Rodent Models of Depression: Forced Swim and Tail Suspension Behavioral Despair Tests in Rats and Mice

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ABSTRACT

The development of antidepressants requires simple rodent behavioral tests for initial screening before undertaking more complex preclinical tests and clinical evaluation. Presented in the unit are two widely used screening tests used for antidepressants, the forced swim (also termed behavioral despair) test in the rat and mouse, and the tail suspension test in the mouse. These tests have good predictive validity and allow rapid and economical detection of substances with potential antidepressant-like activity. The behavioral despair and the tail suspension tests are based on the same principle: measurement of the duration of immobility when rodents are exposed to an inescapable situation. The majority of clinically used antidepressants decrease the duration of immobility. Antidepressants also increase the latency to immobility, and this additional measure can increase the sensitivity of the behavioral despair test in the mouse for certain classes of antidepressant. Testing of new substances in the behavioral despair and tail suspension tests allows a simple assessment of their potential antidepressant activity by the measurement of their effect on immobility. Curr. Protoc. Pharmacol. 49:5.8.1-5.8.14. © 2010 by John Wiley & Sons, Inc.

Keywords: animal models • antidepressants • behavior • depression • mice • rats

INTRODUCTION

Depression is a complex disease with heterogeneous pathology and many of the core symptoms such as feelings of hopelessness and low self-esteem are not easily reproduced in animals (Matthews et al., 2005). Current treatments for depression either fail to produce complete recovery or induce unwanted side effects (Slattery et al., 2004; Baghai et al., 2006), so there is still a large unmet clinical need. Whereas development of novel approaches requires new animal models or intelligent use of existing models (Bosker et al., 2004; Berton and Nestler, 2006), there is still a need for a simple model with good predictive validity for early screening. The behavioral despair test (also named the forced swim test and Porsolt test) was developed using rats and then adapted to mice for just this purpose (Porsolt et al., 1977, 1978). Since then it has become one of the most widely used tests for antidepressant screening (Borsini and Meli, 1988; Petit-Demouliere et al., 2005).

Rodents forced to swim in a narrow space from which there is no escape adopt, after an initial period of vigorous activity, a characteristic immobile posture, moving only when necessary to keep their heads above the water. The animals’ immobility was interpreted as indicating they had learned that escape was impossible and had adopted an immobile position to conserve energy, viewed anthropomorphically as if they had given hope of escaping from this stressful situation. Immobility was therefore given the name “behavioral despair.” It was subsequently found that immobility could be reduced by a wide range of clinically active antidepressants. This simple behavioral procedure has since become a useful test for screening novel antidepressants and continues to...
form the basis for primary screening of psychoactive compounds (Castagné et al., 2006). The Basic Protocol (behavioral despair test) is based on this procedure and describes its application for examining drug effects in the rat. An alternative procedure in the mouse is described (see Alternate Protocol 1), along with a “dry” version of the test where immobility is induced simply by suspending the mouse by the tail (see Alternate Protocol 2).

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.

**FORCED SWIM (BEHAVIORAL DESPAIR) TEST IN THE RAT**

In two sessions separated by 24 hr, rats are forced to swim in a narrow cylinder from which they cannot escape. The first session, lasting 15 min, is conducted prior to drug administration and without behavioral recording. This is done to acclimate the rats to the test situation, thereby providing a stable, high level of immobile behavior during the 5-min test session 24 hr later.

Separating the two sessions allows repeated drug administrations between the initial exposure and the drug test. In the standard protocol, rats are treated with a drug or test compound 24 hr, 4 hr, and 60 or 30 min (the last pretreatment time depending on the route of administration) before the test, the first administration being given immediately after the first session. A minimum of two, but preferably three, pretest administrations provide more stable pharmacological results than a single administration. A variation of the protocol involves chronic treatment programs, even up to 3 or 4 weeks, between the two sessions. The authors do not recommend starting treatments prior to the first session, in order to avoid drug-behavior interaction during the first session. Longer treatment regimes are used to compare animal responses with clinical data for this drug class for which the therapeutic effect appears only after 3 to 4 weeks of treatment.

