Models of Depression: Unpredictable Chronic Mild Stress in Mice

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ABSTRACT

Major depression is a complex psychiatric disorder characterized by affective, cognitive, and physiological impairments that lead to maladaptive behavior. The high lifetime prevalence of this disabling condition, coupled with limitations in existing medications, make necessary the development of improved therapeutics. This requires animal models that allow investigation of key biological correlates of the disorder. Described in this unit is the unpredictable chronic mild stress mouse model that is used to screen for antidepressant drug candidates. Originally designed for rats, this model has been adapted for mice to capitalize on the advantages of this species as an experimental model, including inter-strain variability, which permits an exploration of the contribution of genetic background, the ability to create transgenic animals, and lower cost. Thus, by combining genetic features and socio-environmental chronic stressful events, the unpredictable, chronic mild stress model in mice can be used to study the etiological and developmental components of major depression, and to identify novel treatments for this condition. Curr. Protoc. Pharmacol. 61:5.65.1-5.65.17. © 2013 by John Wiley & Sons, Inc.

INTRODUCTION

The unpredictable chronic mild stress (UCMS) rat model of depression was first proposed by Katz (1982) and developed further by Papp and Willner (UNIT 5.9; Papp et al., 1991, 1992; Muscat et al., 1992; Willner et al., 1992). The model involves exposing the animal at unpredictable times over several weeks to a series of minor-intensity stressors. This results in the development of a number of behavioral alterations in a large majority of animals (some animals can be more stress-resistant), including anhedonia (loss of pleasure) and apathy. These behavioral changes, together with alterations in certain endocrine and neural variables, resemble those found in individuals suffering from major depressive disorder and, as with the clinical condition, are reversed by chronic, but not acute, treatment with antidepressant drugs (Willner et al., 1987; Willner, 1997). This model is considered by many as one of the more useful animal tests for antidepressant activity. The UCMS displays face, construct, and predictive validity, and is one of the few models in which chronic, but not acute, antidepressant administration is effective (Willner and Mitchell, 2002; Belzung and Lemoine, 2011). This model has also been validated in mice (Surget and Belzung, 2009), which have several technical and practical advantages over rats as experimental animals. These include: (1) inter-strain variability which allows for an examination of the contribution of genetic background, (2) the availability of transgenic animals to facilitate the identification of key genes in the development of these behaviors and the response to drugs, and (3) lower cost as compared to rats. The mouse version of this model also has a high translational value as it induces molecular changes that are similar to those observed in depressed patients (Sibille et al., 2009).
NOTE: All protocols using live animals must first be reviewed and approved by an Institutional Animal Care and Use Committee (IACUC) or must conform to governmental regulations regarding the care and use of laboratory animals.

**UNPREDICTABLE CHRONIC MILD STRESS (UCMS) TEST IN MICE**

Mice are subjected to a schedule of mild psychosocial stressors for 9 weeks (Fig. 5.65.1) to induce physical, behavioral, biochemical, and physiological phenotypes. Once these are established, their reversal following the chronic administration of test agents can be used to predict potential antidepressant activity. Physical changes are quantified by measuring the coat state and body weight of the animal. Stressed animals display a worsening of the coat state compared to control, nonstressed animals. This change in coat state is reversed by chronic administration of antidepressants.

**Materials**

- BALB/c mice (64), aged 8 weeks at the beginning of the experiment (e.g., Elevage Janvier)
- Food pellets and water ad libitum
- Stressors (see Table 5.65.1) including:
  - CD of various recordings of birds of prey cries lasting ~10 min (at ambient level); these CDs can be purchased from a variety of commercial suppliers, such as [http://www.wildtones.com](http://www.wildtones.com) or [http://www.amazon.com](http://www.amazon.com)
  - Soiled sawdust of rats from the same or previous day (with feces and urine)
  - Clock for changing the light/dark cycle for the stressed animals with a minimum accuracy of 30 min
  - Spruce sawdust
- Fluoxetine (e.g., Sequoia Research Products, cat. no. SRP01950f) or imipramine (e.g., Sigma-Aldrich, cat. no. I0899) diluted in saline solution (NaCl, 9 g/liter)
- At least two different sound-proof rooms in animal facilities with a stable environment (inverted 12 hr light/dark cycle, temperature 22°C ± 1°C, humidity 55 ± 10%); one room is used to house stressed mice and one room for nonstressed, control mice
- Behavioral testing room
- 32 individual cages for the stressed mice

**Figure 5.65.1**  Experimental design. Four groups of mice (n = 16 mice per group) were created based on the environment (non-UCMS/UCMS) and the treatment (vehicle/fluoxetine). The UCMS regimen lasted 9 weeks. The coat state and the body weight were assessed weekly by two experimenters blind to the treatment. Fluoxetine (20 mg/kg/day) and vehicle (NaCl, 9 g/liter) intraperitoneal administration began after two weeks of UCMS and continued until the end of the experiment. On the seventh week, behavioral tests (cookie test, nest building test and splash test) were initiated.

