

THE INVALIDITY OF THE FORCED SWIM TEST

2025



Summary

The forced swim test (FST) involves placing a small animal in a situation where they fear drowning and using measurements of their behavior to make misguided assumptions about the animal's mood or the potential human efficacy of antidepressant compounds. The test is based on false assumptions, is not required, produces unreliable results, ignores important biochemical realities, is subject to experimental confounds, impedes exploration of human-relevant models, and is cruel to animals. Companies, universities, funding bodies, and legislators are increasingly ending their use and support of the FST. However, the test is still widely reported in scientific literature. This report details the myriad problems with the FST and provides stakeholders with recommendations for ending its use.

Development of the forced swim test

The FST also called the Porsolt Swim Test, has been used since at least the 1950s but was popularized in 1977 by Roger D. Porsolt as a potential method for screening antidepressant drugs. During this test, a small animal, typically a rat or mouse, is placed in a container of water with no way to escape or any place to rest out of the water. Naturally, the animal will spend some time swimming and trying to escape the stressful situation but will eventually become immobile and float. The experimenter records the time that the animal spends swimming and the time that they spend floating in the water. Sometimes, the swimming behavior is divided into two types: climbing behavior, in which the rodent attempts to climb up the sides of a tank or beaker, and swimming behavior, in which the rodent typically swims around but doesn't try to climb out of the container.

A similar test to the FST is the tail suspension test (TST), which operates on analogous principles. An animal (typically a mouse) is held upside down by the tail, typically affixed to a stationary bar or object with a piece of tape. For a while, the mouse will struggle and try to correct this frightening and uncomfortable position but eventually becomes immobile.

Porsolt found that when an experimenter acutely administers some commonly used antidepressant drugs to the animal prior to the FST or TST, the animal may swim (or struggle) for longer and spend less time floating (or remaining still) [1,2]. This was taken to mean that longer swimming times indicate a less "depressed" animal and that the antidepressant is what caused the behavior change. Animals who spent more time immobile were thought to be in "despair," as if they had "given up." However, this interpretation is incorrect for several reasons.

Is immobility a learned behavior?

Evidence suggests that immobility in the FST may be a learned or adaptive behavior, not one representing an internal state of despair. In some FST protocols, typically ones involving rats, the same animal is made to participate in the test more than once, usually before and after administration of a particular substance, so that the animal serves as their own control. In this case, immobility becomes a learned behavior. De Pablo et al. demonstrated that rats generally show less mobility on the second testing day than on the first day [3]. When a group of rats was administered anisomycin, a substance known to disrupt memory consolidation, the anisomycin-treated group stayed more active on the

second day of the test than rats who had not been given the drug, meaning that disrupting the learning process affects behavior during the FST. The untreated rats may have learned that there was no way to escape their situation and that they would eventually be removed from the water by the experimenter, facts that the anisomycin-treated rats did not learn as readily. The anisomycin did not affect the rats' behavior during the first day of the test.

Proponents of the idea that FST immobility is a reflection of behavioral despair equate the behavior to types exhibited in learned helplessness paradigms [4]. To create a state of learned helplessness, the classic experiment involves exposing an animal to a series of inescapable shocks. At first, the animal will actively look for ways to escape the shocks—but over time, they will exhibit fewer or no attempts to escape, even when provided with the means to do so. Experimenters say that these animals have “given up” and resigned themselves to the fate of being shocked.

When the same animal is subjected to the FST more than once, some think that prior exposure to the testing situation acts as a stressor for the animal and that increased immobility on later testing days reflects a sort of learned helplessness caused by the inescapable FST. However, experiments by O’Neil and Valentino showed that prior exposure to the FST had no effect on behavior in other stress paradigms, such as inescapable shock, and that allowing rats a means of escaping from the water container during the first FST didn’t affect their behavior on subsequent exposures [5]. Animals are still more immobile on later days—an observation that is inconsistent with learned helplessness paradigms. This further demonstrates that immobility in the FST may just as easily be interpreted as a learned behavior and not indicative of learned helplessness.

Is immobility an adaptive behavior?

Reviews by West as well as by Molendijk and de Kloet have explained that immobility in the FST is likely a beneficial behavior for these animals [4,6]. Swimming and climbing expend unnecessary energy, and animals who are quicker to realize this have a greater chance for survival in extended submerged situations. In experiments described by Nishimura et al., rats were forced to swim until they sank—as long as two hours. Experimenters found that the amount of time spent immobile within the first 15 minutes of the test predicted sinking—the rats who struggled longer were quicker to sink, while the rats who conserved their energy floated longer before sinking [7]. The experimenters noticed that rats who struggled and swam longer defecated more, potentially signifying increased fear in the “less adaptive” group.