**Materials**

- 180- to 280-g male Wistar rats (e.g., Elevage Janvier)
- Standard rodent diet
- Treatment solutions (see recipe): drug or test compound, reference compound (e.g., imipramine hydrochloride, Sigma or equivalent), and vehicle alone (for control)
- Transparent plastic cages (e.g., Macrolon 44 × 28 × 19-cm), containing wood shavings (e.g., Litalabo, SPPS, http://www.sppsfrance.com)
- Transparent Plexiglas cylinders (20 cm diameter × 40 cm high) containing water (25°C ± 2°C) to a depth of 13 cm (made in house or obtained from commercial suppliers of Plexiglas material)
- Opaque screens for separating cylinders
- Metric balance (e.g., Sartorius model 1401.001.2), accurate to 1 g
- 2-ml syringes for intraperitoneal and subcutaneous injections (e.g., Terumo type BS-025)
- 23-G × 1-in. (0.6 × 16-mm) needles for intraperitoneal injections (e.g., Terumo)
- 21-G × 1.5-in. (0.8 × 40-mm) needles for subcutaneous injections (e.g., Terumo)
- Luer gastric probes with oval extremity (70 mm long × 1.5 mm oval diameter) for oral administration

1. House rats in cages containing wood shavings (six rats per cage), and provide free access to standard rodent diet and tap water, except during the test. House the animals at 21° ± 3°C on a standard (nonreversed) light/dark cycle with illumination from
0700 to 1900. Use six rats per group for a standard experiment (usually five groups: vehicle control, reference compound, and three doses of test substance).

Be sure animals are delivered to the laboratory at least 5 days before the experiment. Experiments are generally performed during the light phase of the cycle, although it is also possible to perform the test under dim red light during the dark phase. If the latter alternative is chosen, preliminary experiments including vehicle and reference substance-treated controls should be performed in order to validate the experimental conditions.

2. Equip the experimental room with standard fluorescent lighting. Set up two transparent cylinders separated visually from each other by opaque screens.

First session (Day 1)
3. On Day 1, 25 hr prior to testing, place the animals in the experimental room at least 60 min before the beginning of the first session. Immediately place an identifying mark on the tail of each animal with indelible ink. Randomly assign animals to a drug treatment, but give all animals within a cage the same treatment. Make food and water available throughout the experiment.

4. Weigh two animals individually, then place one rat in each of the two cylinders for 15 min (first session).

No scoring of immobility is performed during the first session. This session is needed to acclimatize the rats to the experimental situation and to induce a stable, high level of immobility during the test session. Individual weights are used to calculate the dose per animal and for documentation purposes.

5. Remove the rats from the cylinders, dry them with a cloth towel, and place them into a cage adjacent to their home cage.

6. Weigh two additional rats, and place them in the cylinders for 15 min.

7. Administer the appropriate treatment to the first group of two rats (the administration performed 24 hr before the test session) in a volume of 5 ml/kg, and place them back into their home cages. Repeat this sequence until the end of the session. Change the water in the cylinders after every three rats, or more often.

The test should be performed blind, with coded solutions, to avoid bias in evaluating animal behavior.

8. When the Day 1 session is completed, return the animals to the colony room and provide food and water ad libitum.

Test session (day 2)
9. Optional: On the test day, administer drug or test substance to the animals prior to the test session.

Two, or preferably three, pretest administrations at 24 hr, 4 hr, and 30 or 60 min prior to the test provide more stable results than a single administration. Control animals receive the same number of vehicle administrations.

10. Administer the final treatment 30 min (for intraperitoneal or subcutaneous injection) or 60 min (for oral administration) prior to the test.

Drug or test substance should be administered to individual animals in a fixed rotation (A, B, C, etc.) to ensure a regular distribution of the different treatments over time. The same treatment order is maintained during the different phases of the test.

11. Place two animals simultaneously in individual side-by-side cylinders separated by an opaque screen. Observe their behavior for 5 min. Score the duration of immobility by summing the time spent immobile; score as immobile minor movements strictly necessary to maintain the animal’s head above water.
The same observer scores the behavior of two animals simultaneously.

Decoding of treatment group results should be performed after all evaluations are completed.

12. Compare data from treated groups with data from the control group using nonpaired Student t tests (two tailed). Other statistical evaluations (e.g., analysis of variance followed by post hoc tests) can also be employed.

