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Time to Abolish the Forced Swim Test in Rats for Depression Research?

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Abstract

The forced swim test (FST) is a controversial rodent test that has been used for decades, mainly in depression studies. The severity of the procedure makes it ethically questionable and its validity has also been questioned. In this paper we contribute new data to this debate. We identified original research papers related to Major Depressive

Disorder (MDD), using rats as models. We compared the citations received by studies that used the FST and by studies that did not, within subsequent human medical papers. The results show that the number of citations received by both groups was very low, but in the papers describing the FST data the median citation number was zero. Citation analysis indicates that the FST is not contributing significantly to the understanding or cure of MDD. We briefly review other approaches that overcome the ethical limitations of the FST, and which might also surpass its efficacy.

Keywords

3Rs – depression – forced swim test – rat – *in vitro* – *in silico* – alternatives to animal use

1 Introduction

The forced swim test (FST) is a rodent behavioural test developed by Porsolt and colleagues in 1977. It was presented as a test to screen for antidepressants, as according to the authors the animals response to the test resembles depressive illness (Porsolt et al., 1977). The rationale behind this test is that when placing an animal in a vessel filled with water, it will first try to escape, but will eventually give up and exhibit immobility that may be a measure of behavioural despair (Yankelevitch-Yahav et al., 2015).

The FST is widely used as a tool to conduct basic biomedical research, mostly into depression (e.g., Gorlova et al., 2019), but also in other disorders. For example, it was used amongst other tests to verify whether a drug that is widely used to treat Attention Deficit Hyperactivity Disorder has any long-term behavioural effect after its chronic administration (Carias et al., 2019), or to measure differences in coping strategies in mice modelling anxiety, as part of a study aiming to determine the contribution of genetics and social environment in anxiety disorders (Chekmareva et al., 2019).

Over the years, the main use for the FST has been to screen for antidepressant properties of compounds. The merits of this protocol are disputed: a relatively recent meta-analysis of 50 antidepressant studies showed that all drugs tested reduce immobility in the FST (Kara et al., 2018). However, another recent retrospective study exploring how many possible antidepressants tested via the FST, successfully translated into human clinical trials, showed that out of 47 compounds, not even one was considered safe and/or effective as a human antidepressant (Trunnell, 2019).

Another common use for the FST is to study depression's aetiology, pathogenesis and possible behavioural treatments.

The FST attracted public attention relatively recently, after *Nature* published a paper exposing the controversies surrounding the efficacy of this test (Reardon, 2019). Most authors agree that one cannot conclude that a passive rat in the FST, is a depressed rat. Some emphasize that the FST can only be used as a measure of active versus passive behaviours (Commons et al., 2017). Others suggest that other variables such as water temperature (Linthorst et al., 2008) or previous knowledge (Molendijka & Lloet, 2019) may also influence results, and should be controlled.

The claims about the necessity of continuing to use the FST for the study of depression, given the availability of alternative tests, are theoretical at best, and lack empirical evidence. We hope to partly fill that gap with the present study, to enable more evidence-based discussions about the utility of the FST in rats.

If FST studies are contributing meaningfully to current knowledge of depression in humans, then our working hypothesis is that when this protocol is used to study major depressive disorder (MDD – the most severe depression type), the resultant published papers should be well cited by human research papers focused on MDD. To investigate whether this is actually true, we conducted a citation analysis on papers using rats as models within MDD research – either to test pharmacological or non-pharmacological therapies, or to understand possible causes for the disorder or differences in its biology/physiology. We compared the number of citations received by papers using the FST (either alone or in combination with other tests) to those using other test protocols.

Of course, citation frequencies do not definitively indicate the benefits or lack thereof of scientific research. Uncited studies may also contribute to the advancement of biomedical knowledge, through direct transfer of results between scientists. And citation rates may also be affected by factors such as article length, number of authors, their country and university of affiliation (Leimu & Koricheva, 2005). Despite their limitations however, citation frequencies do normally provide reasonably objective approximation of the importance of research results within a field. Research that makes a significant contribution – such as by confirming or refusing important hypotheses – is likely to be cited by subsequent papers. Research that is inconclusive or lacking in significance is much less likely to be cited.

2 Methods

We performed our citation analysis between January and August of 2019 (for more details on the citation analysis applied, see Carvalho et al., 2020). We searched the biomedical bibliographic databases PubMed and SCOPUS for publications using rat models to explore MDD's traits and pathogenesis.