Molendijk and de Kloet argue that the FST lacks essential forms of validity used to assess animal models of human diseases or conditions: construct and face validity [6]. Because the development of depression is a slow process, a test of 15 minutes or even tests conducted over 24 hours cannot be used to determine depression [8]; therefore, the FST lacks construct validity. The FST lacks face validity because “there is no single sign or symptom of depression modeled apart from the anthropomorphic interpretation of floating behavior in terms of despair”[6] and because there is “little similarity between the clinical symptoms of depression in humans and the behaviors measured in the test” [9].

Another way to interpret the potentially adaptive behavior of immobility during the FST is to consider that an animal's actions may represent their individual response to the stressor of being immersed in water, not knowing when or if escape will be possible. Some animals will cope with this situation actively by struggling, and some will cope passively by floating. Commons and colleagues write, "While it could be argued that passive coping strategies to stress are characteristic of depression, the connection between swimming and the human condition begs an abstraction at best. Behavior in the FST is a reaction to the acute stressful stimulus of being placed in a container without an escape route, and human depression reflects a chronic subjective emotional state rather than a reaction to an individual stimulus" [9].

Initial interpretations of the FST are at odds with biochemical reality

The methodology by which the FST was discovered causes doubt that immobility can be equated with "despair." Experimenters noted that acute administration of antidepressants decreased immobility; however, antidepressants do not work in humans to relieve depression when administered acutely. As noted by O'Leary and Cryan, "The FST and TST have been criticised because they are sensitive to acute treatment with an antidepressant drug, whereas several weeks or months of antidepressant treatment is required before a clinical response is reported. Because the inducing factor (acute stress of swimming or suspension) is intrinsically coupled with the readout (time spent immobile), these tests also muddy the water between definitions of test versus model" [10]. The acute immobility response of mice and rats to antidepressant treatment compared with repeated exposure required for humans to note antidepressant effects indicates that these drugs act on—and these types of behavior reflect—different mechanisms between species.

Some experimenters have shown that chronic treatment with the antidepressant fluoxetine also reduces immobility in mice [11]. However, the immobility response also occurs after treatment with drugs that are not used as antidepressants, such as antihistamines and other miscellaneous drugs [12], putting the entire premise on unstable ground. Newer, fast-acting antidepressants show varied and unreliable results in the FST [13–16].

In an analysis published in 2021, PETA neuroscientist Emily R. Trunnell and psychologist Constança Carvalho showed that the use of the FST by major pharmaceutical companies did not reliably predict whether experimental compounds would be effective in treating human depression or ultimately be marketed successfully as antidepressants. Of the 109 compounds identified as having been used in FST experiments by the top 15 most profitable pharmaceutical companies, less than a third were also explored in humans with depression, and, of these, there were only three compounds for which the FST appeared to positively predict antidepressant efficacy [17]. However, not one of the 109 compounds is currently approved to treat any form of depression.

Experimental and strain confounds

Experimental details such as water temperature and depth can alter an animal's behavior during the FST and potentially confound results. Jeffrys and Funder conducted an experiment to test whether water temperature influenced a rat's mobility. They found that when the water was 20°C, rats spent less time immobile and were slower to learn immobility behavior over the course of the experiment

(which included four exposures to the water tank) compared to when the water was 25 or 30°C [18]. A different outcome has been observed for mice, with immobility decreasing in warmer water [12,19].

The depth of water used by experimenters also influences FST results. In one study, placing rats in water with a depth of 35 cm increased swimming and decreased immobility compared to situations in which rats were placed in water with a depth of 15 cm [3]. Presumably, the rats could detect the bottom of the container with their tails at 15 cm.

Importantly, mice show different types of behavior in the FST depending on their strain. When comparing 11 commonly used strains of mice, Lucki and colleagues found that time spent immobile differed more than tenfold between the strain that swam the most and the one that swam the least [20]. Strains also differ in sensitivity to antidepressant drugs administered before the FST. Dulawa et al. noted strain differences in response to chronic fluoxetine treatment, where the drug regimen affected swimming and immobility times in BALB/c mice but not in three other strains, including the ubiquitous C57BL/6 mouse [11].

Other factors that may confound FST results include water cylinder diameter, the age and sex of the animals, the time of day at which the study was conducted, the availability of food, the animals' housing conditions, the laboratory environment, the visibility of other tested animals, and more [21,22]. The reality that these variables can alter FST results so dramatically and have the potential to confound interpretation further invalidates it as a reliable measure of despair or behavior in general.