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5.65.2

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Six to eight cages for the nonstressed mice (four to five mice per cage) with two shelters and one small tube (10-cm length × 6.5-cm i.d.) in each cage

Plastic restraint tubes closed at each end but enabling air circulation via small holes (length 6.5 cm × internal diameter 3.7 cm; see Fig. 5.65.2)

Environmental enrichment (shelters and tubes)

Precision balance 0.1 mg

1-ml syringes with 26-G. 1/2-in. needles for intraperitoneal (i.p.) injections

**Acclimatize the animal**

1. Place the animals in groups of four to five per cage for at least 1 week prior to initiating the protocol.

![Figure 5.65.2](https://example.com) Example of a plastic restraint tube designed, built, and used in our laboratory. This restraint tube (6.5-cm length × 3.7-cm i.d.) is closed at one end by a plastic wall pierced with a hole, allowing the animal to put its snout outside. The other end is closed by a rotating lid pivoting around a screw. Small holes on the tube enable air circulation.

<table>
<thead>
<tr>
<th>Stressor</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Social stress</td>
<td>Each mouse is placed in an empty cage previously occupied by another individual</td>
</tr>
<tr>
<td>Cage change</td>
<td>Each mouse is placed in the empty cage of another individual, and then returned to its original cage</td>
</tr>
<tr>
<td>Sawdust change</td>
<td>In the beginning of the UCMS protocol, the sawdust is changed 2 to 3 times per 24 hr, and up to 6 times per 24 hr at the end of the UCMS regimen. It is also possible to replace clean sawdust by soiled sawdust coming from control mice. The volume of each sawdust change is 250 ml (measured with a beaker).</td>
</tr>
<tr>
<td>Without sawdust</td>
<td>The sawdust is removed during 1 to 6 hr</td>
</tr>
<tr>
<td>Damp sawdust</td>
<td>Place 125 ml water in each cage. The period of damp sawdust can range from 1 to 6 hr.</td>
</tr>
<tr>
<td>“Bath”</td>
<td>The sawdust of each cage is removed and replaced by about 125 ml water at 20°C (about 1 cm water) for 15 to 30 min.</td>
</tr>
<tr>
<td>Cage tilting</td>
<td>Tilt the cages backwards (45 degrees) during 1 to 4 hr</td>
</tr>
<tr>
<td>Rat feces</td>
<td>About 60 ml of rat sawdust is deposited in each cage for a period of 1 to 2 hr</td>
</tr>
<tr>
<td>Restraint stress</td>
<td>The mice are kept in closed and ventilated tubes (6.5-cm length × 3.7-cm i.d.) for 15 to 30 min (mice have the possibility to turn themselves back into the tube)</td>
</tr>
<tr>
<td>Predator sounds</td>
<td>Broadcast a recording of birds of prey cries during 10 min</td>
</tr>
<tr>
<td>Cycle disturbances</td>
<td>Change of the light/dark cycle (e.g., complete reversal of the light/dark cycle, division of the light/dark cycle into four periods of 6 hr, or one to several illumination periods from 30 min to 2 hr during the dark phase and vice versa)</td>
</tr>
</tbody>
</table>
Table 5.65.2 Stressors Schedule

<table>
<thead>
<tr>
<th>Time</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monday morning</td>
<td>Social stress (9:00) + cage tilting (10:00-12:00)</td>
</tr>
<tr>
<td>Monday afternoon</td>
<td>Restraint stress (14:00-14:30) + predator sounds (14:30)</td>
</tr>
<tr>
<td>Tuesday morning</td>
<td>Weight + coat state + sawdust change (10:00, 10:30, 11:00)</td>
</tr>
<tr>
<td>Tuesday afternoon</td>
<td>Rat sawdust (13:00-15:00) + social stress (14:00-16:00)</td>
</tr>
<tr>
<td>Wednesday morning</td>
<td>Damp sawdust (9:30-11:30)</td>
</tr>
<tr>
<td>Wednesday afternoon</td>
<td>Light (12:00-14:00) + dark (14:00-16:00) + light (16:00-18:00)</td>
</tr>
<tr>
<td>Thursday morning</td>
<td>Restraint stress (9:00-9:30) + sawdust change (10:30, 11:00)</td>
</tr>
<tr>
<td>Thursday afternoon</td>
<td>Bath (13:00-13:30) + social stress (15:00)</td>
</tr>
<tr>
<td>Friday morning</td>
<td>Without sawdust (9:30-11:30) + cage tilting (10:00-11:30)</td>
</tr>
<tr>
<td>Friday afternoon</td>
<td>Cage tilting (12:00-14:00) + social stress (15:00-16:00)</td>
</tr>
<tr>
<td>Saturday (day)</td>
<td>Light (9:00-11:00) + dark (11:00-13:00) + light (13:00-15:00) + dark (15:00-19:00)</td>
</tr>
<tr>
<td>Saturday (night)</td>
<td>Light (19:00-21:00) + dark (21:00-1:00) + light (1:00-3:00) + dark (3:00-9:00)</td>
</tr>
<tr>
<td>Sunday (day)</td>
<td>Light (9:00-11:00) + dark (11:00-13:00) + light (13:00-15:00) + dark (15:00-19:00)</td>
</tr>
<tr>
<td>Sunday (night)</td>
<td>Light (19:00-21:00) + dark (21:00-1:00) + light (1:00-3:00) + dark (3:00-9:00)</td>
</tr>
</tbody>
</table>

