Behavioural Brain Research 207 (2010) 84–98

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Distinguishing anxiolysis and hyperactivity in an open space behavioral test

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ARTICLE INFO

Article history:
Received 5 August 2009
Received in revised form
21 September 2009
Accepted 25 September 2009
Available online 2 October 2009

Keywords:
Novelty
Habituation
Fear
Avoidance
Benzodiazepines
GABAA

ABSTRACT

This report describes the emotional responses of mice exposed to an unfamiliar elevated platform that is extended on two opposite sides by downward lowered steep slopes. Balb/c mice were exposed to the test for 12 min per session in 3 successive days. They received i.p. administration of diazepam (0, 0.5, 1 and 3 mg/kg) or amphetamine (0, 1, 2.5, 5 and 10 mg/kg) 30 min prior to test sessions. Separate groups of Balb/c mice were used for each dose of the drugs.

Both drugs increased the number of crossings on the platform, indicating increased motor activity, and the effects were dose-dependent. Diazepam also significantly increased the number and duration of entries onto the slopes indicating an anxiolytic effect, whereas none of the saline or amphetamine-treated mice adventured onto the slopes. Amphetamine and diazepam produced an inverted U-shaped dose–response effect on different parameters of the test and demonstrate that the drug concentration which elicited a peak in mean number of entries is different from the drug concentration which elicited a peak in mean duration of entries. This study demonstrates the sensitivity and discriminatory power of an open space anxiety test for future pharmacological studies.

1. Introduction

Anxiety is an emotional state represented by a feeling of worry, apprehension, nervousness, or unease, typically about an impending or anticipated event with an uncertain outcome, and by self-doubt about one's capacity to cope with it. It can be accompanied by marked physiological signs such as sweating, tension, and increased cardiac pulse rate.

A definition of anxiety has been proposed to facilitate the experimental study of anxiety in both humans and animals. Gray and McNaughton [31] suggested that anxiety results from a conflict between the drive to avoid or escape and the drive to explore the source of threat. They also distinguish between fear which induces or leads to anxiety responses and fear which induces or lead to escape or avoidance responses. It is therefore very important that anxiety is not confused with escape or avoidance responses. When there is an opportunity to escape or avoid, anxiety may not be expressed, or it dissipates rapidly unless the need to explore the source of threat remains pressing or pending.

Numerous behavioral tests have been developed for assessing anxiety in animals [9,10,15,30,33,42,45,50,61]. Most of these tests are based on measures of an unconditioned response to novelty in a spatial environment. However, anxiety can be expressed in various behavioral settings. It can be expressed in a plus-maze, a T-maze and an open-field as well as in a Skinner-box, in a shuttle-box, in a radial-maze, or in a water maze. Not all of these tests, and possibly none of these, may provide specific measures of anxiety. The main issue here is not whether a behavioral test involves anxiety but how a behavioral test can provide specific quantitative measures of anxiety that are not confounded or confused with measures of other behavioral responses to the experimental settings of a test (construct validity) and that these measures can be clearly discriminated from measures of escape or avoidance responses (discriminant validity).

We have argued that animals may experience anxiety when introduced for the first time to the plus-maze, the light dark box and the open-field but what is recorded and measured in these tests are escape and/or avoidance responses [20–22]. In fact, numerous authors clearly indicate that these tests do measure escape and avoidance responses but all of the same authors seem to argue that these measures are specific indicators of anxiety [7,29,42,48,50,55]. It is not possible to infer from the observed behavior in a plus-maze, a light dark box and an open-field that animals experience a conflict between the drive to explore and the drive to avoid or escape. Animals discover very quickly that they have a choice between two alternatives and they show preference for a protected space than for an open space. There are no explicit responses that can be measured which reflect unequivocally and without ambiguity the desire of animals to explore the unprotected space, or that animals are indeed anxious in the protective space. The

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doi:10.1016/j.bbr.2009.09.042

view that animals express a drive to explore an unprotected space remains speculative. To compensate for this flaw, many scientists now rely on ethological parameters [5,6,57,58] which make these tests redundant and defy the purpose of the development of an anxiety behavioral test for animals. A behavioral test needs to be self-contained, self-sufficient. Why would one need a specifically designed apparatus if ethological responses can be obtained in any unfamiliar environment, for instance in an animal cage? Furthermore, these ethological parameters remain ill-defined and ambiguous [6,34,70]. For instance, stretched-attended postures and rearing can be seen in well-habituated animals performing a working memory task in a radial-maze and in a T-maze. It is difficult to determine how and when stretched-attend postures are different from vicarious-trials-and-errors. In addition, rearing and possibly circling in an enclosure may be due to the need of animals to access external visual cues that are obstructed by walls; rearing is hardly observed on an elevated platform. It was also found that risk assessment behavior represents a behavioral dimension independent from anxiety [11,47,59]. Furthermore, Weiss et al. [70] report that ethological measures were unable to dissociate between "anxiety reduction and exploration enhancement, or allow definitive conclusions to be drawn regarding the mechanism by which either a benzodiazepine or amphetamine enhance open-arm exploration".

In order to resolve this issue, we proposed recently two open space behavioral tests of anxiety. In these tests, animals are introduced for a first time to an elevated platform [20,21] or to a radial-maze [22,24] where a protected space is not available to avoid from or to escape to. In these conditions, at any one time, an approach to one place can be considered an escape from or avoidance of another place. The same response can be considered as an approach, an escape or an avoidance response at the same time. The drive to escape/avoid is confounded with the drive to explore; i.e. both cannot be dissociated. It is this un-dissociable expression of approach and escape or avoidance responses that would define anxiety in both humans and animals. We do not agree with the view that discriminates between fear and anxiety [27,38,40,41,43,49,51,54,67]; this distinction implies that there is no fear component in anxiety. Such a view is misleading and it deflects attention from the distinction between fear-induced anxiety and fear-induced escape or avoidance. The distinction should be made between anxiety and escape or avoidance responses; fear is a common denominator. There is an urgent need for a novel conceptualization of the various emotional responses devoid from cultural and/or metaphysical viewpoints and readily adaptable for experimental investigation.

