Thrita J Neu. 2023 December; 12(2): e144117. https://doi.org/10.5812/thrita-144117.

Published online: 2024 March 5. **Review Article**

Direct and Indirect Factors Affecting the Forced Swim Test to Investigate the Level of Depression in Rodents

Mehran Joodaki [1], Nasrin Hosseini [2,*]

[1] Shariati Hospital, Tehran University of Medical Sciences. Tehran, Iran
[2] Neuroscience Research Center, Iran University of Medical Sciences, Tehran, Iran

[*] *Corresponding author:* Neuroscience Research Center, Iran University of Medical Sciences, Tehran, Iran. Email: hosseini.n58@gmail.com

Received 2023 December 20; **Revised** 2024 February 3; **Accepted** 2024 February 20.

Abstract

Context: The forced swim test (FST) is employed to examine depression and depressive-like behaviors in rodents, such as mice and rats. In this test, increased periods of immobility and decreased swimming by the animal indicate heightened despair and depression-like behaviors.

Evidence Acquisition: This review discusses the impacts of the animals' race, gender, age, and weight and environmental factors like light, noise, and smell on the FST.

Results: Our review reveals that racial differences in rats and mice can influence their behavior. Differences in the nervous system structure and sex hormones related to gender are also significant. Additionally, animals that are very young or old, and those that are either very overweight or underweight, are unsuitable for the FST. Environmental factors such as light, noise, and smell were identified as confounding factors that could influence the outcomes and compromise the study's reliability.

Conclusions: It is essential to consider these factors and enhance the conditions and environment to carry out a standardized test. Furthermore, by acquiring more detailed information about these factors and minimizing or eliminating their effects, studies can yield more reliable results.

Keywords: Forced Swim Test, Rodents, Depression, Depressive-Like Behaviors

1. Context

Depression, or major depressive disorder, is linked to mental, physical, and behavioral disturbances (1). Symptoms in patients with depression vary widely and include cognitive and memory impairments, anhedonia, reduced energy, weight loss, a depressed mood, and feelings of hopelessness (2). Similarly, animals exhibit depression-like behaviors, such as despair (3, 4), making animal models valuable for research in this area. The forced swim test (FST), also known as the behavioral despair test, is utilized to assess depressive-like behaviors and the efficacy of antidepressants in mice, rats, and other rodents (3, 5). This test is favored for its low cost, reliability, sensitivity, and specificity to antidepressants. During the FST, researchers place the animal in a transparent tank filled with water and observe its attempts to escape or avoid drowning (6, 7). After being submerged, the animal will vigorously attempt to escape before eventually ceasing to move, remaining stationary except for the movements necessary to keep its nose above water. This immobility reflects a loss of hope in escaping the stressful situation. Key variables measured in the test include immobility, swimming, climbing, and diving (7). These variables can be affected by various factors that may skew the results. Therefore, this study aims to explore the factors influencing animal behavior in the FST, providing researchers with insights to achieve more dependable outcomes by understanding and adjusting these factors.

2. Evidence Acquisition

2.1. Factors Affecting FST

2.1.1. Race

Behavioral variations are observed across a broad spectrum of taxa. Furthermore, distinct behaviors have been identified in different strains of rats, likely attributable to structural and functional brain differences (8, 9). Accordingly, behavior in the FST can vary significantly depending on the animal's strain. For instance, mice of various strains, without interventions or treatments, have exhibited significant differences in immobility times (3).

The Flinders sensitive line (FSL) and Wistar Kyoto (WKY) strains have demonstrated more depressive-like behaviors in the FST (10). However, WKY rats showed a limited response to fluoxetine, a serotonergic antidepressant, and an unusual increase in swimming and climbing behaviors following treatment with desipramine, a noradrenergic antidepressant (11). The Long-Evans strain, commonly used to assess depressive-like behaviors, exhibited more significant immobility in the FST under chronic stress conditions compared to Sprague-Dawley, Wistar, and Fisher 344 rats (12). Some studies have suggested that strain differences indirectly affect behavioral outcomes through their impact on body weight (13). While the exact reasons behind these behavioral variations remain unclear, it is evident that strain differences in both rats and mice can influence animal behavior and the outcomes of related tests (3). Therefore, selecting the most appropriate animal strain based on the study's specific goals and objectives is crucial.

