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Measuring the Effects of Circadian Rhythm-Related Manipulations on Depression-Like Behavior in Rodents: Forced Swim and Tail Suspension Tests

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Abstract

The forced swim and tail suspension tests are commonly used to determine the effects of circadian-related pharmacological, genetic, and environmental manipulations on depression-like behavior in rodents. Both tests involve scoring immobility of rodents in an inescapable condition. Here we describe how to setup and carry out these tests.

Keywords

forced swim test; tail suspension test; depression; antidepressant; immobility; mice; rats

1 Introduction

Sleep and circadian rhythm disruptions frequently occur in depression and bipolar disorder. Phase shifted and dampened activity and hormonal rhythms are observed in individuals suffering from mood disorders [1]. In addition, studies have found associations between circadian gene polymorphisms and mood disorders [2–5]. Circadian rhythm disruptions may play a causal role in the etiology of mood disorders since environmental disruption of circadian rhythms, such as by shift work or jet lag, can trigger or worsen the symptoms of mood disorders [6, 7]. Further supporting that circadian rhythms may directly affect mood, treatments that target the circadian system, such as bright light therapy or agomelatine, are used to treat mood disorders [8, 9]. Therefore, determining the neurobiological mechanisms that underlie the associations between circadian rhythms and mood could lead to improvements in the diagnosis and treatment of depression and bipolar disorder.

Our lab and others are using animal models to understand the role of circadian rhythms in mood-like behaviors. Here, we discuss two widely used behavioral assays, the forced swim test (FST) and tail suspension test (TST), which can be used to assess the effects of circadian gene and light cycle manipulations on depression-like behavior in rodents. Both the FST and TST involve placing animals in an inescapable stressful situation, in a tank of water or suspended by their tail, respectively [10, 11]. Animals will initially struggle to escape,

but by the end of the tests most animals will display an immobile posture. Animals that more quickly become immobile and/or spend more time immobile are considered to show a depression-like phenotype.

Both assays are easy to perform and require minimal time, which allows for reliability across labs and high throughput screening. Importantly, there are differences between the tests that may make one test more suitable for an experimental question. In the TST, there is no potential for inducing hypothermia and mice can be returned immediately to their home cage. Therefore, the TST may be more preferred when testing a compound that affects body temperature. Furthermore, the TST has a higher throughput, compared to the FST, which requires additional time to replace the water between animals and to attain the correct temperature. However, the TST is not recommended for rats and larger mice, as they may be too heavy to be supported by their tail [12]. Therefore, the FST should be used for larger animals. Also, the FST has been more widely used and thus there is more literature to compare FST results to.

The major strength of these assays is their ability to rapidly screen novel compounds and experimental manipulations for antidepressant-like effects. These tests have predictive validity, as treatments that are effective in humans reduce immobility in these assays, including serotonin reuptake inhibitors, agomelatine, electroconvulsive shock, and sleep deprivation [13–18]. Therefore, these tests can be used to determine if novel chronotherapies affect depression-like behavior. For example, our lab has found that an inhibitor of the circadian gene casein kinase 1 was effective in normalizing latency to immobility in the FST in ClockΔ19 mice [19].

These assays can also be used to screen for manipulations that increase depression-like behavior. Factors that increase the risk for depression in humans, such as stress, increase immobility in the FST and TST [20–22]. Thus, latency to immobility and immobility time in these tests may represent a depression-like endophenotype in rodents, although this perspective can be controversial (reviewed in [23]).

It should be noted that both tests are sensitive to acute antidepressant treatment (reviewed in [23]), whereas humans typically do not respond to acute treatment, requiring a span of weeks before antidepressants are efficacious. This suggests that antidepressants have a different underlying mechanism in rodents, in these tests specifically, compared to humans. Comparing the effects of experimental manipulations on immobility in these tests in parallel with behaviors in other paradigms measuring depression-like behavior is a more powerful approach. For example, the novelty suppressed feeding test is not affected by acute antidepressant treatment, instead it requires long-term, chronic treatment [24, 25]. Overall, continuing to determine how environmental, genetic and pharmacological manipulations targeting circadian rhythms effect mood-like behaviors with these assays in combination with other behavioral tests may lead to novel, more effective treatments for mood disorders.

