Chapter 16

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 indispensable 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.

Key words: 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, up

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to 46% of depressed patients do not fully respond to initial monotherapy antidepressant treatments (6), collectively emphasizing the need for newer and more effective drugs (1, 2, 5, 7).

Animal models are widely used to study the neurobiological mechanisms of depression (8–10). Ideal animal depression models must be reasonably analogous to the human symptoms, be able to be monitored objectively, be reversed by the same treatment modalities as humans, and be reproducible between laboratories (3, 5, 11). 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, 12). 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. Examples of both types of animal paradigms, equally important for further progress in biological psychiatry and drug discovery, will be discussed here in detail, based on their common use in behavioral pharmacology research. Finally, automated versions of some of these tests are currently available (13, 14), enabling consistent behavioral measurement, standardization of experimental protocols, and increased throughput and testing.

2. Materials

2.1. Animals

1. Various inbred, selectively bred, and genetically modified (mutant or transgenic (15)) mice (*see* more details in chapter 18 on mouse models of anxiety in this volume). We recommend using most of the inbred strains listed in the T1priority list of the Mouse Phenome Project database (www.jax.org/phenome), especially C57BL/6J, 129S1/SvImJ, and BALB/c mice. We also recommend browsing the Mouse Genome Informatics (http://www.informatics.jax.org/) database by the depression phenotype to find appropriate transgenic or mutant strains (e.g., $Disc1^{Rgsc1393}$/$Disc1^{Rgsc1393}$, Tg(Syn1-ADCY7)11004Btab/0 mice).
2. In general, avoid strains with overt motor or sensory deficits (e.g., vestibular, cardiovascular, visual) when using tests that may be confounded by these factors. In addition, males and females may exhibit different behavioral reactions to

the experimental stimuli (16). Therefore, the sex is also an important factor to consider when choosing an appropriate animal for experimentation (*see* **Note 1**).

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. Young mice recover more quickly from shipping stress (i.e., 1 week) than adult mice, which may require several weeks to acclimate. 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 (e.g., <5 animals per cage). The mouse holding room should be kept at approximately 21°C, on a 12/12 h light cycle. As mice are nocturnal, the light cycle may be inverted if spontaneous activity measures are needed (16).
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. Requirements for Experimental Models

1. Sucrose consumption test:
   4–10% sucrose (*see* **Note 2**)

   Home cage

   Two drinking bottles, one with pure water and the other with a sucrose solution (add sucrose to the animals' standard drinking water, as a change in water type may dissuade animals from drinking).
2. Forced swim test (FST):
   Clean glass cylinder (e.g., height 25 cm, diameter 10–15 cm)

   Water maintained at 23–25°C

   Towels to dry animals after swimming

   Stop watch to calculate the duration of immobility.

   Optional: video camera for subsequent video tracking and data analysis (e.g., (13). For more information on behavioral tracking software, please *see* **Table 16.1**. Video-tracking software requires highly developed algorithmic analysis of input; however, recording typically may be done with a standard video camera. Alternative methods of behavioral tracking include vibration-based (e.g., Bioseb, Vitrolles, France) FST activity monitoring.

Table 16.1
Automated video-tracking system manufacturers

Name	City	Country	Web address
Any-Maze	Wood Dale, IL	United States	www.anymaze.com
Bioseb	Vitrolles	France	www.bioseb.com
CleverSys Inc.	Reston, VA	United States	www.cleversysinc.com
Harvard Apparatus	Holliston, MA	United States	www.harvardapparatus.com
Linton Instrumentation	Diss, Norfolk	England	www.lintoninst.co.uk
Medi Analytika India Pvt. Ltd	Adyar, Chennai	India	www.medianalytika.com
Noldus	Leesburg, VA, or Wageningen	United States or Netherlands	www.noldus.com
Qubit Systems	Kingston, ON	Canada	www.quibitsystems.com
San Diego Instruments	San Diego, CA	United States	www.sandiegoinstruments.com
TSE Systems	Midland, MI	United States	www.tse-systems.com

3. Tail suspension test (TST):
   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. The design of the TST apparatus is usually negotiable, given that the animal is securely attached to a solid suspension apparatus and that this apparatus is at least 35 cm above the nearest surface (18, 19). Several companies provide behavioral tracking software that is flexible with the variations in experimental design and would yield reliable data that would translate between designs (**Table 16.1**). Additionally, there are also prefabricated apparatuses that can be purchased (*see* **Table 16.2** for details).

   Tape measure to determine the height of suspension.