2.1.2. Gender

Behavioral variations between male and female animals have been noted, stemming from differences in the nervous system's structure, physiology, and brain chemistry (14). Research has indicated that gender impacts behavioral assessments in several ways, including variations in serotonergic activity across different brain regions and the influence of distinct sex hormones (15). Such differences are also evident in the FST (3). Female rats exhibited more swimming behavior than males during the pre-test but demonstrated increased floating time during the primary test. Conversely, male rats displayed more climbing behavior in pre-test and main-test sessions (15). In female rats, the induction of chronic stress through isolation and restraint during adolescence reduced active behaviors in the FST, whereas it had no impact on male rats (16). However, results vary, with some studies finding no gender differences in behaviors among Brown-Norway, Fisher 344, Lewis, WKY, and spontaneously hypertensive strains (17, 18). Gender-related differences in response to drug interventions in the FST have also been reported. One study found that the antidepressant effect of desipramine was significantly more significant in male rats than female rats (19). Chronic treatment with clomipramine, another tricyclic antidepressant, proved effective in male rats exhibiting novelty-seeking behaviors but did not affect females under similar conditions (20). Female rats on a creatine-enriched diet showed increased activity in the FST, whereas male rats did not exhibit any changes (21). Therefore, it is recommended to consider the gender of animals when studying the effects of various drugs and, if possible, to include both sexes in the research (3).

2.1.3. Age

Animals of varying ages exhibit distinct behaviors, likely due to the development of the brain and alterations in neuronal communication (22). Age, alongside weight, is a crucial factor to take into account. Young and old animals have demonstrated varied responses in the FST. Rats first displayed floating behavior at 21 days old, with this ability stabilizing as they aged, particularly by 26 days. Sprague-Dawley male rats aged 18 to 20 months showed reduced mobility in the FST compared to younger rats aged 3 to 4 months (3). In another study, adult male rats (90 days old) exhibited greater immobility and less activity than juvenile rats (35 - 37 days old) during the pre-test; similarly, in the main test, both immobility and climbing behaviors increased in the FST (23). It appears that sensitivity to certain antidepressants varies with age. For instance, chronic administration of paroxetine, a selective serotonin reuptake inhibitor, induced a typical antidepressant effect in adult rats in the FST but was ineffective in adolescent rats (24). The efficacy of tricyclic antidepressants, as well as epinephrine and serotonin reuptake inhibitors, in improving depressive-like behaviors was more pronounced in rats aged 4 weeks compared to those aged 40 weeks (25). Social isolation and treatment with reserpine reduced immobility in Swiss Webster rats aged 17 - 21 days but had no impact on rats aged 26 - 30 in the FST (26). Therefore, the selection of animals for the test should avoid very young or ancient subjects, emphasizing the importance of considering the rats' age, particularly in long-term studies.

2.1.4. Weight

Various behavioral studies have employed animals of different weights. Some research utilized rats within the weight range of 150 to 175 g or 160 to 180 g (27), whereas certain guidelines recommend a weight range of 275 -

450 g (28). As such, diverse weight ranges have been suggested for conducting behavioral tests. Notably, the body weight of rats can significantly influence their behavior in the FST. One primary reason weight impacts behavioral assessments is its association with age, with weight and age typically considered in conjunction (3). Employing appropriate statistical analysis is a recommended strategy to mitigate the influence of this confounding factor (29).

2.1.5. Analysis Process

The FST should be administered by researchers who must be made aware of the specifics of the research process. Additionally, the camera placement used for observing and recording animal behavior during the experiments should be disclosed, whether positioned above or to the side. Automated analysis is preferred over manual analysis to minimize researcher bias and enhance efficiency (3). Video recordings are generally processed by software; however, if software analysis is not feasible, manual analysis should be conducted by researchers unaware of the experiment's details.

