Animal Research Review Panel Guideline 30
The forced swim test in rats and mice

Animal Research Review Panel Guideline 30

November 2022

1. What is the forced swim test?

The forced swim test (FST), using mice and rats, involves placing an animal in a cylinder of water too deep for it to stand in and from which escape is impossible. The test measures the time taken until the animal stops swimming and floats or performs only the necessary movements required to keep their head above water.

2. Why is the forced swim test used?

The FST was originally designed as a screening test for novel antidepressant drugs, based on the knowledge that animals dosed with established antidepressants swim for longer than control animals that have not been dosed. In simple terms, administration of a drug that increases the time an animal swims before floating is interpreted as an antidepressant effect (Porsolt et al., 1977), (Petit-Demouliere et al., 2005), (*British Association for Psychopharmacology: Fact sheet on the forced swim test*, 2020).

3. How is the forced swim test performed?

The test relies on placing animals in an inescapable and uncontrollable stress situation.

Original test version

A cylinder of water of radius >20cm and 15 – 18cm depth is used. The water temperature is maintained at 25 degrees. The animal is placed in the cylinder and after initial attempts to escape by swimming, struggling, climbing, or diving, it will then display a progressive increase in the frequency and duration of immobile floating. After 15 minutes the animal is taken out of the water, dried, and returned to the home cage. 24 hours later the animal is again placed in the container of water. This second session usually lasts 5- 6 minutes, with most animals soon displaying passive behaviour – they stop swimming and show little if any attempts to climb the wall of the cylinder or to dive. The time from placement in the water to immobility / floating is the main outcome measure of the FST (Porsolt et al., 1977).

Test modifications

Two major modifications to the original procedure are:

- For rats, the depth of the water is increased to 30 cm, so the animal is not able to remain stable, without swimming, through tail contact with the bottom of the cylinder, which deceases the amount of immobility time. Scoring is based on two types of active behaviours, swimming and climbing, and the use of a time sampling technique, where the predominant behaviour is measured in 5 second intervals (instead of the cumulative time to immobility) (Ferreira et al., 2018).
- For mice, a single swim session of 6 minutes is used and during the last four minutes the duration of immobility is recorded in seconds (Can et al., 2012). A 15 minute pre-test session is not used, as this has been shown to induce stable immobile behaviour (de Kloet & Molendijk, 2016), (Ferreira et al., 2018), (*British Association for Psychopharmacology: Fact sheet on the forced swim test*, 2020).

4. What is the debate about the forced swim test?

The debate about the use of the FST relates to:

- The accuracy of the test as a screen for new antidepressant drugs
- The use of the test as a "model of depression"
- The welfare effects on the animals

4.1 Use as a screen for potential antidepressant drugs

There is some support for the FST being reliable as a screening test for potential antidepressant drugs (Petit-Demouliere et al., 2005), (Ferreira et al., 2018), (*British Association for Psychopharmacology: Fact sheet on the forced swim test*, 2020). A 2018 review article concluded that the FST (along with the tail suspension test and the sucrose preference test) was informative, providing the following were done:

- More than one test is used, with coherent results
- Secondary drug effects are considered and properly controlled for
- Each test and specific protocol are validated with data from at least a "gold standard" antidepressant drug (Ferreira et al., 2018).

The most frequent secondary drug effects which may confound FST results are changes in motor activity and this should be controlled for with specific tests for locomotor activity that are run concomitantly with the FST (Slattery & Cryan, 2012), (Ferreira et al., 2018).

A further limitation of the FST is understanding the effect of sex. Despite established differences in risk and response to treatment for depression in females and males, the original FST protocols were established using male animals only, and very few studies since have compared females and males, and those which do make the comparison show differences in behavioural responses and dose dependency between female and male animals (Kokras et al., 2015).

There are also differences in strains of mice, and to a lesser extent rats, in times to immobility and climbing behaviour. Some of the most commonly used strains display very high levels of

immobility, which makes the detection of drug effects more difficult (Slattery & Cryan, 2012), (Ferreira et al., 2018).

There are some variations in the reliability of the FST with different classes of drugs. For example, selective serotonin reuptake inhibitors (SSRIs) have been much more reliably detected as antidepressants in the mouse FST compared to the rat FST. Most studies find no effect of SSRI treatment using the rat FST (although use of the modified FST in rats is described as reliable for SSRIs) (Ferreira et al., 2018).