*Example of stressors schedule during the third week.

2. Place identification marks on the animals and separate them into two groups of 32 for UCMS exposure, and 32 to serve as unstressed controls. House the stressed and control groups in different rooms but under the same time and temperature conditions, as noted above. Whereas the UCMS mice are housed individually in their home cage, the control mice are group-housed (four to five per cage) with environmental enrichment (shelters and tubes).

*Individual housing is part of the UCMS protocol and is a stressor in itself.

**Apply stressors and assess physical condition**

3. Subject the UCMS mice to stressors detailed on Table 5.65.1. Expose the animals to the stressors throughout the entire circadian period (i.e., in the morning, in the afternoon/evening, and during the night) and randomly (the stressors are applied in an unpredictable manner). To increase stress intensity, stressors can be combined after the first week of the UCMS regimen (e.g., rat sawdust and restraint at the same moment) (Table 5.65.2).

*As it is important to avoid any habituation of the animals to the stressors, the unpredictable aspect of the stress protocol is essential. Therefore, the order of stressors must vary weekly during the stress exposure period.*

4. Assess the body weight and the coat state of mice on a weekly basis. As the coat state is a function of the frequency and extent of grooming behavior, it is a measure of the animal’s motivation toward self-centered activities. The coat state will vary from smooth and clear in normal, control animals to bristling with spikes in those subjects that are most affected by the stressors. Assess the coat on the following seven body areas: head, neck, back, abdomen, tail, forepaws and hindpaws (Fig. 5.65.3) and score the coat state for each area as follows: 0 (good) for smooth and shiny fur,
Figure 5.65.3  Assessment of the coat state for (A) non-UCMS control mouse and (B) UCMS-subjected mouse. The coat state score results from a qualitative scoring of different parts of the body including the head, the neck, the forepaws, the back, the abdomen, the hindpaws, and the tail. Each zone is scored 0 if in a good state (the fur is smooth and shiny, with no tousled, spiky patches), 0.5 if in moderately bad state (the fur is slightly fluffy with some spiky patches), and 1 in bad state (the fur is dirty and fluffy on most of the body with slight staining). On the figure, the non-UCMS control mouse would be scored 0.5 given the presence of a slight degradation of the coat on the neck. The UCMS mouse would be scored 1 on the head, 1 on the neck, 1 on the back and 0.5 on the abdomen (not visible on the picture), and thus obtain a global score of 3.5. Mice were marked with picric acid in these pictures.

with no tousled, spiky patches; 0.5 (moderate) for slightly fluffy fur with some spiky patches; 1 (bad) for fluffy fur on with slight staining. Sum the scores for all seven body parts to obtain an overall score, with a maximum possible score of 7. Weigh the animals and monitor their overall physical condition. As some stressors, such as damp sawdust or restraint stress, may induce a deterioration of the coat state, while “bath” stress can enhance the coat state temporarily, it is very important not to use such a stressor within 24 hr before these measurements.

Administer the test compound
5. Further divide the 32 non-UCMS and 32 UCMS mice into two subgroups: control animals receiving vehicle (9 g/liter NaCl, i.p.) and treated animals injected with antidepressant (fluoxetine, 15 mg/kg/day, i.p., or imipramine, 20 mg/kg/day, i.p.) at a volume of 10 ml/kg (16 mice per group). Administer vehicle and antidepressant to both non-UCMS and UCMS mice at a fixed time of day (e.g., between 13:00 and 15:00) for 2 to 4 weeks to assess the pharmacological effect.

It is recommended that animals not receive pharmacological treatment just before behavioral testing (Basic Protocol 2) to avoid any acute effects of drug administration.
ASSESSMENT OF SELF-DIRECTED ACTIVITY AND ANHEDONIA IN MICE

UCMS should induce a depressive-like state. In humans, this is characterized by symptoms of apathy and anhedonia. In rodents, responses to stress and antidepressant effects can be assessed by measuring spontaneous grooming behavior (splash test), spontaneous motivation (nest building), and appetite for pleasurable food (cookie consumption). Unlike the weekly assessments of physical condition described in Basic Protocol 1, these tests are conducted during the final weeks of the UCMS procedure at least two or three weeks after the beginning of the pharmacological treatments, in order to allow the compounds to induce their therapeutic effects (Fig. 5.65.1).