2 Materials

2.1 Forced Swim Test

1. Transparent tanks of water. In our lab, we use 4L glass beakers (16 cm diameter, 25 cm high) for the FST in mice and a large clear plastic tank from Stoelting (20 cm diameter, 45 cm high, 60160) for the rat FST.
2. Thermometer
3. Dividers
4. Timer
5. Camera, tripod and SD card
6. Heating pads or heat lamp
7. Paper or cloth towels
8. Clean, empty cages

2.2 Tail Suspension Test

1. Visually isolated box and/or oversized cages, at least 25 cm tall.
2. A ledge or a cage top, hopper, or metal rod to suspend over the cage. A metal rod would be ideal so there is very little material for the mouse to attempt to grab.
3. Adhesive tape or a metal clip.
4. Timer and video camera if dividers are being used to test multiple animals.

3 Methods

3.1 Forced Swim Test

1. Bring mice or rats into the testing room and allow them to habituate for 1 h.
2. Position the camera and dividers. The dividers should be tall enough to prevent the mice from seeing each other. The color of the dividers or surface behind the tanks should allow for clear visualization of the mice (e.g. white dividers for black mice).
3. Fill the appropriate number of tanks with water between 23–25°C. Record the measured water temperature for each tank. The depth of the water should be high enough to prevent the animal from sensing the bottom of the tank with their feet or tails. In our lab, we use a depth of 15–20 cm for mice. In rats, it is recommended to use a depth of 30 cm [26]. Most importantly, water depth and tank parameters should remain consistent across animals.
4. Start the video before placing the animals gently into the tanks. Once all animals are in the water, start the timer. For mice, the FST consists of a single 6 min session. For rats, the FST traditionally involves a 15 min preswim followed by a 5 min test the following day.

5. After the test session is finished, animals should be placed in empty cages on heating pads. Mice can be dried with paper towels, whereas large cloth towels may be used for rats. Keep animals from the same homecage separated until all animals have been tested, since exposure to a stressed cagemate may affect behavior in the FST.
6. Water tanks should be replaced with fresh water for each animal.
7. Score videos for latency to immobility and time spent immobile. Total immobility time is determined for only the last 4 min of the video. Immobility is considered no movement except for those necessary to keep the animal afloat. Alternatively, one can score mobility time and report immobility time as the remaining time. It may be easier to determine when the animal is mobile. For rats, mobility can be separated into climbing, swimming and diving. Climbing refers to when the forepaws break the plane of the water as the rat makes thrashing movements along the walls of the tank. Swimming refers to horizontal movements that propel the rat around the inside the tank. Diving typically occurs at the beginning of the test and is when the rat goes below the water surface, searching for escape routes. It is possible that only one type of movement may be affected by an experimental treatment. There is evidence to support that different neurotransmitter systems underlie swimming versus climbing behavior [27]. FST videos should be scored by at least one blinded, trained observer.

3.2 Tail Suspension Test

1. Bring mice into the testing room and allow them to habituate for 1 h.
2. Set up the boxes on a large flat surface.
3. Place dividers between/around the cages so animals are not disturbed by the movements of adjacent test subjects (see FST).
4. Bedding in the cages will help absorb any urine or feces.
5. Set up a cage top, hopper, or metal rod above the empty box, or arrange the boxes under an available ledge. Make sure the animals hang at a minimum of 25 cm above the floor of the cage. This will ensure they cannot reach the ground. Furthermore, the mouse should be 15 cm away from any other object (for example, the sides of the cage). This is particularly important if you use a hopper or cage top. In this case, turn the hopper sideways so the animal cannot reach the sides.
6. Suspend the animal by attaching the end of its tail (approximately 2 cm from the end) to a ledge using a piece of adhesive tape or a metal clip. Leave a few mm of the tip exposed.
7. Keep the animal suspended for a 6 minute test.
8. If scoring in real time, have a blinded, trained observer use a timer to record total immobility time and latency to immobility. Immobility is defined as hanging passively with a lack of voluntary limb movement. There may be some artificial

movement if the animal is swinging due to earlier momentum while they were mobile.

9. If clear cages are being used, sessions can be videotaped and scored by a similarly blind, trained observer at a later date. Make sure to videotape the entire session, beginning before the animal is suspended. Again, measurements can be taken by measuring latency to immobility, as well as total time spent immobile. Sometimes it is advantageous to normalize time spent immobile to a control group.