The accuracy of the FST as an antidepressant screening tool has also been challenged. A retrospective review assessing whether compounds tested in the FST had been further explored for antidepressant effects in humans by major pharmaceutical companies demonstrated that of 109 compounds tested, only 28% had been followed up. Of these, there were only three for which the FST appeared to positively predict antidepressant efficacy, but none were currently approved to treat any type of depression (Trunnell & Carvalho, 2021).

4.2 Use as a "model of depression"

The FST has been used in studies to acutely model aspects of depression, It was assumed that immobility equated with "giving up" or "behavioural despair" akin to learned helplessness, that could be used as a model for depression. However, this interpretation is now considered controversial and too simplistic.

Alternative explanations for the behaviour include that it might represent a shift from an active (and energy-expensive) coping strategy to a passive one, to conserve energy, with immobility during the FST reflecting an adaptive response to an inescapable situation rather than despair (Armario 2021).

Current thinking is that the FST is not an animal model for aspects of depression nor does it reproduce the behavioural characteristic of any other neuropathological condition in humans (Armario., 2021) (Borsini et al., 1986), (Molendijk & de Kloet, 2015), (Molendijk & de Kloet, 2019), (Commons et al., 2017), (Ferreira et al., 2018) (British Association for Psychopharmacology: Fact sheet on the forced swim test, 2020).

4.3 Welfare effects on animals

Negative impacts on the welfare of animals in the FST include:

- The FST relies on placing animals in an inescapable, life-threatening situation, designed to induce a high level of stress on the animal.
- Hypothermia may be experienced during and after the procedure.
- Social isolation stress is likely to be experienced during the test and in the drying period before return to the home cage.
- Fatigue from swimming.
- Handling before and after the procedure may be a stressor.
- Instances of drowning and near-drowning have been reported (via Animal Research Review Panel survey 2021).

Methods to reduce the impacts on the welfare of animals include:

- Maintaining the water temperature at 25 degrees (water that is too hot or too cold may alter the swimming response and time to immobility).
- Dry animals and provide warmth (for example an incubator at 32 degrees) after removal from the water.
- Animals should be tested individually, and continual close monitoring of animals undertaken while in the water to make sure they are not at risk of drowning.
- Limiting the number of swimming episodes and times to those established for the FST:
    - For rats a maximum of two swims, with a maximum of 15 minutes for the first and 6 minutes for the second.
    - for mice a maximum of one swim with a maximum of 6 minutes.
- Positive reinforcement after the procedure, for example by providing a food treat.
- Acclimatise animals to handling.
- Limit the time animals are isolated from their cage mates.
- Carry out follow up monitoring checks on animals after the procedure (for example 10 minutes, 30 minutes, 1 hour, 2 hours, 12 hours, 24 hours).
- Careful experimental design is critical, with studies showing the FST has an extremely large mean effect size, which if included in prospective power estimations indicates significantly less animals are required for the experiment to have adequate power to detect biologically relevant effects (Smalheiser et al., 2021). It is important to note however that extremely large mean effect size has only been demonstrated for the FST in mice.

5. Are there alternative to the forced swim test?

There are currently no 'in vitro' (non-animal) methods of checking whether a potential new treatment is likely to have antidepressant effects (*British Association for Psychopharmacology: Fact sheet on the forced swim test*, 2020).

Alternatives to the FST for antidepressant drug screening that use animals are the **tail suspension test** and the **sucrose preference test**.

The tail suspension test is used in mice and similarly relies on placing animals into an acute, inescapable, and uncontrollable stress situation. The sucrose preference test relies on prior induction of behavioural alterations that impair reward responses, such as chronic exposure to mild but inescapable stressors. These tests are commonly used in combination with the FST for screening potential antidepressant drugs (Ferreira et al., 2018).

6. What does this mean for researchers and Animal Ethics Committees?

The Animal Research Review Panel (the Panel) considers the FST to have a high negative impact on the welfare of animals.

The Panel recommends that use of the FST as an "acute model of depression" in research not be approved by Animal Ethics Committees (AECs).

The Panel recommends that use of the FST for testing potential antidepressant drugs be approved by AECs by exception only, based on compelling justification in the application on the need to use this test and why alternatives with lesser welfare impact cannot be utilised. The

Panel further recommends that AECs should consider these welfare considerations for any procedure involving non-voluntary swimming protocols.