Table 16.2
Selected commercial suppliers of behavioral equipment for depression research

Test apparatus	Manufacturer	Company web site
Tail suspension test	Panlab, Barcelona, Spain Columbus Instruments, Columbus OH, United States Bioseb, Vitrolles, France	www.panlab.com www.colinst.com www.bioseb.com
Forced swim test	Panlab, Barcelona, Spain San Diego Instruments, San Diego CA, United States Bioseb, Vitrolles, France	www.panlab.com www.sandiegoinstruments.com www.bioseb.com

Adhesive tape to secure mice to suspension apparatus (*see* **Notes 13 and 14**).

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 (14, 20–22).

4. Chronic mild stress (CMS):
   Supplementary cages for application of stressors.

   Various stressors, e.g., soiled rat bedding, confinement tube, or predator sounds (*see* **Note 18**).

3. Methods

3.1. 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. Note that strong scents (e.g., perfume) and loud or sudden noises should be avoided in the experimental room.
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.

3.2. Drug Administration

1. All experimental protocols described here are compatible with testing various antidepressants, administered with a vehicle (e.g., saline). A typical experiment may include one or several drug-treated groups (e.g., several doses or several pre-treatment times) compared to a vehicle-treated group of mice. Usually (unless stated otherwise), 10 animals per experimental group will be needed, also providing adequate statistical power (*see* further). However, if the effects of the drugs are particularly robust, a smaller n (e.g., $n = 7–8$) may suffice. For mild effects, a larger number of animals ($n = 15–16$) may be required.
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] or intranasal [i.n.]). Route of administration, dose, and pre-treatment time vary depending on strain sensitivity and the drug being used. Continuous drug infusion (using osmotic pumps, such as Alzet pumps) at a constant rate may be used to improve the availability of the drug, and implantable depots can be used for s.c. drug administration to achieve lasting therapeutic effect.

3.3. 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.
2. Some experiments may require one-way ANOVA with repeated measures, or *n*-way ANOVA depending upon the number of groups tested (*see* more details in the chapter on mouse models of anxiety in this volume).

3.4. Sucrose Consumption Test

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 water and a sucrose solution to drink. Usually, healthy 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 (23). Since various antidepressant drugs reverse the anhedonia-like reduction in preference for sucrose, e.g., (24–26), this test is widely used in the screening of antidepressant drugs. As in most experiments, between 8 and 12 mice may be used per group in this test. However, as few as 6 mice may yield good results, if depression-like phenotypes are robust. C57BL/6J and 129S1/SvImJ mice respond well in this assay (*see* **Note 2**). We recommend finding suitable mouse strains through Mouse Genome Informatics or Mouse Phenome Database based on each laboratory's individual scientific needs.

1. For a set period of time (e.g., 1, 3, or 7 days), allow experimental mice (housed in their standard home cages) 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 (*see* **Notes 2 and 3**).

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/g body weight. In addition, commercially available automated lick-counters (lickometers) may be used (e.g., by Lafayette Instrument Co, Lafayette IN, United States or Columbus Instruments, Columbus OH, United States). Assess the number of licks at each bottle for the duration of the test (i.e., 24–72 h) per 100 mg of body weight, and the preference for sucrose as a percentage of total licks (23, 27, 28) (*see* **Notes 4–6**).

3.5. Coat State Assessment

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 (29–31). Antidepressants have been shown to improve the coat condition of mice while reducing depression-like symptoms (29–31). For example, the reduction of corticotropin-releasing factor (CRF) has been associated with improved coat state (and is implicated in depression) (32). Of importance here, antidepressants (e.g., imipramine) and anxiolytics (e.g., chlordiazepoxide) have been shown to interact with corticotropin-releasing factor (33) (*see* **Note 7**).

1. After removing the animal from the home cage, assess the coat state in each of eight regions: head, neck, forepaws, dorsal coat, ventral coat, hindlegs, tail, and genital region. A coat with a healthy appearance (i.e., unchanged throughout the course of the experiment, normal coat state) should receive a score of 0. Conversely, a coat state that appears damaged or dirty (i.e., noticeably different from a normal coat state) should receive a score of 1. The average of the eight scores for each animal can then be compared among individuals or groups (30, 31). Due to the subjectivity of this assessment, it is beneficial to have more than one observer score each animal (these results should also be compared for inter-rater reliability). One way to minimize bias is to take the animal in question (i.e., the one with a seemingly dirty coat state) and compare it with another animal with an apparently normal coat state. Another way to observe an abnormal coat is to search for mild to severe piloerection, either generally or on specific body parts (32). This can either be in addition to, or independent from, a dirty coat appearance. Taken together, these symptoms signify the animal is not grooming normally and has declining hygiene, implicating overt depressive-like symptoms, (*see* **Notes 8 and 9**).