4 Notes

4.1 Forced Swim Test

1. In rats, the traditional FST paradigm consists of a preswim day. On the preswim day, rats are placed in the tank for 15 min. Then 24 h after the preswim, rats are placed in the tank for 5 min. The preswim is necessary for the rats to quickly show immobility during the 5 min test session. However, since this two-day paradigm could arguably involve a learning and memory component, treatments that affect learning and memory could confound the results [28]. Therefore, some investigators prefer to just use a single 15 min test.
2. Make sure to select the appropriate rodent strain. For example, some strains of mice, such as FVB, show very little immobility in the FST and thus would not be an ideal choice to test if a novel compound has antidepressant-like effects [12].

4.2 Tail Suspension Test

1. Some strains of mice will climb their tails during the TST, particularly C57BL/6 mice [29]. Tail climbing could interfere with the ability to score the TST. Excluding mice that climbed their own tails would also cause a sampling bias. Therefore, strain of mouse should be considered when planning to use the TST.
2. Do not secure the tail at the very end, suspending the tail at least 2 cm from the end will ensure the tail does not break or become damaged.
3. If using simply an oversized cage and top, make sure the cage is not cloudy as this could interfere with scoring.
4. It is not recommended to use the tail suspension test with rats due to their large size and the potential strain that would be placed on their tail.

4.3 Both FST and TST

1. Although a preswim is typically used in rats, some studies do include a preswim, or two day TST protocol, in mice, so that differences between experimental groups can be discerned [30–32]. Specifically, if mice have had a prior surgery, an extra FST can be helpful. Surgery can be a stressor in mice [33] and this can affect how animals respond to swimming for the first time.

2. If you are videotaping more than one animal at a time, be sure to label each container with the identification of each animal. This will make it easy to identify the mice when scoring the behavior at a later date. However, never include the groups of each animal, that way the video scorers will remain blind.

3. Ideally, when examining the effect of a circadian-related manipulation on mood, the behavioral assays should be conducted both in the dark and light phase using separate groups of animals. If studying free-running animals the tests could be conducted during their active and inactive phases. Experiments in constant conditions should be carefully designed, as prolonged exposure to constant dark can increase immobility [34].

4. Experiments should be designed so that the testing groups are counterbalanced across time of testing and testing apparatus. The time of testing should not differ between animals from different experimental groups since immobility time of animals have been shown to vary across the day [35]. If possible, include at least one animal from each experimental group during each testing session.

5. Individual housing of rodents influences immobility time [36]. Animals should be consistently grouped or single housed across experimental treatments.

6. When examining the behavior of animals in multiple assays, it is recommended to test animals in the FST or TST at the end of testing. These tests are stressful and could impact behavior in other tests, such as the elevated plus maze.

7. Make sure to be extremely still and quiet during the behavioral testing. This is particularly important during the FST or if translucent cages are being used during the TST. Otherwise, the mice might show artificial movement due to external distractions.

8. Measuring locomotor activity in parallel to the FST and TST is critical. If immobility is changed in either the FST or TST, it is necessary to determine whether this could be attributed to an overall effect on locomotion.

9. An alternative way to score the videos is to measure percent time immobile by using discrete checks of immobility every 5 seconds.

10. It is possible to use available techniques for automated scoring, but these methods should be compared to results obtained by hand scoring. For the TST, automated systems are available that involve attaching the tail of the mice to a sensor which measures the energy exerted by the mice. Investigators can also use video tracking software to automatically score behavior in the FST or TST. Furthermore, some researchers have used the Nomura water wheel FST [37]. In this modified FST, a fixed wheel is located at the surface of the water. Mice will turn the wheel in attempt to escape from the tank. The number of wheel turns can be used as an unbiased measure of struggling.