We searched PubMed using Medical Subject Heading (MeSH) search terms: "Depressive Disorder, Major" AND "rat" OR "rodent". MeSH terms are a comprehensive list of key terms made available by PubMed, which are designed to identify all relevant studies in an area (Uman, 2011). Hence, searching for "Major Depressive Disorder" retrieves other nomenclatures for the same disorder, such as melancholia. Likewise, the search term "rat" retrieves papers using rats in general. Using PubMed filters we excluded review articles ("review", "systematic review", "meta-analysis", "bibliography") as well as opinion articles ("biography", "auto-biography", "comment", "editorial", "interview"). Since Scopus does not have the MeSH term tool, we used the search terms "Major depressive disorder" AND ("rat" OR "rattus") in the search fields. We included journal papers, books, research reports and conference proceedings written in English or Portuguese, which are within our linguistic fluencies. We restricted our search to publications prior to December 31, 2013, to allow adequate time (at least five years) for citations to occur. We then excluded all papers reporting both animal and human data, so that we could more accurately compare the contribution of papers using the FST with the citations of other papers presenting animal data.

The retrieved papers were divided into papers that used the FST (alone or combined with other behavioural tests), and those using other protocols (whether individually, or combined with additional protocols). We conducted a citation analysis on both groups, using the cited reference search facility within Scopus and Web of Science. More specifically, we determined the number of citations these papers received by human medical papers focused on MDD.

We modelled the number of citations received by papers as the response in a Poisson GLM with a single binary variable being method ("FST" or "other"). A significant coefficient (significance level 0.01) was interpreted as a significant difference in the number of citations between the different methods.

3 Results

We located 178 papers reporting original data obtained from rats used as models for human MDD. Of those, 43 used the FST, and 135 did not. In both cases, citations by human medical papers focused on MDD were low: almost half of the papers were never cited by subsequent human papers on MDD (Fig. 1).

The mean number of citations by the human MDD literature for papers using the FST was 1.4, whilst for papers using other test methods, this was 2.4. Remarkably, the median number of citations was 0 for the former, while it reached 1 for the latter. This means that if you produce a paper using the FST the probability of it never being cited by the human MDD literature is higher than the probability of it being cited.

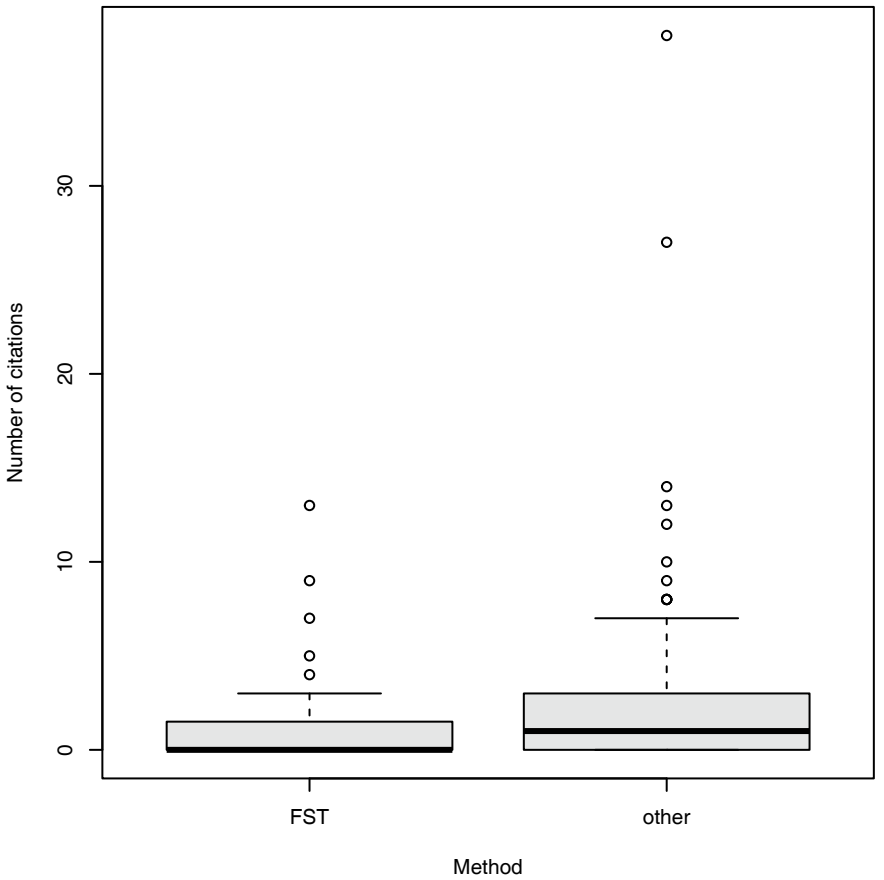

FIGURE 1 Citations received by papers using the FST, and other test methods, respectively

4 Discussion

Our results show that papers that used rats within the FST are generally not cited by human papers on MDD: the median number of citations was 0, while for papers using other test methods, the median number of citations was 1.

