National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

Position paper: Forced swim test (September 2024)

The UK's Animals in Science Committee has published guidance on the use of the forced swim test based on the review of 18 project licences for animal work and questionnaires of researchers and other stakeholders [1]. This paper sets out the NC3Rs' position on the test, its validity and what alternatives currently exist.

The NC3Rs position

We welcome this call to investigate alternatives to the use of the forced swim test as a screening tool for antidepressant drugs and a model of depression. Whilst the test has proved to be sensitive to a wide variety of existing antidepressant treatments, its selectivity has been in question for some time and its sensitivity to novel treatments that differ in their mode of action from earlier generations of antidepressant drugs has not been proven. The forced swim test is also not required for the development of new medicines, as our previous work helped clarify.

The use of the forced swim test as a model of depression, rather than a screening tool for antidepressant treatments, has been debated extensively. With no face validity and a number of different interpretations to explain the behaviour seen in this assay, the use of the forced swim test in this way is indefensible.

The forced swim test has remained in use due to a lack of validated alternatives. We have funded work into refined rodent tests for affective behaviours such as those based on cognitive bias as alternatives to the use of the forced swim test as well as supporting assays considered to be partial replacements because of their use of organisms that are not considered capable of suffering. Further work is also needed into non-animal assays that could be of use in this area.

Background

Summary

The forced swim test continues to be used as a screening tool for antidepressants. Whether it can detect drugs using novel mechanisms is yet to be fully elucidated but it has proved to have predictive validity in the case of currently used clinical drugs that work via the serotonergic and noradrenergic systems. Its predictive power is tempered, however, by known false positives where drugs of different classes have shown an antidepressant-like effect in the forced swim test despite not being used as antidepressants in the clinic. The results obtained are also influenced by multiple factors such as the sex and strain of the animals used and the temperature of the water, making drug effects more difficult to replicate across laboratories.

The forced swim test does not provide a reliable model of depression or any of its symptoms, in part because of conflicting explanations of the immobility behaviour that it the main measure of this test. Alternative measures such as sucrose preference may provide an alternative by focusing on symptoms such as anhedonia, also seen as a core symptom of depression, or measures of psychological processes disrupted by depression such as cognitive bias may also prove useful as screening tools. A wide variety of other symptoms associated with depression in humans, such as changes in weight, appetite and sleep, could easily be measured in a translatable way in animal models.

What is the forced swim test?

The forced swim test is a behavioural assay used in rats [2, 3] and mice [4, 5] that was first developed in the 1970s as a screen for antidepressant drugs. It involves placing rodents into a cylinder of water deep enough that the animals cannot touch the bottom, creating an inescapable aversive situation. If rodents stop swimming, the mouth and nose remain above the waterline, so they can remain immobile without drowning. This immobility is interpreted by some researchers as a measure of "behavioural despair" whilst others see it as an adaptive response to an inescapable situation [6]. Reducing this immobility and delaying the onset of the first bout of immobility are used as the main measures for an antidepressant effect in this test.

The precise methodology used for the forced swim test varies between research groups but commonly involves a test length of five or six minutes which may be preceded one day earlier by a 15-minute pre-exposure. This pre-exposure is more commonly used in rats [7] than mice [8]. Factors such as the circumference of the cylinder used [9], the sex and strain of the animals [e.g. 10] and temperature of the water [e.g. 11] can all impact the results obtained and may vary between studies.

What is it used for and is it reliable?

Antidepressant screening

The forced swim test was first developed as a screen for antidepressant treatments [2-5]. Despite a long association with antidepressant treatment, there is no regulatory requirement to perform the test but it may form part of the body of evidence supporting the passage of a compound into clinical trials [12]. Whilst the forced swim test has been effective in detecting antidepressant-like effects from existing clinical treatments it may not be selective for antidepressant drugs and may not be sensitive to detecting the effects of drugs that act in ways very different from those compounds used to initially validate it.

A wide variety of existing antidepressant treatments have been shown to effectively reduce immobility in the forced swim test, typically those relying on activity on the serotonergic or noradrenergic systems [see 13 for summary]. However, some antipsychotics [14] and anxiolytics [15] have also been shown to decrease immobility in this test, suggesting that false positives are a risk with this assay. This is in addition to a number of putative treatments being identified using the forced swim test only to show no effect in clinical trials [16, 17]. This has led to some questioning its ability to detect potential novel treatments that differ in their mode of action from more traditional antidepressant treatments [17]. Were a novel clinically-effective treatment found to be ineffective in the forced swim test, that is a false negative result, this would further damage the case for its predictive validity.

As the main measure for this test is a lack of locomotor activity, a drug's effect on general locomotion may be a confound and contribute to the rate of false positives. For example, psychostimulants have been seen to be effective in reducing immobility in the forced swim test [e.g. 18] which may represent a false positive. However, psychostimulants have been used in palliative care and the elderly, with or without more traditional antidepressants, and found to be effective in improving their mood [e.g. 19], complicating the view on what these results might mean for the validity of this test. Nonetheless, consideration of changes in general locomotion need to be considered when interpreting results from the forced swim test.