NOTE: The behavioral testing should follow the order presented here, beginning with the test that requires a period of habituation (cookie test). Furthermore, behavioral tests can be stressful, it can be considered as a stressor in itself. Further information regarding the cookie test, the nest building test and the splash test, e.g., pictures and videos, is available at http://www.affective.disorders.sciences.univ-tours.fr.

Materials

Four experimental groups of mice from the previous subdivisions in Basic Protocol 1 (non-UCMS/vehicle, non-UCMS/antidepressant, UCMS/vehicle, UCMS/antidepressant; sixteen mice per group)
Shortbread cookies [e.g., Pepito (LU) buttery shortbread cookies topped with chocolate, or Oreo (Kraft Foods)] cookies with two chocolate wafers separated by cream filling; the cookie must be appetent for mice in being somewhat crispy and sweet
70% alcohol
10% sucrose solution (elaborated with white sugar sold in conventional retail stores)

Device containing three aligned chambers (20-cm length × 20-cm width × 20-cm height); only the colors of the walls and the floor are different between the chambers: white for the first one, gray for the second, and black for the third. The three chambers are linked by two gates with a door controlled by the experimenter (Fig. 5.65.4)
Light dimmer and luxmeter
Stopwatches
32 individual cages for non-UCMS mice (for the nest building test and the splash test)
Cotton nestlets (5 × 5 cm, 2 to 3 g; e.g., SERLAB, D00009 or LBS Biotech Nestlets)
One cage for the splash test
1-liter sprayer (e.g., garden hand sprayer) for the splash test
Lamp with red bulb

The cookie test

1a. Divide each experimental group (non-UCMS/vehicle, non-UCMS/antidepressant, UCMS/vehicle, UCMS/antidepressant; sixteen mice per group) into two subgroups: mice receiving cookies and mice with regular food pellet (eight mice per group).

   The group sizes should not be smaller than eight mice each.

2a. Four and one-half weeks before the start of the assessments, place a piece of cookie (~2 g) in the cage of each mouse of the “cookie subgroup” every 2 days for 2.5 consecutive weeks to familiarize the animals with the palatable stimulus. The last 2 weeks before the test are cookie-free.

3a. Retrieve all food from cages 1 hr before the beginning of the test.
Figure 5.65.4  Example of the apparatus designed, built and used in our laboratory. This device consist of three aligned chambers (20-cm length × 20-cm width × 20-cm height) communicating by two gates (doors are controlled by the experimenters). Only the colors of the walls and the floor are different between the chambers: white for the first one where the mouse is placed, gray for the second, and black for the third chamber where the food is available.

The food is removed to avoid inter-individual differences in the drive for feeding (hunger). If this is not done, some mice are less inclined to feed, which could bias the findings.

4a. Place a piece of cookie (∼2 cm × 2 cm) or a regular food pellet in the center of the black box, and place the mouse at the other end of the device in the white chamber (head facing opposite to the opening).

The observations are made under low-intensity white light (∼200 lux).

5a. During the 5 min test session, measure the time it takes (latency) for the animal to pass through the first and the second gate (when the four legs have crossed the door), to smell the food and to chew it, and the number of times the mouse sniffs and chews the food.

After the mouse has passed through the first gate, close the door. If the mouse has not entered in the gray room after 2 min, gently guide it to this second room and close the door. Do not close the second door.

6a. After the end of this first session, replace the mouse in its home cage.

Clean the device with 70% alcohol between each mouse.

7a. Perform this test every 3 days for each animal (i.e., 4 sessions of testing over 9 days).

The nest building test

1b. As the stressed mice are already individually housed, isolate non-UCMS mice in clean individual cages for 24 hr before the test (Fig. 5.65.1).
Unpredictable Chronic Mild Stress in Mice

5.65.8

It is not necessary to subdivide the animals into groups of eight as was done for the cookie test. The nest building test is performed on four experimental groups (non-UCMS/vehicle, non-UCMS/antidepressant, UCMS/vehicle, UCMS/antidepressant; sixteen mice per group).

2b. One hour before the beginning of the dark phase (active period), place a cotton nestlet in each cage.

To avoid disrupting the mouse and its nesting behavior, drugs or test agents should not be administered during the 24 hr testing period.

