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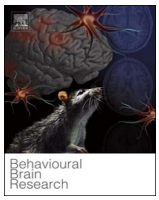

Review

Animals, anxiety, and anxiety disorders: How to measure anxiety in rodents and why☆

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ABSTRACT

Measurement of anxiety is desirable for the benefit of drug development and understanding the brain function and mental well-being. Animal models offer the advantages of detailed neurobiological analysis, experimental manipulation of specific components in the brain circuits that underlie psychopathology, and the possibility of screening novel drugs with clinical potential. A large variety of animal models of anxiety and screening tests of anxiolytics is currently in use. While their value in advancing the knowledge and predicting therapeutic success of drugs is unquestionable, the expectations have grown much higher, and the frustration over absence of novel successful drug concepts is rising. It is argued that the multitude of factors that can interfere with animal behaviour in anxiety tests, and the complexity of neurobiology of the various anxiety disorders, present high demands on validation of each anxiety test within each specific laboratory condition. Anxiety models should be explicitly related to a theoretical paradigm on underlying neurobiology, because there is a diversity in concepts, and validation of the model and the selection of behavioural readouts is critically dependent on the neurobiological model. Environmental conditions during the model production and anxiety testing need more attention, including the less considered factors such as ultrasounds. More attention is required to the differences in anxiety neurobiology between males and females, and inter-individual differences in coping strategies.

1. Introduction

Only little effort is needed to find an explanation why anxiety measurement in animal models is of paramount importance: Anxiety disorders are highly prevalent throughout adulthood [1], have an early onset [2], affect a significant proportion of young adults [1], remain an enormous burden on health care resources [3], and are co-morbid with a variety of medical conditions including neurological and cardiovascular illnesses, with resultant major reduction in quality of life [4]. Furthermore, anxiety is a component in a variety of diseases [5], appears to be associated with accelerated aging [6], current treatment options are perceived as inadequate [7], and our understanding of the pathogenesis is thought of as far from sufficient [8,9]. Owing to the high costs of clinical trials, and especially so with regard to the CNS therapeutics [10], preclinical models of the disorder and drug screening tests that would assure of the relevance of the selected molecular target, or predict clinical efficacy of the substance in development, are of obvious significance.

Consistently with the importance of the theme, many overviews of anxiety tests and anxiety modelling have been published. The reader is suggested to consult with Cryan and Sweeney [9] for a recent comprehensive overview of available tests, as well as other important recent reviews, each with somewhat different focus [11–14]. These treatises present the state-of-the-art while having somewhat different emphasis, and discuss the pertinent issue of predictive, face, convergent, etiological, construct and population validity, eventually concluding that animal models are indispensable in psychiatric research, that much progress has been made, but that much more has been desired. Furthermore, the issue of increasingly uncritical use of the animal tests in attempt to “translate” complex neurobiology and its consequences between species has been clearly raised [15]. This uncritical use may however have real-life incentives, as Georg Wilhelm Friedrich Hegel would have reminded us with his words: “What is reasonable is real; that which is real is reasonable.” [16]. Indeed, a recognition is due that while in the ideal world we pursue pertinent research questions with persistence guided by the best of all accumulated knowledge, in reality modern research is highly fragmented by the temporary nature of grants, student projects, post-doc jobs, increasing administrative burden on senior investigators, the lure of emergent technologies, changes in priorities of the institutions, and else. The resultant shift

☆ Dedicated to the memory of Jaak Panksepp (1943–2017).

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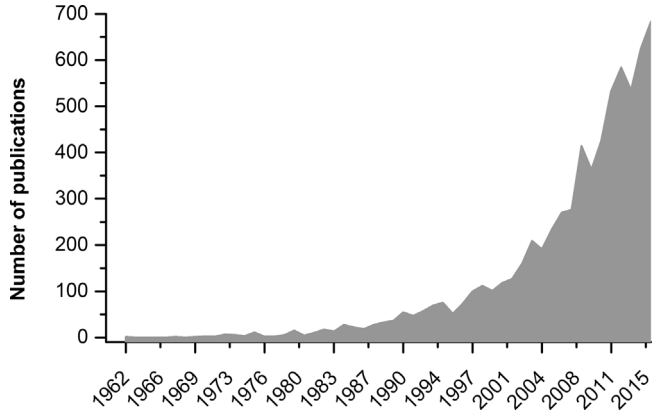

Fig. 1. Annual output of anxiety research in animals: The number of items in PubMed database from the first item in 1962–2015, by using search term "anxiety animal model".

towards short-term goals and the overwhelming increase in the amount of scientific information, including that on anxiety tests and models in animals (Fig. 1) are together implicitly guiding the efforts of research towards what seem to be the low-hanging fruit. Alas, some of the fruits can be part of a large-scale self-created mirage, as evidenced by the fact that anxiolytic drugs with any novel mechanism of action have been slow to emerge, while this is not owing to shortage of good intention. As it has been succinctly put in a characterization of the historical development in the field: "It is somewhat ironic that as the tests employed became more sophisticated, the development of anxiolytic drugs has not greatly increased" [9].