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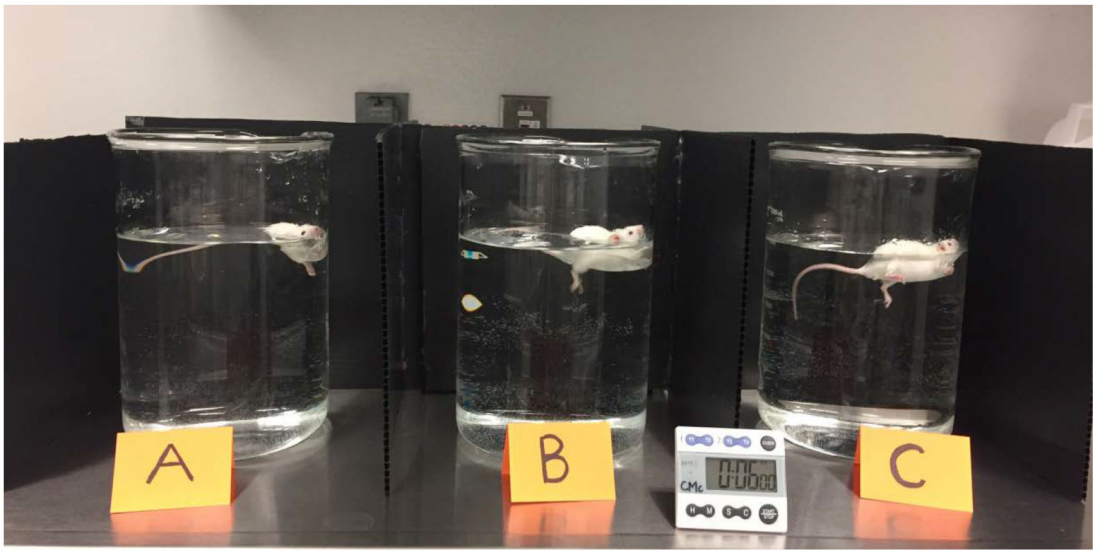

Fig. 1.
FST setup for white mice. Tanks are filled with 15 cm of water. Each mouse has been given a label.

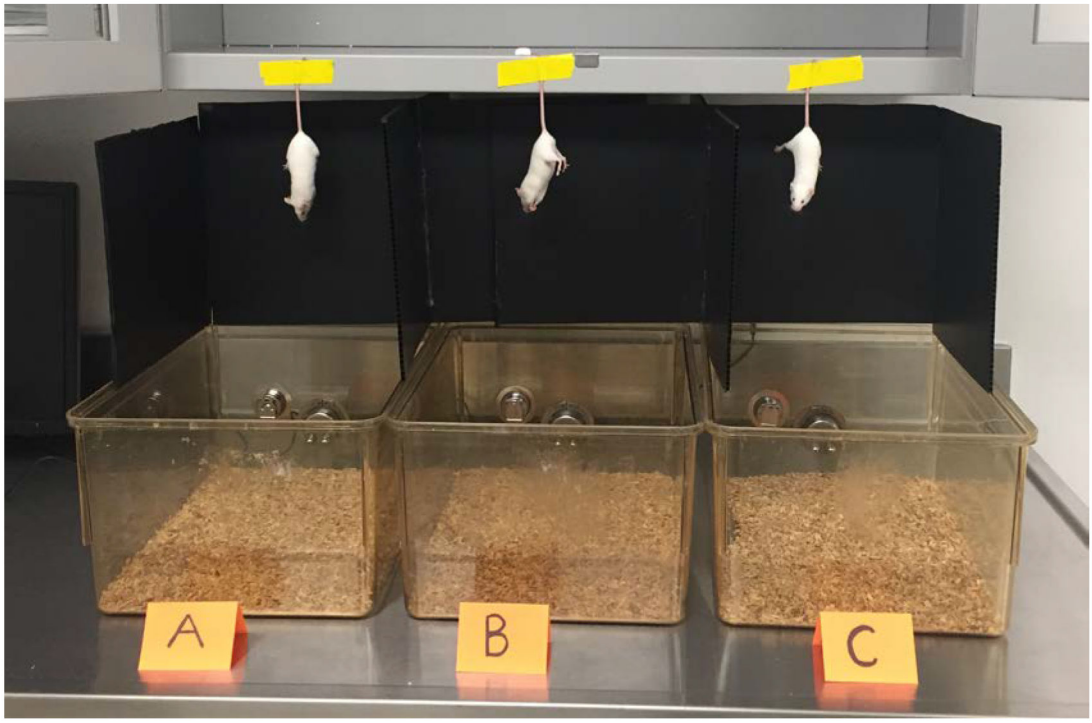

Fig. 2.

TST setup for white mice. Dividers are placed on oversized cages and mice are suspended from a shelf ledge. Each mouse has been given a label.