2.1.6. Light

Standard lighting conditions during the FST have been shown to induce agitation and hyperactivity in rats under chronic stress (30). Circadian rhythms also play a role in influencing FST outcomes (31). For instance, varying the light exposure from 10 - 14 hours increased swimming duration in Wistar rats (32). Moreover, subjecting animals to 14 hours of light and 10 hours of darkness, as opposed to 6 hours of light and 19 hours of darkness, produced an antidepressant effect in male rats (33). Thus, chronic exposure to light without a dark phase, as well as low light exposure during the dark phase, can elevate depressive-like behaviors in the FST (34, 35). Consequently, the lighting conditions within the animal housing facility are crucial for obtaining accurate FST results.

2.1.7. Atmospheric Pressure

Atmospheric pressure can impact behavioral tests. A study demonstrated that reducing air pressure (20 hectopascals below normal atmospheric pressure) led to increased immobility times in rats.

2.1.8. Noise

Findings indicated that exposing male rats to noise for one hour did not affect their performance in the FST, neither immediately nor 24 hours later (3, 36). However, other research has found that noise at 2 000 Hz/120 dB increased the duration of immobility in the FST for female rats, a response not observed in male rats (37). Additionally, exposure to high-volume noise ultimately resulted in heightened anxiety-like behaviors in rats during behavioral assessments (38). Despite these varied outcomes, the influence of noise on behavioral tests must be considered. As such, the laboratory environment should be kept completely silent with minimal external movement. The testing room's door should remain closed, and warning signs to ensure quietness outside the testing area is advisable to reduce the risk of errors. If creating a silent environment during the FST is unfeasible, ambient noise might distract the animals. A white noise generator set to a volume at least 10 dB above the ambient noise level, measured before the animals enter the room, can help mitigate this issue (39).

2.1.9. Smell

Odor is another crucial factor influencing behavioral tests. Rats tested in a water tank previously used by other rats exhibited more immobility than those in a tank with fresh water (40, 41). This behavior is believed not to be due to contamination from feces or urine but rather exposure to stress-inducing factors such as pheromones released by rats during the FST (42). Nonetheless, contamination from feces or urine cannot be disregarded, as another study indicated that exposure to urine from a prior rat increased immobility (43). To prevent mice from coming into contact with urine, feces, or other contaminants, it is recommended to use clean and fresh water for each animal (3).

2.1.10. Time

Some studies suggest that time may also influence the outcomes of the FST, with immobility being shorter between 12:00-2:00 PM compared to 24:00 PM-2:00 AM (44). Given the impact of the experiment's timing on animal behavior, conducting all experiments under consistent timing and conditions is advisable (39). Additionally, the influence of different seasons on this test has been examined. The research found that female rats exhibited greater immobility in February and May (mid-autumn and spring) compared to August and November (mid-summer and fall), highlighting seasonal effects on the test (45).

2.1.11. Pharmaceutical and Chemical Factors

Various drugs and chemicals have been shown to affect the levels of immobility and activity in animals

during the FST. For instance, a dose of 35 mg of ketamine decreased immobility in the test (17). Ketamine's antidepressant effects in the FST are believed to stem from its impact on N-methyl-D-aspartate (NMDA) glutamate receptors, an increase in brain-derived neurotrophic factor, or activation of the mammalian target of rapamycin in the prefrontal cortex (46). Conversely, a 10 mg dose of citalopram did not decrease immobility in the FST, possibly due to the single dosage or the low drug dose (17). Amphetamine and caffeine also reduced immobility in the FST (47) and administering reboxetine for 14 days significantly decreased immobility and increased climbing behavior in rats (6). Furthermore, intraperitoneal injections of 30 mg/kg desipramine and 10 - 30 mg/kg imipramine also reduced immobility (48). Thus, it is important to consider the drugs used during experiments, especially when employing various substances.

2.1.12. Test Room

The testing room must be completely quiet and well-lit (3). It is advisable to leave animals out of the housing facility and go to the test room. Instead, placing them in a separate calm and quiet room (a waiting room) for a few minutes is preferred (28, 39). During the transportation of animals to the waiting and test rooms, all potential stress and anxiety-inducing factors should be eliminated or significantly reduced. If the FST is conducted with a group of animals, they should be brought into the test room individually. The subsequent animal should only enter after the previous one has completed the test and left the room, as the noise and scent left by preceding animals could introduce errors into the testing process (39). Therefore, allowing a break between tests for each animal is beneficial, and ensuring the test room is equipped with an effective ventilation system is beneficial. Additionally, animals should not be housed in the same room after undergoing behavioral tests. Furthermore, to ensure uniformity in the testing conditions, environmental factors such as temperature, lighting, and ventilation in the test room must be consistent for all animals (28, 39).