3b. At two time points (5 and 23 hr after the beginning of the dark phase), evaluate the nest quality (Deacon, 2006; Fig. 5.65.5) using the following criteria:

Score 1: The cotton square is intact.
Score 2: The cotton square is partially used.
Score 3: The cotton is scattered but there is no form of nest.
Score 4: The cotton is gathered but there is no nest ("flat nest").
Score 5: The cotton is gathered into a “ball” with a small passage for entry of the animal (as an igloo, with or without roof).

The second evaluation of the nest quality is performed 1 hr before the onset of the dark phase to avoid destruction of the nest by the awakening of the mouse.
The splash test

1c. Place a mouse in the “splash cage.”

Every animal is individually housed at this step, since non-UCMS mice have already
been isolated at least 24 hr before the nest building test. The splash test is per-
formed on the four experimental groups (non-UCMS/vehicle, non-UCMS/antidepressant,
UCMS/vehicle, UCMS/antidepressant; sixteen mice per group).

2c. With the sprayer, “splash” the back of the mouse with a high viscosity 10% sucrose
solution to stimulate grooming behavior, and quickly place the mouse back into its
home cage.

The sprayer allows delivery of a fixed volume (about 0.7 ml) of sucrose solution. Each
mouse should receive two sprays.

3c. Measure the latency to initiate the first grooming behavior, as well as the frequency
and duration of grooming over a 5-min period. Start the stopwatch when the mouse
is returned to its home cage, immediately after applying the sucrose solution.

The assessment is performed under red light and in the original rack of the individual
home cage.

COMMENTARY

Background Information

Chronic stress disrupts the overall home-
ostasis of the organism and contributes to
the etiology of major depression in causing
cognitive, behavioral, and physiological im-
pairments (Surget and Belzung, 2009). The
environmental factors triggering major depres-
sion include various psychosocial stressors
(e.g., disturbed family environment, childhood
sexual abuse, educational attainment, life-
time traumas, marital problems, etc.) (Kendler
et al., 2002; Caspi et al., 2003). Moreover,
stressful life events can precipitate a depre-
sive episode in vulnerable subjects (Caspi
et al., 2003). Unlike animal models used to as-
se ss a symptom of major depression based on
exposure to a relatively aversive acute stress,
such as the forced swim (UNIT 5.8) and tail
suspension tests, the UCMS model was devel-
oped to study multiple disturbances resulting
from chronic exposure to stress. This model
therefore aims to reproduce a depressive-like
state that develops gradually in response to
the chronic stress that is thought to be a ma-
jor contributor in the development of clinical
depression. Thus, the UCMS model has con-
struct validity because, like human depression,
the phenotype results from environmental and
psychological stressors.

While many of the core features of ma-
jor depression, such as suicidal ideation and
excessive guilt, are uniquely human quali-
ties and therefore not reproducible in mice,
UCMS animals display a long-lasting complex
pathological phenotype resembling many of
the symptoms/endophenotypes of human de-
pression (Surget and Belzung, 2009). These in-
clude behavioral and cognitive alterations such
as anhedonia, loss of interest, learning deficits,
signs of despair, difficulty in decision making,
and sleep disturbances. These animals also dis-
play neurobiological abnormalities, including
reduced levels of monoamines, immune sys-
tem dysfunction, reduced hippocampal den-
dritic branching, increased branching in the
amygdala, and a decrease in hippocampal neu-
genesis. The UCMS subjects also have en-
docrine changes associated with their chronic
stress, such as increase in glucocorticoid levels
(Surget and Belzung, 2009). These behavioral,
neurochemical, and endocrine alterations de-
velop gradually following exposure to differ-
ent stressors, indicating face validity for the
model.

A key feature of an animal model for a psy-
chiatric disorder is its ability to predict clinical
efficacy for chemical substances designed to
treat the condition. This characteristic is esen-
tial to validate new targets for antidepressant
action. The UCMS model displays a variety of
physical, behavioral and neurobiological dis-
turbances that are reversed by both conven-
tional and atypical antidepressants (Surget and
Belzung, 2009). Furthermore, it is notable that,
as is the case with humans, the antidepres-
sant response in UCMS animals is obtained
only after chronic administration of these
drugs. This too suggests the UCMS model is
more relevant as a screening test for antide-
pressants. The UCMS-induced alterations can
be reduced or abolished in mice by chronic
treatment with a variety of conventional
antidepressant agents, including tricyclics such as imipramine and desipramine, selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, and the selective noradrenaline reuptake inhibitor (SNRI) maprotiline (Yalcin et al., 2008).