3.6. Forced Swim Test (FST)

1. This test can be performed manually or with automated video/software systems. Manual labor is not as high throughput as automated behavioral tracking software, and the latency of the observer to react can reduce the accuracy or data acquisition. Regardless of manual or automatic observation, the number of mice that can be tested depends on the duration of the test (6 min is enough to obtain reliable data and to determine significance in this test; therefore, 10 mice could be done per hour).

Although it does not induce experimental depression in mice, the FST is one of the most commonly utilized ethological models of fast high-throughput antidepressant screening. The FST places mice in an inescapable aversive situation and measures their "despair," (learned helplessness) by a measure of increased duration of immobility in the water. Animal FST immobility is markedly reduced by antidepressant drugs. The FST has good predictive validity and is widely used in research investigating acute and chronic effects of antidepressant drugs (20–23, 34) (*see* **Note 10**).

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) (*see* **Notes 11 and 12**).
3. After testing, dry mice thoroughly with towels and return to their home cages.

3.7. Tail Suspension Test (TST)

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 (*see* **Notes 13 and 14**).
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 (*see* **Note 15**).

3.8. Chronic Mild Stress (CMS)

5. 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 (12, 35–37) (*see* **Note 16**).

1. Following a random schedule, expose mice to two or more stressors each day for 4–7 weeks (*see* **Note 17**).
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 (35). Full experimental design must be published (e.g., degree and duration of cage tilt, dimensions of confinement tube and duration isolated in this tube, quality and duration of predator sounds, size of empty cage and duration of isolation), so that it can be compared across studies and between laboratories. Time of day during administration of stressors, as well as for assessing depression (sucrose intake, bouts of fighting or aggressive behavior, loss of body weight) must be recorded and standardized when possible. Additionally, duration of exposure to sucrose to measure intake should be provided by each study utilizing this model. The schedule of stressors should be random; however, they should be recorded and published citing the order in which they occurred to isolate this pattern as potentially manipulating experimental results (*see* **Note 18**).
3. To prevent habituation and enhance the unpredictable nature of the model, stressors should be applied at varying time intervals.
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.

4. Notes

1. Occasionally, mice may have altered cognitive domains that may be easily misinterpreted in models of depression (9). 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 often incorrectly 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 enhanced 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 (9).

2. The use of a 4–10% sucrose solution will usually generate good results for most mouse strains (e.g., C57BL/6J, 129S1/SvImJ mice). However, some strains (especially mutant or transgenic mice) may have abnormally reduced taste sensitivity, which would make assessment of their hedonic responses in this test difficult. Review Mouse Genome Informatics for mice with abnormal taste sensitivity (e.g., Gnat3tm1Rfm/Gnat3tm1Rfm). Most other mice will respond accurately to this test. However, always check taste sensitivity prior to performing a sucrose consumption test by using a standard taste sensitivity test (*see* specific mouse phenotyping literature (8–10) for details). Consider using a different strain if the problem persists. Alternatively, higher concentrations of sucrose (e.g., 20–35%) may be required.
3. To avoid 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. For example, this test may be unsuitable for diabetic (e.g, $Hk2^{tm1Laak}/Hk2^{+}$) or obese strains with altered water consumption.
4. 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 3-day test will be more appropriate in most cases). Note that this protocol must have flexibility and adaptability to be useful as general guidance across laboratories and countries. Rigidly stated specifics can deter novice experimenters from implementing their own research ideas and techniques.