In contrast to the acute nature of assays such as the forced swim test, antidepressant treatments in humans are typically given chronically and may take some time to reach the peak of their clinical effectiveness [20], the recent discovery of ketamine as a rapid-onset treatment for otherwise intractable depression being a prominent exception to this [21-23]. More traditional antidepressants have been found to have acute effects in humans on related processes such as emotional processing [24], including in healthy volunteers [25, 26], so a predictive assay that is sensitive to acute treatments could still have some validity. That these effects can be seen in healthy volunteers also suggests that the use of "non-depressed" rodents in the forced swim test may not present a further limiting factor in the validity of this test [27].

Swim stress

The forced swim test is also used as a stressor by research groups interested in the response and resilience to stress [e.g. 28]. In these situations, a clear justification for the choice of swim stress over other forms of stress should be made and considerations of the length of the stress needed to reach the scientific goals of the study as recommended by the UK's Animals in Science Committee [1]. This includes both the length of individual sessions as well as the number of exposures to the stressor required for the study.

A number of interventions have been found to effectively increase stress-related markers in rodents such as levels of corticosterone, not only swim stress but also foot shock and restraint. These all reliably increase levels of acute corticosterone in mice, as does exposure to a predator, the scent of a predator or an acoustic startle cue [29]. Frequently these stressors are used repeatedly over a prolonged period to model chronic stress. In the case of restraint stress, this can involve restraint for multiple hours each day for several days [e.g. 30]. However, increases in corticosterone can be seen within 15 minutes following a single 15-minute session of restraint [29]. The choice of intervention should therefore be dependent on the scientific question and what data are required but it is essential that potential refinement opportunities are regularly reviewed and actively embedded into study plans to ensure impacts on animal welfare are kept to a minimum.

A model of depression

Some groups have relied on the forced swim test as a model of depression itself. Depression is a multifaceted human psychiatric disorder, characterised by a variety of symptoms which include lowered mood, anhedonia, loss of energy, changes in weight and appetite, changes in sleep, a difficulty in concentrating, and suicidal ideation [31, 32]. Whilst some of these symptoms have clear analogues in common laboratories species, accurately assessing mood is complex and may be impossible, and symptoms such as suicidal ideation are likely to always be beyond what can be modelled in non-human species.

In this context, immobility is seen as a measure of "behavioural despair" which in turn is seen as being related to lowered mood. However, this interpretation of immobility in this assay and the use of the forced swim test as a measure of depression has been controversial for some time [8, 33, but see 34 for a response] which has been reasserted in guidance from the UK's Animals in Science Committee [1].

This may reflect a wider debate about the role of serotonin in the pathology of depression; whilst many clinically effective antidepressants alter serotonergic function, the idea that alterations in the serotonin system underlie depression itself has been challenged [35]. However, some lines of evidence still suggest it has a role in the pathophysiology of the condition [e.g. 36] and the parsimonious view is that this multifaceted disease results from multiple factors. The forced swim test lacks the construct validity to tease out what might be the result of changes in individual neurotransmitter systems as well as the face validity to be easily compared to any of the symptoms that make up this disorder.

What other related behavioural tests are there?

The forced swim test has continued to be used as a behavioural screen for antidepressants and model of depression despite its questionable validity due to a lack of alternatives. The closely-related tail suspension test [37] has the same limited face validity as well as further complications such as the ability of C57Bl/6 mice to be able to climb up their own tails and thus not provide reliable data in this test [38].

Anhedonia is considered a core symptom of depression alongside lowered mood [31, 32]. This can be measured in rodents using assays such as sucrose preference [39]. This may provide an alternative to the use of the forced swim test by focusing on a different core symptom of depression, one that can be more directly translated to this complex human disorder.

Exploring endophenotypes seen in humans that relate to depression such as cognitive bias can also be tested in animals in a similar way to humans [40-42]. Such tests establish "good" and "bad" stimuli then present novel stimuli intermediate to these. For example, a high-pitched tone might be associated with a reward, a low-pitched tone with a negative outcome (or absence of reward), then a variety of tones that fall between these two are presented to see which are judged to be more likely to be positive and those more

likely to be negative. A negative cognitive bias is seen as a hallmark of depressive-like behaviour and a shift away from this would be expected from antidepressant treatment [e.g. 24, see 43 for a critique of the related idea of depressive realism]. This method also has the advantage of probing antidepressant action in a way that relates to modern neuropsychological theories on how they exert their effects in humans [44, 45] and has been found to be sensitive to both traditional antidepressants and the rapid-acting antidepressant ketamine whilst also distinguishing between these two modes of action [46].

Depression is a complex disorder and so will always be a challenge to model in experimental animals. The nature of the condition also means that any model will likely impact on the welfare of the animal. This needs to be minimised and reviewed regularly to ensure that the scientific goals are met without unduly impacting animal welfare. Continuing to be informed of the clinical picture and developments in the treatment of depression with also ensure that pre-clinical work continues to be relevant.

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