2.1.13. Water Tank

A cylindrical tank of glass or Plexiglas is needed to conduct the FST. However, a Plexiglas tank is preferred due to its higher resistance to the animal's frequent movements inside the tank. It is also advisable to mark the water level with a marker or similar tool to ensure consistency across tests without repeated measurements, maintaining the same water height for all animals (39). The tank's diameter and the water's depth are critical factors that influence rat behavior. Various protocols have recommended the water height and tank diameter to be 30 - 60 cm and 20 - 30 cm, respectively (3, 6, 7, 46). The water depth must be adjusted according to the animal's size to prevent the rat or mouse from touching the bottom of the tank with its tail or legs or from escaping the tank. In the FST, researchers should meticulously manage details and minimize animal stress to achieve optimal results. If multiple tanks are placed in proximity, dividers should be used to prevent the animals from seeing each other. Additionally, these separators should not reflect light to avoid compromising the quality of recorded footage (39). Contrary to previous practices, cleaning the tank with detergents after each test is not recommended, as this may introduce more variability in the results (3).

2.1.14. Water Temperature

Various studies have reported that the ideal temperature for tank water ranges from 22 - 26°C, with an average optimal temperature of 25°C (39, 46, 49). At this temperature, immobility in mice decreases, while at 35°C, it increases. Conversely, rats exhibit increased activity in cooler water temperatures and become more inactive as the temperature rises. However, rats show greater immobility at 19°C compared to the 25 - 30°C range (3). It is crucial to ensure that water at the appropriate temperature is available before commencing the test. If not, the necessary equipment, such as ice, cold water, or hot water, should be prepared to adjust the tank to the desired temperature. A simple mercury thermometer can be used to measure the water temperature (39). When using both hot and cold water to adjust temperature, it's essential to first equalize the tank's temperature by stirring, then place the thermometer in the center of the tank at a depth of 25 cm (7). However, a water-resistant infrared thermometer is preferred for its ability to quickly measure temperature, thereby reducing the time needed to conduct the test (39).

2.1.15. The Video Recording Device

The video recording device (camera) should be positioned above or to the side of the FST area, and the recording methodology must be detailed in reports (3). To enhance video quality, positioning the camera closer to the tank is advisable. If room light reflection is intense, using a polarized lens filter is necessary. Real-time scoring of rats without a video camera is

discouraged due to the high difficulty, time consumption, and increased likelihood of errors (39).

2.1.16. Paper Towels and Heating Device

Before returning the animals to their cages, it is crucial to gently dry them with paper towels. While using a heating device or lamp can be beneficial, care must be taken to ensure the temperature does not exceed 32°C (39). Consequently, a heating device or lamp should be available in the test room, and animals should be placed near it immediately after the test. Research indicates that 30 minutes is an appropriate duration for warming the animals, although the temperature should be adjusted according to the animal's size (17). It is advisable to position the heating device in a separate room. Warming the animals right after the test helps to prevent additional physical stress due to cold.

3. Conclusions

Numerous factors can serve as confounding variables and influence the animals' responses during behavioral tests like the FST. Factors such as age, gender, strain, weight, and stress levels of the animals are crucial considerations, along with environmental factors like noise, light, odor, as well as water and ambient temperatures, which must be carefully managed in study designs. When a factor cannot be eliminated, it is imperative to ensure consistent conditions across all animals to prevent data distortion. Therefore, attention to these factors is essential, as well as utilizing animals in optimized conditions and an appropriate environment for conducting standardized tests. Furthermore, by gathering comprehensive information about these factors and minimizing or eliminating their effects, studies can yield more reliable outcomes.

Acknowledgements

We sincerely thank all those who helped us with this review article.

Footnotes

Authors' Contribution: Study concept and design: Mehran Joodaki and Nasrin Hosseini; acquisition of data: Mehran Joodaki; drafting of the manuscript: Mehran Joodaki; critical revision of the manuscript for important intellectual content: Nasrin Hosseini; study supervision: Mehran Joodaki.