Even though citation analysis does not conclusively indicate the value of research for the reasons described previously, our results strongly indicate that current reliance on the FST – at least when applied to rats – not only consumes valuable scientific resources, with a low likelihood of benefit for the understanding and ultimately the cure of human MDD, but it also submits animals to stressful procedures with low prospects of any benefit. This contravenes most applicable ethical review guidelines, including Directive 2010/63/EU on the protection of animals used for scientific purposes.

Animal-free, human biology-based approaches appear more promising when studying MDD and other neuropsychiatric disorders. These include the use of patient-specific, induced pluripotent stem cell (iPSC)-based tissue cultures (Haggarty et al., 2016; Vadodaria et al., 2016; Xu et al., 2016; Wang et al., 2020), neuroimaging to identify relevant biomarkers (neuromarkers) and employ them in clinical assessments (Jollans & Whelan, 2018), and *in silico* modeling of psychiatric disorders, Computational Psychiatry, which describes in computational terms the relationship between the neurobiology of the brain, its environment and mental symptoms (Adams et al., 2016). A major obstacle to the study of brain disorders is that brain tissue of living patients cannot be biopsied, and limited access to diseased tissues makes it difficult to research disease aetiology and to find effective treatments. In the past decade however, the iPSC technology, first developed by Takahashi and Yamanaka (2006), has been used to further research into a number of neuropsychiatric illnesses such as schizophrenia (e.g., Brennand et al., 2011; Larijani et al., 2021), bipolar disorder (Madison et al., 2015), and depression (Vadadoria et al., 2016; Xu et al., 2016). Disrupted serotonergic neurotransmission is expected to be involved in MDD. Human serotonergic neurons can be differentiated from fibroblasts (Vadadoria et al., 2016) or directly converted from fibroblasts (Xu et al., 2016). Selective serotonin reuptake inhibitors (SSRIs) are the first treatment option for MDD but not all patients respond to this treatment. Thus, Vadodaria and colleagues (2019) used this new patient-specific *in vitro* model system to investigate the SSRI resistance of some patients. Their results indicate that altered serotonergic wiring may give rise to maladaptive circuitry which contributes to SSRI resistance in MDD patients (Vadadoria et al., 2019). Non-invasive neuroimaging has been used to identify relevant neuromarkers, that represent risk factors, or indicators of disease progression or of treatment-associated changes, for the various psychiatric illnesses. This approach demands the use of machine learning analysis, and the adoption of regression-type approaches (Jollans et al., 2018). Computational models of cognition are promising new tools that simulate how the brain infers the state of the environment, to assess possible future actions (Adams et al., 2016). Computational Psychiatry has been described as a bridge from neuroscience to clinical applications (Huys et al., 2016). With the availability of high-capacity computing platforms and information generated by basic neuroscience research, including the promising use of patient-derived cell models, this human biologically based computational framework is a powerful research tool (Siekmeier, 2015). Neuromarkers can be identified and validated more easily using *in silico* modeling, and novel treatment targets determined, relevant to the prevention, treatment and recovery from relevant to prevent, treat, and recover from

psychiatric disorders can be determined. These human biology-based models demonstrate a clear advantage over traditional animal models as they recreate key aspects of human disease, increase our understanding of the underlying disease mechanisms, and thus, can assist in finding new, effective treatments.

We would also like to stress the critical contribution of non-invasive studies with humans. Randomized controlled trials (RCTs), observational longitudinal studies with human patients, studies comparing effectiveness of different courses of treatment (for a review of different kinds of human studies, see, for example Carvalho et al., 2020), are crucial to advance our understanding of the aetiology, pathogenesis and treatment of these disorders.

Based on these results, we propose a ban on the use of the FST in rats. If it is of such limited use for its main purpose – studies on depression – we cannot assume it provides significant benefit for the study and treatment of other human disorders. It would be interesting to conduct a wider citation analysis of published papers utilising the FST, to determine whether similar results would occur for other species (e.g., mice), and for other human disorders. Should this be the case, the FST should be totally banned.

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