**FORCED SWIM (BEHAVIORAL DESPAIR) TEST IN THE MOUSE**

This protocol describes an equivalent procedure for primary screening studies using mice instead of rats. The use of mice has certain advantages because it requires less test substance and mice are considerably less expensive than rats. The authors’ experience with this method has demonstrated that mice show a sufficiently stable level of immobility after a single exposure and that a single substance administration is generally sufficient to detect antidepressant-like activity, thereby rendering the mouse procedure more suitable as a primary screen. The mouse protocol can also be employed for studies using transgenic animals.

**Materials**

- 20- to 25-g male NMRI (Naval Medical Research Institute) mice (e.g., Elevage Janvier)
- Standard rodent diet
- Treatment solutions (see recipe): drug or test compound, reference compound (e.g., imipramine hydrochloride, Sigma or equivalent), and vehicle alone (for control)
- Transparent plastic cages (e.g., type Macrolon 25 × 19 × 13 cm) containing wood shavings (e.g., Litalabo, SPPS, http://www.sppsfrance.com)
- Transparent Plexiglas cylinders (13 cm diameter × 24 cm high) containing water (22°C ± 2°C) to a depth of 10 cm (made in-house or obtained from commercial suppliers of Plexiglas material)
- Opaque screens for separating cylinders
- Metric balance (e.g., Sartorius type 1401.001), accurate to 0.1 g
- 1-ml syringes (e.g., Terumo type BS-01-T)
- 25-G × 0.625-in. (0.5 × 16–mm) needles for intraperitoneal injections (e.g., Terumo)
- 23-G × 1-in. (0.6 × 25–mm) needles for subcutaneous injections (e.g., Terumo)
- Luer gastric probes with oval extremity (25 mm long × 1.2 mm oval diameter) for oral administration

1. House mice (10 per cage) in cages containing wood shavings. Provide free access to standard rodent diet and tap water, and maintain a controlled temperature of 21°C ± 3°C and a standard (nonreversed) light/dark cycle with illumination from 0700 to 1900. Use five groups of ten mice per group for a standard experiment (vehicle control, reference compound, and three doses of test substance).

   *Make sure animals are delivered to the laboratory at least 5 days before the experiment. Experiments should be performed during the light phase of the cycle.*

2. Equip the experimental room with standard fluorescent lighting. Set up two transparent cylinders separated visually from one another by an opaque screen.

3. Place the animals in the experimental room at least 60 min before the beginning of the experiment, and immediately place an identifying mark on the animals’ tails with indelible ink. Make food and water available throughout the experiment.
4. Weigh the animals and immediately administer the appropriate treatment in a volume of 10 ml/kg. Wait a predetermined time after administration to begin the test, usually 30 min for intraperitoneal and subcutaneous injections, and 60 min for oral administration.

   *The test should be performed blind with coded solutions to avoid bias in evaluating the animal behavior.*

5. Place two animals simultaneously in individual side-by-side cylinders separated by an opaque screen. Measure the latency to immobility from the start of the test and the duration of immobility for the last 4 min of the 6-min test session.

   *With the single-session mouse procedure, the animals show more stable levels of immobility during the last 4 min of the session, thereby providing a more suitable baseline for detecting antidepressant-like activity.*

   *The latency to immobility corresponds to the delay between the start of the test and appearance of the first bout of immobility, defined as a period of at least 1 sec without any active escape behavior.*

6. Score the duration of immobility by summing the time spent immobile (i.e., the time not spent actively exploring the cylinder or trying to escape from it); score as immobile minor movements strictly necessary to maintain the animal’s head above water.

   *The same observer scores the behavior of two animals simultaneously.*

   *The first 2 min of the session are useful for preparing other animals.*

7. Compare data from treated groups with data from the control group using non-paired Student t tests (two tailed). Other statistical evaluations (e.g., analysis of variance followed by post hoc tests) can also be used.