We suggested that anxiety is heavily expressed by animals on a large open elevated platform [20,21], however it was difficult in this setting to define a parameter of the test that would determine a state of anxiety and discriminate between individuals or groups of animals. The presence of anxiety could only be inferred from its interference on memory performance when animals had to discriminate between a novel and a familiar object on an unfamiliar elevated platform [20]. In the present report, we propose an improved version of the elevated platform, now extended on two opposite sides with steep downward slopes. In this apparatus, all animals would experience anxiety but those that are less anxious are expected to adventure onto the slopes. The criterion for the measure of anxiety in animals is based on the crossings from the platform onto a slope. This criterion is similar to the one we used in our 3D maze test of anxiety [22,24]. Animals that crossed from a bridge onto an arm were considered less anxious than those who stopped on the bridges. In the present test, anxious animals may adventure onto the edges of the platform and spend longer time there but those that are less anxious are expected to enter onto the slopes. We expect also that a drug with specific anxiolytic properties rather than one which increases locomotor activity and exploration would facilitate the entry onto the slopes. The two drugs of reference that we used for this purpose are diazepam and amphetamine. Diazepam is the main stream anxiolytic compound and gold-standard in drug discovery of novel drug targets to treat anxiety [46,59,60]. Amphetamine is a psychostimulant; it has been reported to increase motor activity or exploration which has been confused with a reduction of anxiety [13,70]. The effects of these drugs were examined on the behavior of Balb/c mice. This strain of mice was shown to be more anxious than CD-1 (also albino though outbred strain) and c57/BL6 (pigmented inbred strain) mice in the 3D maze [22,24] and in this new test [23], and therefore they are more appropriate for detecting the anxiolytic effects of a drug compared to CD1-1 or c57 mice which might be unaffected or possibly impaired as their anxiety is at a floor level. We performed two separate experiments, one with different doses of amphetamine and the other with different doses of diazepam to assess the detailed pharmacological effects of these drugs in this new test. We used a large number of spatio-temporal parameters that would describe the behavior of mice in the test and would account for differences between drugs and dose–responses. The description of this set of parameters is justified by the fact that a novel behavioral test needs to demonstrate content validity, that these parameters can account for specific and non-specific effects of experimental manipulations, and that the test is self-sufficient, self-contained and does not need to be complemented with other tests or ethological observations.

2. Methods

2.1. Animals

74 male Balb/c AnNCrl mice obtained from Charles River (UK) were used in the experiments described in this report. They were 56–62 days old at the date of arrival and were left to acclimatize to local laboratory conditions for 2 weeks. They were housed in colony room that was held under a 12 h light/12 h dark cycle (light 07:00–19:00 h at 180 lx) and at 23 ± 1 °C. In order to avoid unequal light exposure, the upper shelf was occupied with plastic cages filled with clean sawdust. Mice were housed in a group of 5 mice per cage. Individual mice could be identified by their cage number and their ear tag code. All mice had *ad libitum* access to food and water. During their stay they were removed twice a week from their cages for cleaning the cages and renewing their food and water supply. Animal treatment and husbandry were in accordance with approved use of animals in scientific procedures regulated by the Animals (Scientific Procedures) Act 1986, UK.

2.2. Drugs and treatments

Amphetamine (AMP) used in the first experiment and diazepam (DZP) used in the second experiment were purchased from Sigma–Aldrich (UK). Amphetamine was dissolved in physiological saline whereas diazepam was dissolved with 0.3% Tween-80 in 0.9% physiological saline. Both drugs were freshly prepared on the days of the test and administered i.p. 30 min before the start of a session in a volume of 10 ml/kg body weight. Animals were tested once a day in 3 successive days. Control mice in the amphetamine experiment received physiological saline whereas those in the diazepam experiment received physiological saline with 0.3% Tween-80. Experiment 1 consists of 5 groups comprising one group treated with saline ($n = 9$) and separate groups treated with amphetamine at 1 mg/kg ($n = 8$), 2.5 mg/kg ($n = 8$), 5 mg/kg ($n = 8$), and 10 mg/kg ($n = 7$). Experiment 2 consists of 4 groups comprising one group treated with saline ($n = 9$) and separate groups treated with diazepam at 0.5 mg/kg ($n = 9$), 1 mg/kg ($n = 8$) and 3 mg/kg ($n = 8$). In each experiment, individual mice were tested in a random order. Rats and mice have a much greater metabolic capability than humans across a wide range of drugs. They eliminate benzodiazepines much more quickly than humans [8]. The half-life of diazepam is about 60 min in rats and mice [32,69]; therefore it is unlikely for the pharmacokinetic plasma level to accumulate (carryover) from daily injections of the drug.

2.3. Apparatus and testing procedures

It consists of a platform 80 cm × 80 cm wide, elevated 75 cm from the ground. Steep inclined panels (width: 80 cm × 25 cm, slope angles: 77°) made of rigid wire mesh are attached on two opposite sides of the platform (see Fig. 1). The platform was divided into a central area covered with a white tile (16 cm × 16 cm wide and 0.4 cm thick), an inner area surrounding the central area (16 cm wide and 2048 cm^2),