Conflict of Interests: The authors reported no conflicts of interest.

Funding/Support: This manuscript did not receive any funding/ support.

References

1. Villas Boas GR, Boerngen de Lacerda R, Paes MM, Gubert P, Almeida W, Rescia VC, et al. Molecular aspects of depression: A review from neurobiology to treatment. *Eur J Pharmacol.* 2019;**851**:99-121. [PubMed ID: 30776369]. https://doi.org/10.1016/j.ejphar.2019.02.024.
2. Joodaki M, Radahmadi M. Depression and Different Brain Areas: Neural Activity and Potential Mechanisms. *Avicenna J Neuro Psycho Physiology.* 2022;**9**(4):150-62. https://doi.org/10.32592/ajnpp.2022.9.4.102.
3. Bogdanova OV, Kanekar S, D'Anci KE, Renshaw PF. Factors influencing behavior in the forced swim test. *Physiol Behav.* 2013;**118**:227-39. [PubMed ID: 23685235]. [PubMed Central ID: PMC5609482]. https://doi.org/10.1016/j.physbeh.2013.05.012.
4. Bagot RC, Labonte B, Pena CJ, Nestler EJ. Epigenetic signaling in psychiatric disorders: stress and depression. *Dialogues Clin Neurosci.* 2014;**16**(3):281-95. [PubMed ID: 25364280]. [PubMed Central ID: PMC4214172]. https://doi.org/10.31887/DCNS.2014.16.3/rbagot.
5. Joodaki M, Radahmadi M, Alaei H. Comparing the Therapeutic Effects of Crocin, Escitalopram and Co-Administration of Escitalopram and Crocin on Learning and Memory in Rats with Stress-Induced Depression. *Malays J Med Sci.* 2021;**28**(4):50-62. [PubMed ID: 34512130]. [PubMed Central ID: PMC8407799]. https://doi.org/10.21315/mjms2021.28.4.6.
6. Flores-Serrano AG, Vila-Luna ML, Alvarez-Cervera FJ, Heredia-Lopez FJ, Gongora-Alfaro JL, Pineda JC. Clinical doses of citalopram or reboxetine differentially modulate passive and active behaviors of female Wistar rats with high or low immobility time in the forced swimming test. *Pharmacol Biochem Behav.* 2013;**110**:89-97. [PubMed ID: 23769836]. https://doi.org/10.1016/j.pbb.2013.06.003.
7. Mezadri TJ, Batista GM, Portes AC, Marino-Neto J, Lino-de-Oliveira C. Repeated rat-forced swim test: reducing the number of animals to evaluate gradual effects of antidepressants. *J Neurosci Methods.* 2011;**195**(2):200-5. [PubMed ID: 21167866]. https://doi.org/10.1016/j.jneumeth.2010.12.015.
8. Stohr T, Schulte Wermeling D, Weiner I, Feldon J. Rat strain differences in open-field behavior and the locomotor stimulating and rewarding effects of amphetamine. *Pharmacol Biochem Behav.* 1998;**59**(4):813-8. [PubMed ID: 9586836]. https://doi.org/10.1016/s0091-3057(97)00542-x.
9. Pare WP, Tejani-Butt SM. Effect of stress on the behavior and 5-HT system in Sprague-Dawley and Wistar Kyoto rat strains. *Integr Physiol Behav Sci.* 1996;**31**(2):112-21. [PubMed ID: 8809595]. https://doi.org/10.1007/BF02699783.
10. Malkesman O, Weller A. Two different putative genetic animal models of childhood depression--a review. *Prog Neurobiol.* 2009;**88**(3):153-69. [PubMed ID: 19545781]. https://doi.org/10.1016/j.pneurobio.2009.03.003.
11. Lopez-Rubalcava C, Lucki I. Strain differences in the behavioral effects of antidepressant drugs in the rat forced swimming test. *Neuropsychopharmacology.* 2000;**22**(2):191-9. [PubMed ID: 10649831]. https://doi.org/10.1016/S0893-133X(99)00100-1.
12. Bielajew C, Konkle AT, Kentner AC, Baker SL, Stewart A, Hutchins AA, et al. Strain and gender specific effects in the forced swim test: effects of previous stress exposure. *Stress.* 2003;**6**(4):269-80. [PubMed ID: 14660059]. https://doi.org/10.1080/10253890310001602829.

