stress (CMS):

Materials:

1. Supplementary cages for application of stressors.
2. Various stressors (e.g., soiled rat bedding, confinement tube, or predator sounds).

Chronic mild stress (CMS) presents mice with an unpredictable barrage of stressors to induce (rather than simply measure) a depressed state. CMS reduces sucrose or saccharin intake in mice, a symptom of anhedonia (see above). CMS may also be responsible for decreases in sexual and aggressive behavior, changes in sleeping habits, loss of body weight, pituitary-adrenal hyperactivity, an increased threshold for brain stimulation reward, and an abolishment of place conditioning, making it a valid ethological model of depression. These behavioral deficits can be reduced through chronic treatments of antidepressants, accentuating the pharmacological sensitivity of CMS procedure (11, 37-39).

Procedure:

1. Following a random schedule, subject mice to two or more stressors each day for 4-7 weeks.
2. Typical stressors may include: cage tilting (e.g., 45°), predator sounds, placement in an empty cage, placement in an empty cage with water on the bottom, damp sawdust, inversion of light/dark cycle, lights on during dark cycle, switching cages, food or water deprivation, short-term confinement in a tube, soiled cages with rat odors, and an inescapable footshock (37).
3. To prevent habituation and enhance the unpredictable nature of the model, stressors should be applied at varying time points.

4. Following the period of stress, mice can be tested with behavioral models of depression such as: coat state assessment, sucrose consumption test, FST, or TST (see these protocols above), to determine the effectiveness of the test.

Notes:

1. Consider strain differences in this paradigm. Some stressors may not affect all strains homogenously, and similarly, some models of depression may not accurately reflect depression in specific strains. For example, C57 mice are not sensitive to CMS affects on coat state (25).
2. While CMS is a valid model of depression in mice, it is labor intensive, long in duration, and demanding of space. A practical recommendation for this model is thorough planning of all experiments and consistent completion of the entire CMS battery.

3. Special Notes:

Occasionally, mice may have altered cognitive domains that may be easily misinterpreted in models of depression (8). For example, mice with elevated learning and memory abilities may display active initial locomotion that decreases significantly over time. While this reduction in locomotion may be attributable to heightened learning and habituation, it is easily wrongly assessed as behavioral despair. Likewise, mice with particularly low levels of memory and learning may be misinterpreted as persistently hyperlocomotive. The lack of habituation and decreased sensitivity to repeated stressors may be a result of a reduced learning phenotype, not hyperlocomotion.

Similarly, mice displaying hypoactivity and increased sensitivity to repeated stressors (incorrectly categorized as anxious) may be associated with an increased level of depression and better memory. Additionally, sustained hypoactivity coinciding with a decrease in habituation and sensitivity to repeated stressors may not be the result of increased anxiety or decreased

despair. Rather, these behaviors may indicate reduced learning and memory, but heightened depression. Overall, cognitive functions may strongly modulate animal performance in ethological models of depression. To diminish the likelihood of incorrect interpretation of behavioral data as depression, it is recommended that mice are carefully tested in memory and learning specific tests (8).

Acknowledgements:

This work is supported by NARSAD YI Award to AVK.

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