The FST is used to draw false conclusions

The problem with misinterpretation of the FST is that it has led to a false assumption that it can be used to measure depression in animals. Frighteningly, it has sometimes led to the assumption that the FST can serve as the sole measure used in a study to describe an animal's mood and thus to make inferences about human mood. In a commentary in *Psychoneuroendocrinology*, Molendijk and de Kloet estimate that in the 4,300 papers reporting use of the FST at the time of publication, "[n]o less than [2,020] papers label the phenotype of the floating rodent as depression-like behavior—sometimes with a remark that the validity of the test is debated but often without discussion" [6]. Additionally, 7.5% of these (320 papers) had "used the FST to monitor the outcome of genetic manipulations of signaling pathways suspected to be involved in the precipitation of depression-like symptoms. Most of these studies (we estimate 70%) indeed infer a depression phenotype from the immobility response displayed by the rodent" [6]. In a 2019 follow-up to this analysis, Molendijk and de Kloet found that "the popularity of the FST [was] still increasing" [23] in the three years prior. Of the papers they analyzed, 72% qualified the behavior of a floating mouse or rat as "depressive-like, but without evidence for face, predictive, or construct validity" [23].

The FST in stress research

The use of the FST in stress research is on the rise [24]. It's clear that the FST is stressful, as demonstrated in the compilation video of the test here. When an experimenter places an animal into the water, the animal's stress is clearly visible, and they sometimes defecate in the water. However, the

FST should not be used to make inferences about stress in humans.

Humans typically experience stress due to the workplace, comparing themselves to others, health problems and access to care, national and world events, financial pressure, discrimination, and relationship issues [25]. These types of stressors, which are typically chronic, stand in stark contrast with the acute stress of potential drowning—something that, thankfully, few humans are forced to cope with in their lifetime. When acute, life-altering stressors do occur, they can result in post-traumatic stress disorder, which is difficult to assess in animals since many of the psychological symptoms, such as flashbacks, emotional numbness, and detachment, are not measurable. Many of the symptoms that experimenters can observe could be attributed to other types of mood disturbances [26] or species-specific factors entirely unrelated to stress or emotion.

The increased baseline stress levels experienced by animals held and used in laboratories further undermine the relevance of the FST for human stress research. Unnatural laboratory settings inherently do not meet the ethological needs of any animal and introduce confounding variables stemming from confinement-induced stress, undermining the value of the data collected from these animals.

Several specific factors contribute to baseline stress in the experimental setting:

- Experimenters keep mice and rats in unnaturally cold temperatures for the duration of their lives [27].
- Experimenters force animals to live in solitary confinement [28] inside small cages devoid of any meaningful enrichment [29], which, along with feeding them an unnatural and unvaried diet, impacts their metabolic health [30].
- Experimenters make animals perform complicated and distressing behavioral tasks at times that are biologically irrelevant to when they would normally be active [31,32].
- Abnormal behavior is common in animals in laboratories and is considered a direct result of living in that environment. Abnormal behavioral patterns have even been linked to long-term effects in abnormal physiological development and brain functioning, with some of these patterns thought to reflect permanent brain dysfunction [33].

These factors increase stress-related morbidity and mortality [34] and result in experiments being conducted on animals fundamentally different from their wild counterparts and even further removed from humans.

Philosopher and bioethicist Monika Piotrowska put it this way: “When rodents are stressed in the lab, all of them—as a genetically homogenous group—swim less and float more in the FST than rodents in the control group that have not been stressed. Hence, there is a significant disanalogy between the model rodent population and the target human population. If the rodent population were representative of the human population, only a small fraction of the stressed rodents would swim less and float more” [35].

Industry, academia, and funders abandon the FST

Following discussions with PETA scientists beginning in December 2018, 18 companies have committed not to conduct, commission, or fund the FST [36]. These are:

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| • Abbvie | • Creative Biolabs | • NutriFusion LLC |
| • Amgen | • DSM Nutritional Products | • Pfizer |
| • Astraea Therapeutics | • GSK | • Roche |
| • AstraZeneca | • Johnson & Johnson | • Sage Therapeutics |
| • Bayer | • Melior Discovery | • Sanofi |
| • Boehringer Ingelheim | • Novo Nordisk A/S | |
| • Bristol Myers Squibb | | |

Since January 2020, research universities have confirmed to PETA entities that they either stopped using the test or would not permit it. These include:

Australia	United Kingdom
La Trobe University Macquarie University University of Adelaide University of South Australia University of Western Australia	Exeter University Kings College London Newcastle University University of Bath University of Brighton University of Bristol University of Glasgow University of Leeds University of Liverpool University of Manchester University of Nottingham University of St. Andrews University of Southampton University of Warwick University of York
Colombia	
Universidad del Valle	
United States	
Berea College Butler University Hamilton College Marian University University of Indianapolis	

The following funding bodies and charities have also stated that they will not fund projects that include the FST or will place restrictions on its use:

Australia	United Kindom
Australian Research Council National Health and Medical Research Council	Brian M. Anselmo Foundation Medical Research Scotland The Dunhill Medical Trust

Regulatory opinions on the FST

No national or international bodies require the FST. The test has been discouraged by scientists with the U.K. Medicines and Healthcare products Regulatory Agency, who published a paper discussing the its irrelevancy to drug efficacy and the danger that its use could erroneously filter out potentially effective antidepressants [37]. The U.S. Food & Drug Administration has also confirmed that it does not require the FST for regulatory submissions [38].

In 2019, the European Medicines Agency’s Committee for Medicinal Products for Human Use issued its public assessment report on Spravato (esketamine), a recently approved antidepressant. The assessment revealed that no “animal models of depression” (a category including the FST) were performed by the applicant Janssen and that the agency agreed that “animal models of depression or antidepressant-sensitive behavioural tests are poorly predictive for the human situation” and “would not add further value to the overall assessment” [39].

In 2024, PETA scientists and collaborators published a paper in Regulatory Toxicology and Pharmacology recommending that the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) issue guidance against the use of the forced swim test for regulatory purposes, listing specific actions that ICH could take to address the continued use of this test by some pharmaceutical companies [40].

Regional and national policy changes

Australia and New Zealand

Thanks to legislation introduced by the Animal Justice Party and advanced by Animal-Free Science Advocacy, the FST is illegal in New South Wales as of 2024 [41].

In 2023, the New Zealand (Aotearoa) government research institute AgResearch, which oversees the use of animals in experimentation for more than 40 other institutions in the country, revised its code of conduct—a legally binding document—to state that its ethics committees “will not consider an application” that includes the forced swim test [42]. This development results from a campaign by the New Zealand Anti-Vivisection Society.

Elected officials in New Zealand have a low opinion of the FST and support a transition away from its use. In a report from a meeting in which the FST was reviewed, the government's Economic Development, Science and Innovation Committee discussed the disadvantages of the test and called an expert witness who commented on the FST's "ethical cost" and "lack of utility"[43]. The FST is now considered obsolete in New Zealand.

United Kingdom

In 2024, the Home Office stated its intention to eradicate the FST from the U.K. Lorde Sharpe, the parliamentary under secretary of state for the Home Office, accepted the recommendations set forth by the Animals in Science Committee in 2022, which means that the FST as a model of human depression or for studies of anxiety and its treatment in the country. Lord Sharpe stated his intent to go further and "completely eliminate the use of the forced swim test" [44].

What can be used instead of the FST?

It should be clear by this point that the FST does more harm than good. When a test produces unreliable and misleading data, it is cruel to animals, and there are no requirements to conduct it, ending use of the test is a no-brainer. While there is nothing to be gained from waiting for a one-to-one alternative to the FST to be validated, there is a significant need for better methods to predict antidepressant efficacy in humans. Some options may include assessing changes to BDNF and other neurotransmitters in human serum [37] or human brain organoids [45,46], using human induced pluripotent stem cells to test the functional effects of drugs on neurons from patients with depressive disorders,47 and making better use of human neuroimaging data [48].

Conclusions and recommendations

Animal experimentation has been cited as the primary source for attrition, or drug failure, in human neurobehavioral clinical trials [49], signaling that there is something fundamentally wrong with this field. For decades, experimenters have been subjecting mice, rats, and other small animals to a stressful procedure in which they are forced to swim in water with no way to escape. The FST has been used to make uninformed determinations about an animal's mood and to use these determinations to make potentially false inferences about biology related to human health.

The use of the FST has wasted public funds, animal lives, and research hours. The onus to correct this poor science is on several major players that can take action immediately:

- Institutional, regional, and national legislative and ethical bodies can prohibit specific animal tests like the FST.
- Regulators can issue guidance discouraging the FST from regulatory applications.
- Funders can reject proposals that use the FST.
- Journals can prevent spurious conclusions based on the FST or TST from being reported and circulated in the literature by rejecting or rigorously scrutinizing manuscripts including animal behavioral protocols.

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