13. Vogel H, Kraemer M, Rabasa C, Askevik K, Adan RAH, Dickson SL. Genetic predisposition to obesity affects behavioural traits including food reward and anxiety-like behaviour in rats. *Behav Brain Res.* 2017;**328**:95-104. [PubMed ID: 28389340]. https://doi.org/10.1016/j.bbr.2017.02.037.
14. Kokras N, Dalla C. Sex differences in animal models of psychiatric disorders. *Br J Pharmacol.* 2014;**171**(20):4595-619. [PubMed ID: 24697577]. [PubMed Central ID: PMC4209934]. https://doi.org/10.1111/bph.12710.
15. Drossopoulou G, Antoniou K, Kitraki E, Papathanasiou G, Papalexi E, Dalla C, et al. Sex differences in behavioral, neurochemical and neuroendocrine effects induced by the forced swim test in rats. *Neuroscience.* 2004;**126**(4):849-57. [PubMed ID: 15207320]. https://doi.org/10.1016/j.neuroscience.2004.04.044.
16. Bourke CH, Neigh GN. Behavioral effects of chronic adolescent stress are sustained and sexually dimorphic. *Horm Behav.* 2011;**60**(1):112-20. [PubMed ID: 21466807]. [PubMed Central ID: PMC3112263]. https://doi.org/10.1016/j.yhbeh.2011.03.011.
17. Sheikh S, Sonone P, Verma V, Tripathi CD, Karim BA, Meshram GG. Ketamine; A better anti-depressant? An animal study evaluating the efficacy of citalopram, ketamine and their combination in animal models of depression. *Journal of Neurology, Neurological Science and Disorders.* 2021;**7**(1):19-23. https://doi.org/10.17352/jnnsd.000043.
18. Armario A, Gavalda A, Marti J. Comparison of the behavioural and endocrine response to forced swimming stress in five inbred strains of rats. *Psychoneuroendocrinology.* 1995;**20**(8):879-90. [PubMed ID: 8834094]. https://doi.org/10.1016/0306-4530(95)00018-6.
19. Simpson J, Ryan C, Curley A, Mulcaire J, Kelly JP. Sex differences in baseline and drug-induced behavioural responses in classical behavioural tests. *Prog Neuropsychopharmacol Biol Psychiatry.* 2012;**37**(2):227-36. [PubMed ID: 22353173]. https://doi.org/10.1016/j.pnpbp.2012.02.004.
20. Pitychoutis PM, Pallis EG, Mikail HG, Papadopoulou-Daifoti Z. Individual differences in novelty-seeking predict differential responses to chronic antidepressant treatment through sex- and phenotype-dependent neurochemical signatures. *Behav Brain Res.* 2011;**223**(1):154-68. [PubMed ID: 21549763]. https://doi.org/10.1016/j.bbr.2011.04.036.
21. Allen PJ, D'Anci KE, Kanarek RB, Renshaw PF. Chronic creatine supplementation alters depression-like behavior in rodents in a sex-dependent manner. *Neuropsychopharmacology.* 2010;**35**(2):534-46. [PubMed ID: 19829292]. [PubMed Central ID: PMC2794979]. https://doi.org/10.1038/npp.2009.160.
22. Shoji H, Takao K, Hattori S, Miyakawa T. Age-related changes in behavior in C57BL/6J mice from young adulthood to middle age. *Mol Brain.* 2016;**9**:11. [PubMed ID: 26822304]. [PubMed Central ID: PMC4730600]. https://doi.org/10.1186/s13041-016-0191-9.
23. Martinez-Mota L, Ulloa RE, Herrera-Perez J, Chavira R, Fernandez-Guasti A. Sex and age differences in the impact of the forced swimming test on the levels of steroid hormones. *Physiol Behav.* 2011;**104**(5):900-5. [PubMed ID: 21658399]. https://doi.org/10.1016/j.physbeh.2011.05.027.
24. Karanges E, Li KM, Motbey C, Callaghan PD, Katsifis A, McGregor IS. Differential behavioural and neurochemical outcomes from chronic paroxetine treatment in adolescent and adult rats: a model of adverse antidepressant effects in human adolescents? *Int J Neuropsychopharmacol.* 2011;**14**(4):491-504. [PubMed ID: 21329552]. https://doi.org/10.1017/S146114571100006X.
25. David DJ, Bourin M, Hascoet M, Colombel MC, Baker GB, Jolliet P. Comparison of antidepressant activity in 4- and 40-week-old male mice in the forced swimming test: involvement of 5-HT1A and 5-HT1B receptors in old mice. *Psychopharmacology (Berl).* 2001;**153**(4):443-9. [PubMed ID: 11243491]. https://doi.org/10.1007/s002130000588.
