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A comparative study with two types of elevated plus-maze (transparent vs. opaque walls) on the anxiolytic effects of midazolam, one-trial tolerance and fear-induced analgesia

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Abstract

The phenomenon known as one-trial tolerance (OTT) to the anxiolytic effects of benzodiazepines observed in rats submitted to the elevated plus-maze test (EPM) is considered to be due to the emergence of phobic states across the test/retest sessions. Antinociception is a usual component of the defense reaction. Until now, no study has examined antinociception and OTT together in freely behaving rats in the EPM. This work is a new approach looking at the sensorimotor gatings underlying OTT through the examination of the changes in reactivity to noxious stimuli during OTT development. We used the tail-flick test to assess the reactivity of rats to noxious stimulus during the effects of midazolam in test/retest sessions using two types of EPM, one with opaque (standard EPM) and another one with transparent walls (modified EPM). The authors had previously shown that this modified test caused an overall stressful situation more related to anxiety while the standard test coursed with a mixture of anxiety and high fear levels. In both plus mazes, the study was conducted in two experiments: (i) midazolam before the first trial, and (ii) midazolam before the second trial. In each experimental condition the effects of midazolam were tested under two doses (0.5 and 1.0 mg/kg) against a control group that received injections of saline. The anxiolytic effects of midazolam were more pronounced in animals tested in the modified EPM than in the standard EPM. Stressful stimuli present in both types of maze were able to elicit one-trial tolerance to midazolam on re-exposure. However, anxiolytic-insensitive behaviors in the first and the reduction in exploratory activity in the second trial are more pronounced in the standard EPM indicating that this test is more prone to transfer fear-related states across trials than the modified maze test. Antinociception is not present upon the re-exposure of rats to the EPM. These findings show that animals tested in the modified EPM showed higher sensitivity to the anxiolytic effects of midazolam than the standard EPM. Antinociception was not a concomitant of the shift in the emotional state present in the retest sessions of the EPM. These results are in agreement with the premises that repeated stressful experience leads to anxiolytic-insensitive fear state different from anxiety.

Keywords: Antinociception; Elevated plus-maze; Midazolam; One-trial tolerance; Transparent walls

1. Introduction

The traditional elevated plus maze test (EPM) with two open and two closed (opaque) arms involves mixes of conditioned, innate, proximal and distal aversive mechanisms such that aversive cues detected at a distance could function as a negative incentive that activates a fear system which guides the organism from danger present in the open arms of the maze (Graeff and Deakin, 1991). Thus, the use of the EPM as an animal model of anxiety is based on the measures of all ethological categories that reflect the conflict resulting from the natural tendency of the animals to approach and avoid dangerous situations (Gray and McNaughton, 2000). Accordingly, the nature of the threat

Abbreviations: BZD, benzodiazepines; EPM, elevated plus-maze; IA, index of antinociception; M, midazolam; OTT, one-trial tolerance; S, saline; TFL, tail-flick latencies.

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i.e. whether learned or innate and the nature of the appropriate response (emission or suppression of an action) has a bearing on drug responses (Handley and McBlane, 1993). In this context, while benzodiazepines (BZD) injected in rats upon initial exposure to the EPM increase the percentage of entries and the time spent in the open arms of the maze, a single previous undrugged experience in the EPM renders these compounds inefficacious (Lister, 1987; File, 1990). This phenomenon, known as 'one-trial tolerance' (OTT) or 'one-trial learning', appears to be dependent on learning from the first trial of the location of relative safety of the EPM (Rodgers and Johnson, 1995; Holmes and Rodgers, 1999). This phenomenon has also been associated to the fact that upon the initial exposure the animals experience concurrent states of anxiety and fear in the EPM. Behavioral studies have proposed that the submission to trial 1/trial 2 in the EPM results in a qualitative shift in emotional state, probably with phobia acquisition (File et al., 1990; Nutt, 1990; File and Zangrossi, 1993; Rodgers and Shepherd, 1993; Holmes and Rodgers, 1998; Cruz-Morales et al., 2002). The basic idea is that trial 1 may represent the acquisition of a phobia-like response to the open arms, and the lack of anxiolytic-like effects of BZD in the second exposure to the EPM may be related to the well-known insensitivity of phobic behaviors to the anxiolytic action of BZDs and other classes of compounds with anxiolytic action (Bertoglio and Carobrez, 2000). In this way, a modified EPM test with closed arms made of transparent walls induces moderate stress and is more sensitive to the anxiolytic effects of BZDs than the standard test which presents concurrent states of anxiety and fear in the EPM (Anseloni et al., 1995; Anseloni and Brandão, 1997). Indeed, previous reports have described freezing, defecation, and increases in plasma corticosteroids as behavioral and physiological expressions of fear when the animals are restricted to the open arms of the standard maze (Pellow et al., 1985; Treit et al., 1993). In view of these findings the authors examined whether one-trial learning could also be observed in the modified EPM test in which anxiety-like behaviors predominate over fear.