Other agents that have been reported to display antidepressant-like activity in UCMS animals include the opioid tramadol, which has a venlafaxine-like structure (Yalcin et al., 2005), the corticotropin-releasing factor 1 (CRF1) receptor antagonists antalarmin and SSR125543 (Ducottet et al., 2003; Alonso et al., 2004; Surget et al., 2008), the vasopressin 1b (V1b) receptor antagonist SSR149415 (Griebel et al., 2002; Alonso et al., 2004; Surget et al., 2008), the cannabinoid CB1 receptor antagonist rimonabant (Griebel et al., 2005), and the β3-adrenoreceptor agonist SR58611A (amibegron) (Stemmelin et al., 2008). Psychoactive agents that do not display antidepressant activity in the clinic, such as pindolol, yohimbine, chlordiazepoxide, sulpiride, MK801, morphine, and 3, 4-methylenedioxyamphetamine (MDMA) are ineffective in the UCMS model (Surget and Belzung, 2009). Together, these findings substantiate the predictive validity of this model of major depression.

Critical Parameters and Troubleshooting

Stress procedure

The stressors used, which are mainly psychosocial, are mild in their intensity, with the essential features being their chronic nature and unpredictability. For ethical and scientific reasons, severe stressors, such as food and water deprivation or painful stress, should not be employed. From a technical standpoint these stressors may not parallel the etiology of human pathology, and their generalized effects on the animal’s physical state and wellbeing might interfere with the measures of the UCMS-induced effects. Since the UCMS test requires a large number of animals, it is possible to design a “staircase-shaped” protocol by dividing the animals into different sets that do not begin the UCMS regimen at the same time, with up to a one week lag between groups (Fig. 5.65.6). While this facilitates the behavioral testing phase by minimizing the number of mice involved, it means the animal groups are not subjected to the same stressors simultaneously, which is an additional variable when interpreting the results. Therefore, without several experimental rooms to isolate the different groups of mice, some stressors, such as cycle disturbances or predator sounds, cannot be used.

Coat state

In rodents, auto-grooming behavior is very sensitive to stress (Kalweff and Tuohimaa, 2004a,b). In the UCMS model, the deterioration of the coat state can be related to a decrease of grooming and, in consequence, to a disturbance of self-directed behavior. The human correlate would be the poor personal hygiene displayed by many depressed patients. The deteriorations of the coat state are mainly observed on the head, the neck, and the back of mice, as well as on the abdomen and the hindpaws. Thus, the maximum score rarely exceeds 4. However, since all stressed mice do not cope with stress in the same way, a complete assessment of the coat state is still necessary to have the most accurate measurement possible. It is important to note that the deterioration of the coat in the UCMS mice is dependent on their genetic background. Certain strains, such as BALB/c and DBA/2, are more sensitive to the UCMS-induced effect on
Assessing the coat condition in mice with colored fur is more challenging than in albinos. Moreover, the subjective nature of the assessment made when judging coat condition is a limitation of this test. To address this issue, it is best if two investigators, both of whom are blinded to treatment, make independent assessments of coat condition.

**Body weight**

Weight gain is another physical sign of the effect of chronic stress and pharmacological treatments. In the case of the “staircase-shaped” UCMS protocol, the body weight must be expressed relative to the initial weight of the mice prior to exposure to stress (week 0). Although a decline in the rate of body weight gain, along with a reversal of such an effect following chronic administration of an antidepressant, are generally observed in UCMS animals (Surget et al., 2009; Nollet et al., 2011), others have been unable to detect differences in body weight gain between control and UCMS mice (Surget et al., 2008), even though other depression-like changes developed. Since major depression is often associated with weight changes, the lack of a UCMS-induced effect on body weight reported by some is perplexing. It should be kept in mind, however, that human depression may be accompanied by weight gain or weight loss, and that UCMS-induced body weight disruption appears to be dependent on the duration, timing and type of stressors employed (Fig. 5.65.7B). Given these findings, changes in body weight should not be the primary end-point in the UCMS test.

**Test compound administration**

In the UCMS model, compounds are usually administered at the beginning of the third week of stress exposure and maintained continuously for 4 to 7 weeks thereafter (i.e., weeks 6 to 9 of the UCMS regimen). Acute administration of antidepressants is ineffective in this model of major depression, with compound-induced effects typically appearing within 2 to 4 weeks of treatment (Surget and Belzung, 2009). Intraperitoneally (i.p.) (10 to 20 mg/kg/day) or orally (p.o.) (20 to 30 mg/kg/day) administered fluoxetine or
imipramine are the most widely used treatments for verifying this assay. When the model is used to screen test agents for potential antidepressant activity, one of these drugs should be included as a reference agent.

When compounds are being evaluated in vivo, it is imperative that some preliminary pharmacokinetic data be generated to ensure that the compound is known to be present at the time of testing and in what amount. Increasingly, in vivo studies rely more on the measured plasma level of a compound, rather than the dose administered, to construct accurate dose-response curves. For peptides, a half-life of less than 1 min can be incompatible for a test procedure where animals are measured for behavior or a performance phenotype 30 or 60 min after compound administration. Many compounds can, however, produce their effects via alterations in gene expression which can be long lasting, such that their biological half-life is many times longer than the actual presence of the compound in the plasma. If a short-acting compound produces an effect beyond its plasma half-life, this can provide valuable information on its potential mechanism of action. Conversely, the behavioral effect of a compound that parallel its plasma half-life provides a direct cause-and-effect relationship that is proportional to the plasma concentration. If there is no pharmacokinetic information on a compound, this can seriously compromise the intent and outcome of the experiment.