26. Yates G, Panksepp J, Ikemoto S, Nelson E, Conner R. Social isolation effects on the "behavioral despair" forced swimming test: effect of age and duration of testing. *Physiol Behav.* 1991;**49**(2):347-53. [PubMed ID: 2062907]. https://doi.org/10.1016/0031-9384(91)90055-s.
27. Castagne V, Moser P, Roux S, Porsolt RD. Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. *Curr Protoc Pharmacol.* 2010;**Suppl 49**:5.8.1-14. [PubMed ID: 22294373]. https://doi.org/10.1002/0471141755.ph0508s49.
28. Slattery DA, Cryan JF. Using the rat forced swim test to assess antidepressant-like activity in rodents. *Nat Protoc.* 2012;**7**(6):1009-14. [PubMed ID: 22555240]. https://doi.org/10.1038/nprot.2012.044.
29. Brenes JC, Rodriguez O, Fornaguera J. Differential effect of environment enrichment and social isolation on depressive-like behavior, spontaneous activity and serotonin and norepinephrine concentration in prefrontal cortex and ventral striatum. *Pharmacol Biochem Behav.* 2008;**89**(1):85-93. [PubMed ID: 18096212]. https://doi.org/10.1016/j.pbb.2007.11.004.
30. Strekalova T, Spanagel R, Dolgov O, Bartsch D. Stress-induced hyperlocomotion as a confounding factor in anxiety and depression models in mice. *Behav Pharmacol.* 2005;**16**(3):171-80. [PubMed ID: 15864072]. https://doi.org/10.1097/00008877-200505000-00006.
31. Gomes KM, Souza RP, Inacio CG, Valvassori SS, Reus GZ, Martins MR, et al. Evaluation of light/dark cycle in anxiety- and depressive-like behaviors after regular treatment with methylphenidate hydrochloride in rats of different ages. *Braz J Psychiatry.* 2011;**33**(1):55-8. [PubMed ID: 20602012]. https://doi.org/10.1590/s1516-44462010005000018.
32. Prendergast BJ, Kay LM. Affective and adrenocorticotrophic responses to photoperiod in Wistar rats. *J Neuroendocrinol.* 2008;**20**(2):261-7. [PubMed ID: 18047552]. https://doi.org/10.1111/j.1365-2826.2007.01633.x.
33. Molina-Hernandez M, Tellez-Alcantara P. Long photoperiod regimen may produce antidepressant actions in the male rat. *Prog Neuropsychopharmacol Biol Psychiatry.* 2000;**24**(1):105-16. [PubMed ID: 10659987]. https://doi.org/10.1016/s0278-5846(99)00084-6.
34. Fonken LK, Kitsmiller E, Smale L, Nelson RJ. Dim nighttime light impairs cognition and provokes depressive-like responses in a diurnal rodent. *J Biol Rhythms.* 2012;**27**(4):319-27. [PubMed ID: 22855576]. https://doi.org/10.1177/0748730412448324.
35. Fonken LK, Finy MS, Walton JC, Weil ZM, Workman JL, Ross J, et al. Influence of light at night on murine anxiety- and depressive-like responses. *Behav Brain Res.* 2009;**205**(2):349-54. [PubMed ID: 19591880]. https://doi.org/10.1016/j.bbr.2009.07.001.
36. Armario A, Gil M, Marti J, Pol O, Balasch J. Influence of various acute stressors on the activity of adult male rats in a holeboard and in the forced swim test. *Pharmacol Biochem Behav.* 1991;**39**(2):373-7. [PubMed ID: 1946578]. https://doi.org/10.1016/0091-3057(91)90194-7.
37. Bulduk S, Canbeyli R. Effect of inescapable tones on behavioral despair in Wistar rats. *Prog Neuropsychopharmacol Biol Psychiatry.* 2004;**28**(3):471-5. [PubMed ID: 15093953]. https://doi.org/10.1016/j.pnpbp.2003.11.012.
38. Zhvania M, Gogokhia N, Tizabi Y, Japaridze N, Pochkidze N, Lomidze N, et al. Behavioral and neuroanatomical effects on exposure to White noise in rats. *Neurosci Lett.* 2020;**728**:134898. [PubMed ID: 32224224]. https://doi.org/10.1016/j.neulet.2020.134898.
39. Can A, Dao DT, Arad M, Terrillion CE, Piantadosi SC, Gould TD. The Mouse Forced Swim Test. *J Vis Exp.* 2012;(59). e3638. [PubMed ID: 22314943]. [PubMed Central ID: PMC3353513]. https://doi.org/10.3791/3638.
40. Abel EL, Altman HJ, Commissaris RL. Maudsley reactive and nonreactive rats in the forced swim test: comparison in fresh water