The Panel requires that use of the FST be classified as **Procedure Category P7: Major Physiological Challenge** (see Guidance on completing Form L). AECs must also determine if other non-voluntary swimming protocols should be similarly classified. This means AECs must provide detailed information in their annual reports to their establishments on the approval of the FST. The information should include the justification for the use of the FST, the species and number of animals used, as well as measures implemented to reduce the number animals used for the project. (See ARRP Policy 5: Annual reporting by Animal Ethics Committees to accredited animal research establishments).

Further guidance on matters to consider is in Animal Research Review Panel Guideline 24: Consideration of high impact research projects by Animal Ethics Committees

References

Armario, A. (2021) The forced swim test: Historical, conceptual and methodological considerations and its relationship with individual behavioral traits, *Neuroscience & Biobehavioral Reviews, 128*, 74-86, https://doi.org/10.1016/j.neubiorev.2021.06.014.

Borsini, F., G, V., & A, M. (1986). Does the behavioral "despair" test measure "despair". *Physiology and Behavior, 38*, 385 - 386.

British Association for Psychopharmacology: Fact sheet on the forced swim test. (2020). https://www.bap.org.uk/pdfs/news_Forced_Swim_Test_Factsheet.pdf

Can, A., Dao, D. T., Arad, M., Terrillion, C. E., Piantadosi, S. C., & Gould, T. D. (2012, Jan 29). The mouse forced swim test. *J Vis Exp*(59), e3638. https://doi.org/10.3791/3638

Commons, K. G., Cholanians, A. B., Babb, J. A., & Ehlinger, D. G. (2017, May 17). The Rodent Forced Swim Test Measures Stress-Coping Strategy, Not Depression-like Behavior. *ACS Chem Neurosci, 8*(5), 955-960. https://doi.org/10.1021/acschemneuro.7b00042

de Kloet, E. R., & Molendijk, M. L. (2016). Coping with the Forced Swim Stressor: Towards Understanding an Adaptive Mechanism. *Neural Plast, 2016*, 6503162. https://doi.org/10.1155/2016/6503162

Ferreira, M. F., Castanheira, L., Sebastiao, A. M., & Telles-Correia, D. (2018). Depression Assessment in Clinical Trials and Pre-clinical Tests: A Critical Review. *Curr Top Med Chem, 18*(19), 1677-1703. https://doi.org/10.2174/1568026618666181115095920

Molendijk, M. L., & de Kloet, E. R. (2015, Dec). Immobility in the forced swim test is adaptive and does not reflect depression. *Psychoneuroendocrinology, 62*, 389-391. https://doi.org/10.1016/j.psyneuen.2015.08.028

Molendijk, M. L., & de Kloet, E. R. (2019, May 17). Coping with the forced swim stressor: Current state-of-the-art. *Behav Brain Res, 364*, 1-10. https://doi.org/10.1016/j.bbr.2019.02.005

Petit-Demouliere, B., Chenu, F., & Bourin, M. (2005, Jan). Forced swimming test in mice: a review of antidepressant activity. *Psychopharmacology (Berl), 177*(3), 245-255. https://doi.org/10.1007/s00213-004-2048-7

Porsolt, R. D., Le Pichon, M., & Jalfre, M. (1977). Depression: A new animal model sensitive to antidepressant treatments. *Nature, 266*, 730 - 732.

Slattery, D. A., & Cryan, J. F. (2012, May 3). Using the rat forced swim test to assess antidepressant-like activity in rodents. *Nat Protoc, 7*(6), 1009-1014. https://doi.org/10.1038/nprot.2012.044

Smalheiser, N. R., Graetz, E. E., Yu, Z., & Wang, J. (2021). Effect size, sample size and power of forced swim test assays in mice: Guidelines for investigators to optimize reproducibility. *PLoS One, 16*(2), e0243668. https://doi.org/10.1371/journal.pone.0243668

Trunnell, E. R., & Carvalho, C. (2021, Dec). The forced swim test has poor accuracy for identifying novel antidepressants. *Drug Discov Today, 26*(12), 2898-2904. https://doi.org/10.1016/j.drudis.2021.08.003

Acknowledgements

The Animal Research Review Panel acknowledges survey feedback from establishment AECs across NSW regarding the use of the forced swim test and other swimming procedures.

INT22/157139