The cookie test

Since anhedonia is a symptom of major depression, the cookie test was designed to evaluate the motivation for a palatable stimulus. This is accomplished by assessing three behavioral dimensions: (1) anxiety-like state and exploration of the novel environment (latency to pass through the doors during the first session); (2) habituation to a novel environment (latency to pass through the doors over the sessions); (3) anhedonia (latency to chew the object and the number of cookie chews versus the number of chews of the regular food pellet). By assessing the latter parameters over the sessions it is possible to evaluate the interaction between anhedonic features and environmental habituation. This behavioral test has been validated in BALB/c mice exposed to UCMS and chronically treated with fluoxetine or the CRF1 receptor antagonist, SSR125543 (Isingrini et al., 2010; Surget et al., 2011). Inasmuch as the sucrose preference test has not been successfully adapted to measure UCMS-induced anhedonia in mice (Pothion et al., 2004; Ducottet and Belzung, 2005), the cookie test appears to be a useful way to investigate the motivation for a reward. Furthermore, compared to other assays, the cookie test takes advantage of multiple measures and sessions to provide a more accurate analysis of the UCMS-induced effects on behavior, and particularly on anhedonia. Although the data on the consumption of the regular pellets are not used as part of the statistical analysis in assessing UCMS-induced impairment of anhedonia, the regular food control is needed to establish the baseline consumption for all experimental groups in the cookie test, and to avoid any interpretation biases. As separate control subgroups potentially decrease the statistical power of this assay, it may be possible to design the cookie test so that each animal is its own control. This could be accomplished by adding a control session with a regular food pellet instead of the cookie, or by placing both types of food in the black box, giving the animal a choice. It is noteworthy that once the data on the consumption of the regular pellets have been collected for the validation of the cookie test, it is not absolutely necessary to add these subgroups in the subsequent experiments.

Nest building test

A major advantage of the nest building test is the ease of its execution and interpretation. Another advantage of this test is that it can, like the coat-state analysis, be performed repeatedly. This makes it possible to define more precisely the onset of any antidepressant effects. The second score, which is obtained 24 hr after placing the cotton squares into the cage, appears to be the most relevant for evaluating nest quality, while the first score, which is taken 6 hr after placing the cotton squares into the cage, provides an estimate of nest building speed.

Splash test

Unlike the coat state assay, the splash test is a direct quantitative measure of grooming behavior. The palatability and viscosity characteristics of the sucrose solution sprayed on the dorsal coat of mice are what induce the auto-grooming behavior. The splash test is usually performed a single time at the end of the UCMS procedure and/or of test compound or antidepressant administration. Repeated measures can be contemplated, as this test is not based on novelty. Nevertheless, a validation is needed, since repeated exposure
Figure 5.65.8 Effects of unpredictable chronic mild stress (UCMS) and 6-week fluoxetine treatment (20 mg/kg/day, i.p.) on food consumption in the cookie test. (A) As a control experiment, the consumption of a regular food pellet (number of bites) was quasi-null during the four sessions for all groups. (B) Compared to the UCMS/vehicle group, the consumption of the cookie increased in both non-UCMS/vehicle group (* p < 0.05 and ** p < 0.01) and UCMS/fluoxetine group (## p < 0.01) during the third and the fourth session. (C) No significant difference of the latency to chew the regular food pellet was observed between the experimental groups over the four sessions. (D) Compared to the UCMS/vehicle group, the latency to chew the cookie decreased in both non-UCMS/vehicle group (* p < 0.05 and ** p < 0.01) and UCMS/fluoxetine group (## p < 0.01) during the third and the fourth session (mean ± standard error; n = 8 mice per group).

to the splash test may in itself modify animal behavior. Because all mice must be isolated for the test, control group-housed mice have to be placed singly in a novel home cage containing fresh sawdust 24 hr before testing (if it had not already been done before). The sawdust is changed at the same time in the UCMS mice cages as well. This delay allows the animal to become familiar with a novel environment, minimizing novelty-induced behavioral changes.