**TAIL SUSPENSION TEST IN THE MOUSE**

This protocol describes a procedure in mice that is conceptually similar to those previously presented in this unit, but differs in that immobility is induced by suspending the animal by its tail. A mouse will initially try to escape from tail suspension by engaging in vigorous movements and then, after a few minutes, become immobile. In a manner analogous to the forced swim test, immobility is reduced by a wide variety of antidepressants. However, this procedure has several advantages over the forced swim procedure (see Basic Protocol and Alternate Protocol 1). First, no hypothermia is induced, and the animals, once removed from the experiment, resume normal spontaneous activity immediately. As a consequence, no special post-experimental treatment (rubbing down, maintenance in a warmed environment) is required. Experimenter comfort is also increased. Furthermore, the procedure lends itself readily to automation, permitting the testing of several animals simultaneously, with a resulting increase in throughput for screening purposes. Another characteristic of the tail suspension procedure is that it displays a different spectrum of pharmacological sensitivity than the forced swim procedures, thus providing a complementary approach to the behavioral screening of antidepressant and other psychotropic activity. A final advantage, shared with the mouse version of the forced swim test, is its potential utility for studying transgenic animals.

**Materials**

- 20- to 25-g male Swiss or NMRI mice (e.g., Elevage Janvier)
- Standard rodent diet
- Treatment solutions (see recipe): drug or test compound, reference compounds (e.g., imipramine hydrochloride and diazepam, Sigma or equivalent), and vehicle alone (for control)
Transparent plastic cages (e.g., Macrolon 25 cm × 19 cm × 13 cm) containing wood shavings (e.g., Litalabo, SPPS, http://www.sppsfrance.com)

Automated tail suspension apparatus (e.g., TST System; Bioseb, http://www.bioseb.com) consisting of plastic enclosures (20 cm × 25 cm × 30 cm) fitted with a ceiling hook connected to a strain gauge and computer assembly with Windows compatible software.

Metric balance (e.g., Sartorius type 1101601), accurate to 0.1 g

1-ml syringes (e.g., Terumo type BS-01-T)

25-G × 0.625-in. (0.5 × 16-mm) needles for intraperitoneal injections (e.g., Terumo)

23-G × 1-in. (0.6 × 25-mm) needles for subcutaneous injections (e.g., Terumo)

Luer gastric probes with oval extremity (25-mm long × 1.2-mm oval diameter) for oral administration

1. House mice (10 per cage) in cages containing wood shavings, and provide free access to standard rodent diet and tap water. Maintain a controlled temperature of 21° ± 3°C and a standard (nonreversed) light/dark cycle with illumination from 0700 to 1900. Use six groups of ten mice per group for a standard experiment (vehicle control, two reference compounds, and three doses of test substance).

   Make sure animals are delivered to the laboratory at least 5 days before the experiment.

   Experiments should be performed during the light phase of the cycle.

2. Equip the experimental room with standard fluorescent lighting.

3. Place the animals in the experimental room at least 60 min before beginning the experiment, and immediately place an identifying mark on each animal’s tail with indelible ink. Remove food and water for the duration of the test.

4. Weigh the animals and immediately administer the appropriate treatment in a volume of 10 ml/kg. Wait a predetermined time after administration to begin the test, usually 30 min for intraperitoneal and subcutaneous injections, and 60 min for oral administration.

   The test should be performed blind with coded solutions to avoid bias in evaluating animal behavior.

   Drug and test substance treatments should be administered to individual animals in a fixed rotation (A, B, C), to ensure a regular distribution of the different treatments over time. Automated equipment, such as the Bioseb system, provide randomization sequences, permitting balanced distribution over time and over the different positions in the apparatus.

5. Wrap adhesive tape around the animal’s tail in a constant position three quarters of the distance from the base of the tail. To avoid injury, suspend the animals by passing the suspension hook through the adhesive tape as close as possible to the tail (1 to 2 mm) to ensure the animal hangs with its tail in a straight line.

   Different numbers of animals can be tested simultaneously depending on whether the apparatus is automated. For nonautomatic observation, the same observer can comfortably monitor two animals simultaneously. For an automated procedure, the number of animals measured simultaneously depends on the configuration of the system. The authors recommend testing six animals simultaneously during the same measurement period, with all animals being placed in the apparatus before starting the measurement. This ensures a relatively high throughput without significant dispersion in the duration of suspension before starting the measurement. Whatever the configuration, the animals should be visually shielded from one another during the test.