Given the sheer volume of publications on anxiety testing and all the complexity of findings in the available literature, it is likely that a newcomer to the field increasingly feels pressed toward the realization that there is too much of previous thoughts and practice to consider, and the best is to just start experimenting right away. With this recognition in mind, this article will first 1) present a condensed overview of the often used animal tests of anxiety, providing historical and current key references and including a few field notes that may be relevant to further discussion and for testing refinement; and only then 2) discuss conceptual issues that arise in development of tests of anxiety and models of human anxiety disorders; 3) focus on potential sources of failure and success in using animal models; and 4) consider some recent issues in model development such as sex, genetic background, inter-individual differences, environmental factors etc that have the potential of either confound or, if properly addressed, enrich further studies on anxiety. While recognizing that research on anxiety, screening for anxiolytics and indeed attempts to understand the whole spectrum of psychiatry is being conducted on many different species, and increasingly on "simpler" organisms such zebrafish [17,18] this paper will heavily rely on rodents not only owing to the fact that most of the literature published to date has been dealing with rats, mice and other members of this order (that comprises about 40% of all mammal species) but also because with inclusion of the potential of the whole animal kingdom, the conceptual issues as discussed below would further broaden to unnecessary extent.

2. Major animal tests and models of anxiety

2.1. Tests and models

In the closely related field of depression models much discussion has been directed at the need to distinguish models of depression and antidepressant screening tests. Curiously, while reading the anxiety literature it appears that such a discussion on distinction between drug screens and anxiety models is largely absent. Could it be owing to the perception that anxiety is a way simpler phenomenon and better reflected in a single dimension, or because of the realism of the anxiety researchers who have observed that studies on depression, more often than not, stubbornly refuse to accept the criticism on over-interpretation of the findings in screening tests? What may be commonly assumed but rarely made explicit is the logic that the difference between tests and models lies in the way how anxiety is brought about: In anxiety tests, the assumption is relatively similar anxiety level in all animals before the experiment; in anxiety models, some organisms are known – or thought of – in advance as having persistently higher anxiety owing to genetic background, developmental factors, or adverse environmental events.

Obviously, anxiety tests must be used to make the assessment of anxiety in anxiety models, and if testing itself would produce lasting changes in the brain that will lead to persistent expression of anxiety upon repeated measurement, we have arrived at transforming a test into a model of anxiety.

2.1.1. Tests for the measurement of anxiety

2.1.1.1. Geller-Seifter test. Geller-Seifter test is the prototypical "conflict" test that provides an animal the option to obtain food when hungry by pressing a lever that can also elicit electric footshock [19]. Hence, the response rate to food is inhibited by response-contingent punishment. This test was found to predict clinical efficacy of anxiolytic drugs and further refined by introducing incremental shock levels [20]. The Geller-Seifter test excellently predicts not only clinical efficacy but also the clinically effective dose among benzodiazepines and barbiturates. Under standard conditions it nevertheless has low sensitivity for other anxiolytic treatments. The Geller-Seifter test requires training of the animals and can be affected by other effects of the drug on motivations, such as an analgesic or orexigenic action. These are accounted for by measuring drug action during time slots for unpunished responding, but it should be noted that responding under conflict situation is usually proportionally very much lower, and hence the impact of strong motivational effects (e.g., orexigenic) may not be entirely under control.

2.1.1.2. Vogel test. Another conflict test developed for anxiolytic screening, the Vogel water-lick suppression test, uses thirst motivation instead of hunger [21]. Here, less training is needed as compared to the Geller-Seifter test, but sensitivity to pain and the potential analgesic effect of drugs remain to be controlled for. Numerous procedural variations exist of the Vogel test and have been discussed in detail [22].

The Geller-Seifter, Vogel, and other classic tests involving training of and learning by the animals are time-consuming and have appeared to be less sensitive to systemic administration of drugs other than those acting directly on molecular targets in the GABA-ergic system. For these reasons it is only natural that many attempts have been made to devise principally different and apparently more simple methods. Spontaneous behaviour based tests have been spearheaded by the elevated plus-maze test and the redefinition of the open field paradigm.

2.1.1.3. Open field test. In this context, open field is meant to be a circular or rectangular well-lit arena that is several times larger than the home-cage and is supposed to be novel, strange and mildly aversive [23]. What is measured is the locomotion in terms of the number of squares crossed and rearings, and several refinements made to the open field test pay particular attention to more central areas of the arena and quantitate freezing. The open field test principle has been used to assess anxiety in an amazing variety of living creatures such as rats, mice, gerbils, hamsters, ferrets, foxes, dogs, cattle, sheep, pigs, chicken, quail, and several species of fish [24]. This has however not always been meant to study anxiety in terms translatable to contemporary psychiatry. Indeed, the PubMed database reveals very few references to explicitly anxiety-related open field research until the 90ies (Fig. 2; see 3.2.1). Of course the open field test had been around before that,