Statistics
Considering the relatively small sample sizes, and that assumptions for parametric statistics may not be valid (normality and homoscedasticity), it is recommended that the nonparametric Kruskal-Wallis ANOVA by
Anticipated Results

Coat state deterioration occurs within the first two weeks of stressor exposure. It generally begins on the neck and on the back, and then goes on to involve the head, abdomen and the hindpaws (Fig. 5.65.7A). As noted above, the deterioration is rarely observed on the other parts of the body. Any decrease in the rate of body weight gain usually takes 4 to 5 weeks of stress to become apparent (Fig. 5.65.7B). As these measurements can be repeated, they can be used to determine the time of onset of the drug action. This typically occurs after 2 to 4 weeks of continuous, daily compound administration.

In the cookie test, considering the robust reduction of the chewing latency and the large increase in the chewing number found over the sessions with the UCMS animals compared to controls on regular food, the results indicate that the drive for chewing after a 1-hr food deprivation is stronger with the palatable cookie than with the regular food pellet (Isingrini et al., 2010; Surget et al., 2011). We also found that, during the last two sessions, the UCMS mice display (1) no significant effect on the latency to pass through the doors in all the sessions, (2) an increase in the latency to chew the cookie, (3) and a decrease in the number of chews of the cookie (Fig. 5.65.8). No UCMS-related differences were found when the regular food pellet was used, indicating that the stressors induced anhedonia. Furthermore, the UCMS-induced alterations were reversed during sessions 3 and 4 by chronic administration of fluoxetine (20 mg/kg/day, i.p.), and during the last session by daily administration of SSR125543 (20 mg/kg/day, i.p.). It is noteworthy that other parameters, such as the latency to pass through the gates or the latency to sniff the food, were not affected by drug administration and had not yielded relevant results.

The UCMS mice typically display a decrease in nest quality, a behavior that is counteracted by antidepressant treatment (Fig. 5.65.9A). In the splash test, the latency to groom is increased in the UCMS mice (Fig. 5.65.9B), and its duration and frequency are reduced in these animals (Fig. 5.65.9C,D). The most reliable of these measures is frequency. That is, the stressed animals most consistently exhibit a decrease of grooming behavior, which is restored by the chronic administration of an antidepressant.

Other well-established assays such as the novelty-suppressed feeding test, forced swim and tail suspension tests (UNIT 5.8) to assess behavioral despair or the open field, elevated plus maze, or light-dark test (UNIT 5.38) to assess anxiety can also be performed at the end of the UCMS procedure to provide additional insight. The same applies for biochemical measures (e.g., levels of corticosterone or of pro-inflammatory cytokines) and physiological measures.

Results obtained from a UCMS regimen are dependent on many parameters, which may explain the variability observed between laboratories (and sometimes between protocols within a laboratory). Indeed, UCMS procedures often vary in terms of duration, stressors, unpredictability, species, strains, and treatments. Inter-strain variability (Pothion et al., 2004; Ducottet and Belzung, 2005; Ibarguen-Vargas et al., 2008) is a critical issue in optimizing the effectiveness of the UCMS regimen. We use the BALB/c strain as it displays high stress vulnerability. There can also be variability in the response to UCMS among mice in the same procedure, as some animals are more resilient while others are more vulnerable. However, when using inbred strains of mice, variability is typically low. These differences illustrate the importance of standardizing the procedure in a given laboratory to ensure consistent, reproducible results.

Time Considerations

The UCMS protocol is very costly to perform in terms of the time required to complete the procedure. A typical experiment in which mice are chronically administered a conventional antidepressant requires between 6 and 9 weeks to complete. This involves the time to adapt to the laboratory conditions (1 week) and the application of stressors, including the following phases: the drug-free period (2 weeks), the pharmacological treatment phase (3 to 4 weeks), and the potential behavioral testing period (1 week). As the UCMS must be continued until the animals are sacrificed, extra time might be planned to, for example, to perform intracardiac perfusion or microdissections if other assays are being conducted.
Figure 5.65.9 Effect of unpredictable chronic mild stress (UCMS) and 6-week fluoxetine treatment (20 mg/kg/day, i.p.) on behavior in the nest building and splash tests. (A) In the nest building test, nesting behavior was decreased in UCMS/Vehicle mice compared to non-UCMS/vehicle group (* p < 0.05), while chronic treatment with fluoxetine reversed this UCMS-induced alteration (data show the score of the nest quality at the end of the 24-hr nesting period; UCMS/vehicle group versus UCMS/fluoxetine group, *p < 0.05). (B, C, D) In the splash test, UCMS induced an increase in the latency to groom, and a decrease in grooming frequency, as well as the amount of time spent grooming (non-UCMS/vehicle group versus UCMS/vehicle group, ** p < 0.01), which was reversed by chronic treatment with fluoxetine (UCMS/vehicle group versus UCMS/fluoxetine group, ** p < 0.01) (mean ± standard error; n = 16 mice per group).

Literature Cited
Unpredictable Chronic Mild Stress in Mice

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**Internet Resources**


*Our laboratory Web site providing pictures and videos of the cookie test, the nest building test, and the splash test.*