6. Observe the animals continuously for 6 min. If an automated testing apparatus is not available, use separate stopwatches for each animal and sum the time spent immobile by each over the 6-min observation period.
In contrast with Alternate Protocol 1, the occurrence of immobility is not so clearly concentrated during the last 4 min of the test.

In contrast to the forced swim test, where the animals sometimes have to make small movements to maintain their heads above the water, immobility in the tail suspension test is easier to distinguish from periods of movement.

In contrast to the forced swim test, the tail suspension test causes no long lasting hypothermia or behavioral disturbance.

7. Compare data from treated groups with data from the control group using non-paired Student t tests. Other statistical evaluations (e.g., analysis of variance followed by post hoc tests) can also be used.

REAGENTS AND SOLUTIONS

Use deionized, distilled water in all recipes and protocol steps. For common stock solutions, see APPENDIX 2A; for suppliers, see SUPPLIERS APPENDIX.

Treatment solutions

Soluble compounds: Dissolve compound (drug/test substance or reference compound) in distilled water (for oral administration) or sterile physiological saline (for intraperitoneal or subcutaneous injections).

Insoluble compounds: Disperse compound in 0.2% (w/v) hydroxypropylmethylcellulose in distilled water (for oral administration) or physiological saline (for intraperitoneal or subcutaneous injections).

For substances that cannot be prepared as recommended above, it is possible to use other vehicles (e.g., cyclodextrins, low concentrations of Tween 80, DMSO, or ethanol). In these cases, preliminary verification of the effects of each special vehicle should be performed before any evaluation of the effects of a substance. If the vehicle has its own effects in the test, it is strongly recommended to add an experimental group treated with a neutral vehicle as defined above. Otherwise, interpretation of the effects of a test substance can be confounded by the effect of the vehicle.

Reference compound: Imipramine hydrochloride, 32 mg/kg for intraperitoneal or subcutaneous injections, 64 mg/kg for oral administration. Other clinically effective antidepressants (e.g., 64 mg/kg fluoxetine or 32 mg/kg venlafaxine as maximal doses) can be employed for this purpose. In the tail suspension test, diazepam (4 mg/kg for intraperitoneal or subcutaneous injections, 8 mg/kg for oral administration) can be used as reference myorelaxant.

All compounds are administered in a volume of 10 ml/kg.

COMMENTARY

Background Information

The first tests for screening antidepressants were based on pharmacological interactions. The most common of these was reserpine antagonism. Not only had reserpine sometimes been associated with the induction of depression in humans, it was also known to cause depletion of neuronal stores of monoamines. During the 1960s and early 1970s, monoamine depletion was thought to be a major cause of depression. Thus, the reserpine test possessed a certain degree of construct validity. It was also effective in detecting antidepressants with mechanisms of action similar to those already in clinical use (inhibitors of monoamine uptake and metabolism), thereby demonstrating the predictive validity of reserpine antagonism. Other antidepressant screens include potentiation of the central responses to amphetamine, L-3,4-dihydroxyphenylalanine (L-DOPA), or 5-hydroxytryptamine (5-HT), or antagonism of the central actions of tetrabenazine or neuroleptics.

The advent of atypical antidepressants, such as iprindole and mianserin, that showed little or no activity in these tests, necessitated...
the search for new screens that were not based on pharmacological interactions. One of the earliest was the forced swim or “behavioral despair” test, in which rodents become immobile when forced to swim in a restricted space from which there is no exit. This phenomenon is reminiscent of behaviors displayed by dogs exposed to inescapable shock (Seligman, 1975), or infant monkeys separated from their mothers or peers (Harlow and Suomi, 1974), suggesting that depressive-like states could be induced in animals by exposure to an uncontrollable, aversive environment (Porsolt et al., 1977). Because the forced swim test is much simpler to perform than these other tests, it is much more amenable to drug screening. 

