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Review

Tests of unconditioned anxiety — Pitfalls and disappointments

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HIGHLIGHTS

- Review of the discriminant validity of the current tests of unconditioned anxiety
- Issue with translation of the operational definition of anxiety into behavior tests
- There is no concordance between spatiotemporal and ethological parameters
- Pharmacology is neither sufficient nor necessary in the validation of behavioral tests
- Novel open space anxiety tests are proposed as alternatives to the current one.

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ABSTRACT

The plus-maze, the light–dark box and the open-field are the main current tests of unconditioned anxiety for mice and rats. Despite their disappointing achievements, they remain as popular as ever and seem to play an important role in an ever-growing demand for behavioral phenotyping and drug screening. Numerous reviews have repeatedly reported their lack of consistency and reliability but they failed to address the core question of whether these tests do provide unequivocal measures of fear-induced anxiety, that these measurements are not confused with measures of fear-induced avoidance or natural preference responses — i.e. discriminant validity. In the present report, I examined numerous issues that undermine the validity of the current tests, and I highlighted various flaws in the aspects of these tests and the methodologies pursued. This report concludes that the evidence in support of the validity of the plus-maze, the light/dark box and the open-field as anxiety tests is poor and methodologically questionable.

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1. Introduction

The elevated plus-maze (EPM) or zero-maze (EZM), the light–dark box (LDB) and the open-field (OF) are the main current tests of unconditioned anxiety for mice and rats. They are all intensively used, particularly the EPM, in the study of the neurobiological basis of anxiety and in screening for novel targets and anxiolytic compounds. The validity of these tests has been questioned in numerous reports [29,45,51,64,86,101,183,314,329,321,390]. However, these tests, in particular the EPM, are considered very popular and elected as the reference standard for their sensitivity to benzodiazepines. Hence, introduction of a novel methodology and approach is systematically rejected if it does not include one of these tests for comparisons. But, do these tests really measure the construct of anxiety, and not something else? What standard of reference status do they provide for enforcing their comparisons with novel alternative tests? Does pharmacological validity have any relevance to the construct validity of a behavioral test?

In this review, I will examine various aspects of the current tests of unconditioned anxiety and highlight the numerous issues that in my view undermine the validity of these tests. The review is divided in two major parts; one concerns the spatio-temporal parameters of these tests and the second part concerns the ethological parameters. The lack of concordance between these two and the ambiguity in the interpretations of the observed animal responses are discussed.

I hope that this critical assessment will initiate a constructive debate about the process of validation of behavioral tasks in animal studies. I would like to emphasize here that my concern is not whether or not animals experience anxiety in the EPM, the LDB and the OF. It is likely that they do. My concern is whether these tests provide unequivocal measures of anxiety.

2. Definitions of fear and anxiety

Fear and anxiety are “overlapping, aversive, activated states centered on threat” [296]. The distinction between the two has been difficult and controversial [80,427] due to their overlapping nature. Fear and anxiety have been considered to refer to the same [256,370,428] or different constructs [23,68,152,253,305,319,323] or regarded as parts of the same continuum [128,232,288,354].

Generally, fear is defined as a negative emotional state associated with the perception of imminent or present threat to wellbeing or survival. It is a defensive reaction that motivates and/or facilitates the detection, escape, and avoidance of impending identifiable danger. Anxiety, on the other hand, is defined as a negative emotional state associated with the perception of potential or ambiguous threat. Like fear, it is a defensive reaction, but is characterized by a feeling of apprehension, uncertainty, worries, uneasiness or tension stemming from the anticipation of potential threat or negative outcomes [23,98,152,295,296]. Hence, in fear conditions, humans and animals face an unambiguous situation; they can avoid the threatening stimulus or escape to safety. The aversive stimulus does not carry a positive incentive that diminishes or moderates the need to avoid or escape. However, in anxiety conditions, humans and animals face an ambiguous situation. They are unable to avoid/escape or approach the perceived threat stimulus [but see, Sections 4 and 11]. They experience a high level of uncertainty and unpredictability as the threat stimulus appears to be associated with both positive and negative outcomes [248,342].

3. Issue with aversion, natural preference, conflict and security

The EPM consists of four arms radiating from a central platform forming a plus sign shape; it is elevated from the ground with two opposed walled arms and two opposed open arms [168]. The EZM is a modification of the EPM. It consists of a circular runway divided in two enclosed quadrants opposite to two open quadrants [359,409]. The LDB consists of two chambers one lit and the other dark connected through a small opening or a tunnel [19,87,175]. The OF consists of either a cylindrical, rectangular or a square box with open top [54,163,412]. In all these three tests, animals seem to avoid the open and/or lit space of the open arms of the EPM, the lit chamber of the LDB and the central area of the OF. This avoidance response or “natural aversion” [242] is used as an indicator of anxiety in animals. It is based on the assumption that anxiety involves a conflict between the drive to avoid and the drive to explore a perceived threatening stimulus (i.e. an open space and/or a lit area of a test apparatus), and that the current tests set into play these conflicting drives [46,90,169,281,346]. However, one can also view that animals demonstrate a “natural preference” for dark and/or protected spaces [177,261,272,279,355,389,413], or that such preference optimizes safety and security [12,272,290,414]. Animals are offered a choice between aversive and non-aversive stimuli, and they choose the latter; they are not compelled to venture into the open and/or lit space. Animal scientists appear to hold a paradoxical attitude. They recognize that only the open and/or lit space is anxiogenic while at the same time attributing anxiety to animals that naturally prefer and choose the protected and/or unlit space. The present paradox arises from a confusion between a conflict that emerges from a visual contrast formed by two physical entities presented opposite or side by side (i.e. open vs. enclosed, light vs. dark, white vs. black) and a conflict that results from the action of two opposite drives (i.e. approach vs. escape or avoidance). In the current tests of unconditioned anxiety, there is no evidence of conflicting drives, neither in the case of entry into the protected/unlit space nor in the case of avoidance of the unprotected/lit space. For a conflict to occur, each available choice option needs to