and soiled water. *Physiol Behav.* 1992;**52**(6):1117-9. [PubMed ID: 1484869]. https://doi.org/10.1016/0031-9384(92)90469-i.

41. Abel EL, Bilitzke PJ. A possible alarm substance in the forced swimming test. *Physiol Behav.* 1990;**48**(2):233-9. [PubMed ID: 2255725]. https://doi.org/10.1016/0031-9384(90)90306-o.
42. Abel EL. Alarm substance emitted by rats in the forced-swim test is a low volatile pheromone. *Physiol Behav.* 1991;**50**(4):723-7. [PubMed ID: 1775546]. https://doi.org/10.1016/0031-9384(91)90009-d.
43. Gutierrez-Garcia AG, Contreras CM, Mendoza-Lopez MR, Garcia-Barradas O, Cruz-Sanchez JS. Urine from stressed rats increases immobility in receptor rats forced to swim: role of 2-heptanone. *Physiol Behav.* 2007;**91**(1):166-72. [PubMed ID: 17408705]. https://doi.org/10.1016/j.physbeh.2007.02.006.
44. Dubocovich ML, Mogilnicka E, Areso PM. Antidepressant-like activity of the melatonin receptor antagonist, luzindole (N-0774), in the mouse behavioral despair test. *Eur J Pharmacol.* 1990;**182**(2):313-25. [PubMed ID: 2168835]. https://doi.org/10.1016/0014-2999(90)90290-m.
45. Aksoy A, Schulz D, Yilmaz A, Canbeyli R. Brief Communication: Seasonal variability in behavioral despair in female rats. *Int J Neurosci.* 2004;**114**(12):1513-20. https://doi.org/10.1080/00207450490509131.
46. Réus GZ, Stringari RB, Kirsch TR, Fries GR, Kapczinski F, Roesler R, et al. Neurochemical and behavioural effects of acute and chronic memantine administration in rats: Further support for NMDA as a new pharmacological target for the treatment of depression? *Brain Res Bull.* 2010;**81**(6):585-9. [PubMed ID: 19954760]. https://doi.org/10.1016/j.brainresbull.2009.11.013.
47. Lino-de-Oliveira C, De Lima TC, de Pádua Carobrez A. Structure of the rat behaviour in the forced swimming test. *Behav Brain Res.* 2005;**158**(2):243-50. [PubMed ID: 15698890]. https://doi.org/10.1016/j.bbr.2004.09.004.
48. Kitamura Y, Araki H, Gomita Y. Influence of ACTH on the effects of imipramine, desipramine and lithium on duration of immobility of rats in the forced swim test. *Pharmacol Biochem Behav.* 2002;**71**(1-2):63-9. [PubMed ID: 11812508]. https://doi.org/10.1016/s0091-3057(01)00625-6.
49. Gutiérrez-García AG, Contreras CM. Stressors can affect immobility time and response to imipramine in the rat forced swim test. *Pharmacol Biochem Behav.* 2009;**91**(4):542-8. [PubMed ID: 18851989]. https://doi.org/10.1016/j.pbb.2008.09.008.