Early studies revealed that monoamine uptake blockers and monoamine oxidase inhibitors are active in reducing immobility, even when the drugs display sedative effects. Furthermore, the atypical antidepressants, mianserin and iprindole, are also active in this test. These results suggested this procedure may be capable of detecting antidepressants different from those already in clinical use. During the past 20 years, the behavioral despair test, in both the rat and the mouse, has become one of the most widely used antidepressant screens in industrial pharmacology (Borsini and Meli, 1988; Castagné et al., 2006). In the rat, measurement of active behavior (swimming, climbing and, to a lesser extent, diving) facilitates discrimination between antidepressants acting on serotonin and norepinephrine reuptake (Detke and Lucki, 1996). Adding measurement of latency to immobility to the standard procedure increases the sensitivity of the behavioral despair test in the mouse to some antidepressants (Castagné et al., 2009). In addition to the latency data presented here for the mouse, other reports also use the latency in the rat (Contreras et al., 2001; Carlezon et al., 2002). The tail suspension test described by Stéru et al. (1985) is a conceptually related procedure. In this case, instead of being forced to swim, mice are simply suspended by the tail. As in the forced swim test, mice rapidly become immobile in the tail suspension test. Immobility is reduced by a variety of antidepressants, rendering the tail suspension procedure, especially when automated (Porsolt et al., 1987), highly useful for routine drug screening (Cryan et al., 2005). Other murine models of depression are discussed in Dalvi and Lucki, 1999). 

Critical Parameters and Troubleshooting

Strain of animals

Early experiments indicated that behaviors and results varied with animal strain. Even Wistar or Sprague-Dawley rats from different suppliers yielded different findings (Porsolt et al., 1978). Indeed, some rat strains have been specifically designed to maximize or minimize the level of immobility in an attempt to more closely model genetically determined endogenous depression (Overstreet et al., 1992; Lahmame and Armario, 1995). A recent comparison of the behavioral effects of antidepressants among different rat strains in the forced swim test is given in Lopez-Rubalcava and Lucki (2000). The strain of mice used for test substance evaluation is also of particular importance in the behavioral despair test (David et al., 2003; Petit-Demouliere et al., 2005). It was recently shown that NMRI mice are more sensitive to the effects of fluoxetine than C57Bl6 mice, although the two murine strains display comparable baseline duration and latency to immobility (Castagné et al., 2009).

Strain differences are also observed with the tail suspension test; animals have even been selectively bred for high levels of immobility, thereby rendering them more sensitive to antidepressant treatment (Vaugeois et al., 1996). Nonetheless, the general level of reproducibility between laboratories using numerous strains of mice is remarkably high (Cryan et al., 2005).

Number of sessions

The basic two-session procedure for the rat was designed to induce a high and stable level of immobility during the test session on the second day. It is nonetheless possible to repeatedly expose the animals to the test situation and still obtain a clear reduction in immobility with conventional antidepressants (Porsolt et al., unpub. observ.). With repeated drug treatments, it is preferable to schedule the administration period between the two sessions and compare the observed effects with those from a single treatment given immediately before the second session, preceded by a similar number of vehicle administrations (Vazquez-Palacios et al., 2004). It is not advisable to repeatedly test animals (>3 times) receiving drug regimens because it is difficult to distinguish repeated drug effects from drug-behavior interactions.
**Test session duration**

The basic procedure was designed to ensure a maximum of immobility during the 5-min test session, while maintaining the possibility of also observing a drug-induced increase in immobility. In fact, the amount of immobility can almost be titrated as a function of the length of exposure to the hostile environment. That is, the longer the animal is in the water or is suspended by the tail, the more immobile it becomes, whether in repeated short sessions or in a prolonged single session. It should be noted, however, that a prolonged single exposure to water induces marked hypothermia (Porsolt et al., 1979). Even with relatively warm water (30°C), the body temperature rapidly decreases toward that of the water. This problem is avoided using the tail suspension procedure, where no hypothermia is observed (Cryan et al., 2005).

**Dimensions of the test apparatus**

The apparatus dimensions of the forced test swim are designed to induce a maximum of immobility during the 5-min test session. Early experiments indicated that immobility occurs more rapidly if the dimensions are such that the animal learns quickly that there is no escape. Furthermore, with the rat protocol, the depth of the water is adjusted so the rat can touch the bottom with its hind paws. This enables the animal to steady itself and thereby facilitates the occurrence of immobility. It also prevents the rat from drowning. Despite these considerations, a modified version of the test uses a greater depth of water to allow measurement of active behaviors (climbing and swimming) in addition to immobility (Detke and Lucki, 1996). The modified version of the rat behavioral despair test was initially designed to facilitate detection of the effects of selective serotonin reuptake inhibitors, although it is also possible to observe an anti-immobility effect in the standard version of the test (Castagné et al., 2006). With the mouse protocol, the animals adopt a more horizontal position and do not need to touch the bottom of the cylinder. The dimensions of the apparatus are of less importance in the tail suspension procedure, where freedom of movement is limited by being suspended by the tail.