We also thought that we could contribute to this field of inquiry if we also looked at the reactivity to noxious stimuli during one-trial learning instead of only examining the exploratory behavior of the animals in the test. Indeed, fear triggered either by innate or conditioned stimuli inhibits behavioral responses to pain. Because of this, antinociception has also been considered to be part of the defense reaction and is proportional to the magnitude of the fear states (Bolles and Fanselow, 1980; Miczek et al., 1982; Fanselow, 1991; Castilho and Brandão, 2001; Castilho et al., 2002). Indeed, brief exposure to an elevated plus-maze has been shown to induce antinociception in male mice (Lee and Rodgers, 1990; Lee and Rodgers, 1991; Conceição et al., 1992; Rodgers et al., 1992). It has also been shown that this reaction is fully blocked by benzodiazepines (Lee and Rodgers, 1991). In the second trial the animals remain most of the time of the test in the closed arms and the emergence of phobic behaviors has been considered to be responsible for the one-trial tolerance. If the assumption is made that the amount of fear an animal experiences in the EPM is negatively correlated with its tendency to explore the open arms, then a simple relationship might be expected between this activity and tail-flick latency. That is, as exploration of open arms decreases, the index of antinociception should increase. To examine this hypothesis the authors decided to look at the changes in reactivity to noxious stimuli during OTT development in rats. To this end, we used the tail-flick test to assess the reactivity of rats to noxious stimulus during the development of OTT to midazolam in two sessions with the use of two distinct types of EPM, one with opaque (standard) and another one with transparent walls (modified).

2. Materials and methods

2.1. Animals

One-hundred twenty male Wistar rats, weighing 230–260 g, from the animal house of the Campus of Ribeirão Preto of the University of São Paulo, were used. These animals were transported to a room adjacent to the test laboratory 72 h before the test. They were housed in groups of six per cage under a 12:12 dark/light cycle (lights on at 07:00 h) at 23±1 °C, and given free access to food and water. The animals were taken to the test laboratory at least 30 min prior testing. The experiments reported in this article were performed in compliance with the recommendations of the SBNeC (Brazilian Society of Neuroscience and Behavior), which are based on the US National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2. EPM testing

Two EPM devices were used in this work. They consisted of two open arms (50×10 cm) and two enclosed arms of the same size, with 50 cm high walls. The closed arms were made of wood or Plexiglas for the standard and the modified mazes, respectively. The level of illumination was 100 lx on the floor-level of the open arms of the mazes. The level of illumination was 30 and 90 lx on the floor-level of the closed arms of the opaque and transparent mazes, respectively. The maze was configured such that arms of the same type were opposite each other, and the whole maze was raised 50 cm from the floor. A raised edge (0.5 cm) on the open arms provided additional grip for the rats.

All testing was conducted during the light phase of the LD cycle, between 09:00 and 13:00. Rats were placed individually in the center of the maze facing a closed arm and allowed 5 min of free exploration. The behavior of the animals was recorded by a video camera positioned above