5. 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 two bottles, each with the sucrose solution, for 72 h before the test, or with one water and one sucrose bottle for 1 h per day for 1 week. Also, consider lengthening the period of the test to at least 24 h. Researchers may choose to utilize video recording to document all water intake, however, end point analysis of overall sucrose consumption (as described earlier) is acceptable.
6. 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.
   Some mouse strains may develop a metabolic syndrome-like phenotype or have pathologically high reward-related phenotype (*see* Mouse Phenome Project or Mouse Genome Informatix database for these phenotypes of interest: e.g., metabolic syndrome-like phenotypes in $Neil1^{tm1Rsld}/Neil1^{tm1Rsld}$ mice). Thus, their sucrose consumption may be abnormally affected and alternative methods of depression testing or other mouse strains may be required.
7. Various mouse strains may have different sensitivity in this test. For example, C57BL/6J mice can be somewhat resistant to the deleterious effects of chronic stress on the coat state (38). Strain differences may result in differing levels of grooming activity. For example, some inbred strains may be inherently poor (e.g., BALB/cJ) or excellent (e.g., A/J) groomers, regardless of stress levels. Some genetically modified mouse strains also display "compulsive" grooming behavior (39) that may mask any alterations in the animal's coat state. Consider a more suitable strain if floor or ceiling effects occur.
8. 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. Typically use 8–10 subjects per group in order to obtain reliable data. The nature of this specific test is to be used as complementary assessment of depression to supplement other models of depression. This test is relatively simple; however, it may be more useful when performed adjunct to another test, such as the tail suspension test (e.g., in the tail suspension test, before releasing the animal, simply score the coat state in addition to the other endpoints measured in this test). Similarly, coat state can be assessed while animals are still in the home cage, or with little manipulation outside of the home cage.

9. Some mouse strains may display pronounced balding patches due to alopecia (40) or increased auto- or hetero-barbering behavior (41–43), 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 (44), thereby further confounding the coat assessment protocol.
10. Consider strain and individual differences in baseline immobility duration. C57BL/6J, BALB/cJ, and 129/SvEmJ strains have all been shown to provide reliable data and good sensitivity to pharmacological manipulations in this test (45). There is a growing number of mouse models with metabolic syndrome-like phenotypes, as well as with altered bone physiology (15). Mutant strains with calcium or bone deficiency (search Mouse Genome Informatics for specific examples) can potentially confound data in this respect, so this test would not be reliable in an experiment utilizing such mice. Similarly, obese mice may also confound data, as they could either be too buoyant or simply become exhausted during the test. 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.
11. 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., most 129 mouse substrains) are poor swimmers and develop spastic behaviors in FST situations that complicate their swimming.
12. Some mice exhibiting increased levels of FST immobility may be suffering from fatigue rather than depression per se. Evaluate the fatigability of animals in separate tests. If

mice display high fatigability phenotypes, consider shortening the length of the test.

13. 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.
14. Note that a moderately adhesive tape will be required (preferably, use vinyl tape or medical tape). Since most mice weigh about 25–35 g, duct tape is too strong and would not be required; also, tape of this grade would likely tear hair, skin, and possible part of the tail off the animal.
15. Some strains (e.g., C57BL/6J mice) display specific tail climbing behaviors and may not be an appropriate mouse model for this test (46). In contrast, BALB/cJ, DBA/2J, and BTBR strains were all shown to be reliable in this test and simultaneously responsive to drug effects (e.g., citalopram) (47). The growing number of mice with vestibular deficits (e.g., MRL/MpJ, Ce/J, and SJL/J inbred strains) (48); BDNF knockout mice (49) require further consideration, since 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. Some mutant mice display other specific neurological abnormalities relevant to their TST performance. For example, mutation or deletion of the Pafah1b1 gene of mice on a mixed 129SvEv-NIH Black Swiss background showed a marked increase in "hind leg clutching" behavior (50) whereas hind leg clasping behavior is common in including serotonin transporter knockout mice on 129S1/SvImJ background (own observations). Such phenotypes may result in abnormally high immobility in this test (which can incorrectly be interpreted as low depression). In contrast, spontaneous mild seizures in some mice (*see* Mouse Genome Informatics database for examples) may lead to reduced TST immobility, again confounding depression-related data.
16. 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.
17. 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, C57BL/6J mice are not sensitive to CMS affects on coat state (38).

18. Some suppliers (e.g., Keystone Country Store, www.keystonecountrystore.com) provide electronic devices that emit predator sounds, although rat vocalizations can also be recorded from live specimen. To standardize this, "predator sounds" should be published according to decibel, frequency, pitch, and length of recording. Unfortunately, soiled rat bedding cannot be obtained through a vendor. However, to standardize this stressor it would be possible to use a metabolic cage, collect, and measure the amount of urine and defecation of the rat, and then combine this with fresh rat bedding. A confinement tube is admittedly not descriptive. To isolate the mouse and cause a mild level of anxiety, insert the mouse into a restraint tube. These are typically similar in size and shape, although the exact design and specifications (dimensions) should be published in each manuscript utilizing this stressor. Some manufacturers include ITP (www.intoxproducts.com), AD Research (www.adinstruments.com), and ONARES (onares.com).

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