**Environmental conditions and baseline behavior**

The rat is extremely sensitive to changes in the external environment. Any small movement by the experimenter, or by another rat in a neighboring cylinder, can cause the animal to start swimming again. Accordingly, it is recommended that the experiment be performed in very quiet conditions, with constant illumination and a minimum of movement during the behavioral measures. When animals are being tested in parallel, they must be visually separated by an opaque screen. Mice appear less sensitive to environmental effects. On the other hand, there is greater variability between mice than rats, necessitating the use of more mice (n = 10 per group) than rats (n = 6 per group) in the forced swim test. As the tail suspension test is also affected by variability between individual mice (Cryan et al., 2005), there should be at least 12 mice per group, ideally 15 mice per group.

**Anticipated Results**

**Baseline behavior**

Rats exposed to a 15-min acclimation session remain immobile for about two-thirds of the 5-min test on the second day. With mice, the average duration of immobility is similar during the measurement period (~2 min), whether forced to swim or suspended by the tail. This level of immobility provides a suitable baseline for testing drugs that reduce the duration of immobility, while also allowing for detection of an increase in the duration of immobility. The duration of immobility observed in a particular experiment depends, of course, on a number of factors, including the animal strain, the dimensions of the cylinder, the depth of the water, the illumination of the experimental room, and, in the rat protocol, the duration of the first and second sessions. Neither the interval between the sessions in the rat (up to 4 weeks) nor the temperature of the water (between 22° and 35°C) appears to influence significantly the duration of immobility in the rat behavioral despair test (Porsolt et al., 1979). The latency to immobility in the behavioral despair test in vehicle-control mice generally lies between 60 and 90 sec.

**Drug and test compound effects**

The predictive value of the forced swim and tail suspension procedures for different classes of pharmacological agent has been reviewed (Porsolt et al., 1991; Cryan et al., 2005, Petit-Demouliere et al., 2005).

For the forced swim test, psychostimulants, some antihistamines and anticholinergics, some atypical antipsychotics (e.g., clozapine, sulpiride, amperozide), and a whole range of other compounds including angiotensin-converting enzyme (ACE) inhibitors, calcium
antagonists, γ-aminobutyric acid (GABA) agonists, and several neuropeptides, including enkephalin, oxytocin, and corticotropin-releasing factor (CRF), may yield false-positive results (Porsolt et al., 1991; Petit-Demouliere et al., 2005; Castagné et al., 2006).

It is by no means established, however, that all of these substances are devoid of antidepressant activity in humans. Recent clinical data suggests that some symptoms of depression are decreased by short-term treatment with psychostimulants, suggesting that the terms “false positive” may be misleading or at least exaggerated (Candy et al., 2008). However, the sensitivity of the test to established antidepressants can be improved by judicious choice of the strain of animal to be tested. For example, using NMRI mice improves the selectivity of the test for established antidepressants over psychostimulants and demonstrates that hyperactivity per se does not necessarily compromise the results of the forced swim test, although experimenters should be alert to this possibility (Castagné et al., 2009).

Fewer substances have been evaluated in the tail suspension procedure, but the results obtained are generally similar to those found with the forced swim test (Cryan et al., 2005). One major difference between the forced swim and tail suspension tests is that the latter is particularly sensitive to the sedative effects of some test compounds. For example, several 5-HT1a agonists, such as flesinoxan and alnespirone, generally decrease the duration of immobility in the forced swim test, whereas they increase the duration of immobility in the tail suspension test (Castagné et al., 2006). The most important false negatives for the forced swim test, particularly with the rat, are produced by a number of 5-HT uptake inhibitors, including clomipramine, citalopram, and fluvoxamine, although others, such as fluoxetine, indalpine, and paroxetine, do display antidepressant-like activity in this assay. In this respect, the tail suspension test shows greater sensitivity than the forced swim test to the antidepressant effects of 5-HT uptake inhibitors (Steru et al., 1987). As mentioned above, modification of the behavioral despair test by measuring active behavior in addition to immobility increases the sensitivity of the test to 5-HT uptake inhibitors (Detke and Lucki, 1996).

![Figure 5.8.1](image-url)  
**Figure 5.8.1** The effects of four different reference antidepressants on the duration of immobility in the forced swim test in the rat. Comparison with appropriate vehicle groups using the Student t test: *, p <0.05; ***, p <0.001 (two-tailed test). These substances can all be obtained from Sigma-Aldrich or equivalent.
More important for showing the general validity of the test are the various atypical agents and substances from different chemical classes that are antidepressants and that are active in these assays, but are not detected by pharmacological interaction procedures. In addition to mianserin and iprindole, there are 5-HT\textsubscript{1a} agonists, such as flesinoxan and alnespirone, NMDA antagonists, including 1-aminocyclopentane carboxylic acid (ACPC), phosphodiesterase inhibitors, such as rolipram, and idazoxan, an \(\alpha_2\)-adrenoceptor antagonist. Finally, several drug classes known not to possess clinical antidepressant activity, including classical neuroleptics, benzodiazepines, and analgesics, also do not show activity in the forced swim test. Overall, Borsini and Meli (1988) estimated that 87% of clinically active antidepressants display antidepressant-like activity in the behavioral despair test in the rat, with a somewhat higher hit rate (94%) in the mouse. Recent reports reveal that the behavioral despair and the tail suspension tests are the major tools for preclinical screening of new substances acting on various targets, including, but not limited to, monoaminergic systems (Cryan et al., 2005, Petit-Demouliere et al., 2005).

Representative data depicted in Figure 5.8.1 show the duration of immobility in the forced swim test in rats treated with imipramine and desipramine, two tricyclic antidepressants mainly acting as norepinephrine uptake blockers, fluoxetine, a 5-HT uptake inhibitor, and venlafaxine, a serotonin/norepinephrine uptake inhibitor. Figure 5.8.2 shows the

![Figure 5.8.2](image-url)
Figure 5.8.3  The effects of three different antidepressants on the duration of immobility (upper panels) and the power of the movements (lower panels) in the tail suspension test in the mouse. Comparison with appropriate vehicle groups using the Student t test: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ (two-tailed test). These substances can all be obtained from Sigma-Aldrich or equivalent.

dose-dependent effects of six reference antidepressants on the latency and the duration of immobility in the forced swim test in the mouse. Displayed in Figure 5.8.3 is the duration of immobility in the tail suspension test in mice treated with imipramine, fluoxetine, and venlafaxine.

**Time Considerations**

A standard behavioral despair test using rats (five treatment groups of $n = 6$) requires $\sim 2.5$ hr of experimental time on Day 1 (Session 1, without observations) and $\sim 2.5$ hr on Day 2 (Session 2, with observation of the animals). This does not include the time needed ($\sim 45$ min) to prepare the substances for administration. More generally, the rat test occupies two consecutive afternoons, with 1 hr in the morning of Day 2 for the 4-hr pretest drug administration. If necessary, one extra group can be tested within the same time frame. That is, the maximum number of rats tested per experiment is 36, with experiments requiring more animals performed in sub-experiments.

A standard mouse behavioral despair test (five treatment groups of $n = 10$) requires $\sim 2.5$ hr of experimental time. Measurement of the latency to immobility does not increase the experimental time. This does not include the time needed ($\sim 45$ min) to prepare the substances for administration. Generally, the mouse test occupies a morning or an afternoon. If necessary, two extra groups can be tested within the same time frame. In this case, the maximum number of mice tested per experiment is 70.

A standard tail suspension test in the mouse using an automated device (five treatment groups of $n = 12$ or 15) requires $\sim 2$ hr of
experimental time, not including substance preparation. Generally, the mouse test occupies an afternoon. If necessary, three extra groups can be tested within the same time frame, making it possible to test a maximum of 96 animals per experiment. If an automated device cannot be used, the throughput becomes similar to the mouse behavioral despair test.

**Literature Cited**


