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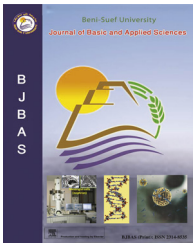

Full Length Article

Behavioral and biochemical characterization of elevated "I-maze" as animal model of anxiety

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ABSTRACT

The elevated I-maze is a modification of the elevated plus-maze model of anxiety in mice. The design of I-maze comprises a straight wooden passage, resembling the English letter "**I**," divided equally into three areas; two enclosed areas (close arms) at both ends of the "maze" and an open area in the center of two enclosed areas. The I-maze completely avoids the central platform of elevated plus-maze, removing any ambiguity in time spent on central platform and allowing uninterrupted animal exploration. In this model, diazepam (1 mg/kg) and gabapentin (10 mg/kg) significantly increased the percentage of time spent in the open areas (%TO) and the number of unprotected head dips (uHDIPS), and reduced the number of protected head dips (pHDIPS) and stretch attend postures (SAP) from close to open arm. Similarly, fluoxetine (5 mg/kg) significantly increased %TO and uHDIPS, and significantly decreased SAP from close to open arm, but it did not have any significant effect on pHDIPS. The 5-HT_3 receptor antagonist, ondansetron (0.1 mg/kg), did not produce any significant change in all the behaviors, observed, as compared to vehicle-treated control mice. On the other hand, the anxiogenic agent, caffeine (15 mg/kg), did produce a significant decrease in %TO and uHDIPS, and significantly increased pHDIPS and SAP from close to open arm. Mice confined in open area of I-maze bring the relevant biochemical changes associated with anxiety behavior, showing significant increase in the levels of plasma nitrate and plasma corticosterone. These data indicate that a combination of novel design of elevated I-maze and a detailed behavioral analysis provides a sensitive model for the measurement of anxiety.

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1. Introduction

Human anxiety is defined as a feeling of apprehension, uncertainty and tension stemming from the anticipation of an imagined or unreal threat (Ninan, 2001). Pharmacological evaluation of anti-anxiety agents is done using different exclusively designed behavioral paradigms. A model of anxiety is an experimental system or paradigm to simulate some aspects of anxiety disorders in which the effects of anxiolytic drugs are examined. Animal behavioral profiles such as locomotion, self grooming, defecation and urination have long been used to detect effects on anxiety, and a number of models, based on animal emotional reactivity, have been proven to be sensitive to stressful manipulations. Many of these models have been successfully used to test new anxiolytic drugs by simple, rapid and inexpensive ways of evaluating animals' conditions. In this class of tests environmental stimuli or contexts are used to induce anxiety states in animals. Studies on antianxiety compounds generally involve exposing the animal to a situation that has not been experienced before, or after some time. Most of them involve putting an animal into a novel enclosure such as maze, open field or other test chamber.

Several experimental paradigms have been constructed to test anxiety in the rodent (Belzung, 1999). In particular, the elevated plus-maze has been used and validated as a tool to assess anxiety in rodents, as well as to determine the efficacy of various anxiolytic compounds. In this paradigm, subjects are placed on the central platform of the elevated plus-maze at the start of the test, and the amount and distribution of time spent in the open and closed arms are measured. Increased time spent in the open arms is indicative of a low level of anxiety, whereas increased time spent in the closed arms is indicative of a high level of anxiety. Validation of this procedure has been confirmed by the effects of anxiolytic compounds. Earlier modifications in the design of elevated plus-maze are (a) elevated Zero-maze (EZM) (Shepherd et al., 1992a, 1992b) and (b) elevated T-maze (ETM) (Graeff et al., 1993). These mazes were designed and proposed as animal models of anxiety, which lack the inherent deficiency in the design of elevated plus-maze; there exists an inherent limitation in the plus maze design, of the unavoidable ambiguity, which is associated with time spent on the central square of plus-maze. It is argued by various workers that period spent on central platform compromises interpretation of time spent in open/close arms. Further, mice have been observed to spend 20–30% of the test period on central square (Lee and Rodgers, 1990). The elevated T-maze was designed to offer the provision of testing conditioned and unconditioned fear in the single apparatus, whereas elevated zero-maze was utilized for detailed behavioral analysis including risk assessment by animal, in addition to measuring anxiety. Though, these mazes offer clear advantages over elevated plus-maze *viz.* avoidance of central platform (in case of both ETM and EZM) and uninterrupted exploration of the apparatus (in case of EZM), the I-maze design facilitates (a) expedited exploration of the maze because, as the animal reaches the end of the closed end, it spontaneously turns back and encounters a much clear view (option) of open space, present inherently between two closed arms (choices), as compared to all other mazes like EPM, ETM and EZM. (b) Authors opine that I-maze offers a rather more robust measure of anxiety as compared to the designs of EPM, ETM and EZM, because during the whole period of animal stay (300 sec) in the maze, the design of I-maze facilitates a clear view of all the portions of the maze. Due to such structure of the maze, during its stay in any one arm (open or close), the animal has always a clear vision of other arm, where it can enter as per its own preference. This feature adds to the reliability of the I-maze, because the animal during its stay in open arm still has a clear view of close arms, on both sides of its stay on the straight open platform of the maze. In this situation, still, if the animal does not opt to enter close arm, then it may be taken as a more robust measure of animal preference for open arm i.e. anxiolytic-like behavior of animal or drug action, rather than an artifact or an unexplained stay of animal in any one arm (out of animal's own confusion and retardation of decision making, which is an inherent feature of anxiety or due to lack of a clear view of another arm during its stay in one arm). (c) This further adds to the utility of I-maze to facilitate a significant interpretation of more subtle drug effects. (d) Further, similar to ETM and EZM, I-maze also avoids the central platform in its design.

Various categories of drugs, like benzodiazepines, barbiturates, alcohol, tri-cyclic antidepressant, have been used for long time to treat anxiety. Apart from these established categories of drugs, there are certain categories, which have been explored for their anxiolytic potential. These include selective serotonin reuptake inhibitors (SSRIs) like paroxetine, citalopram and fluoxetine. These categories have been considered as possible therapeutic replacements for some of traditional anxiolytics because they are found to have comparable anxiolytic effect to diazepam. Further, some anti-epileptics like tiagabin, gabapentin and pregabalin have been explored for anxiety treatment.

The present study was undertaken to examine the possible anti-anxiety activity of selected categories of drugs on a proposed model of anxiety, named as "I-maze." A model is said to be behaviorally validated, if a model facilitates the expression of a significant pharmacological activity of selected drugs in mice, thereby showing a clear behavioral change in mice, as a result of administration of selected drug(s).

In the brain, NOS has been localized in regions involved with anxiety, such as hypothalamus, amygdala and hippocampus (Vincent, 1994). There is evidence suggesting the role of NO/cGMP signaling pathway in effect of NO on anxiety (Eroglu and Caglayan, 1997). Inhibition of nitric oxide–cGMP pathways by inhibition of NOS has been reported to produce antianxiety effect (Spolidório et al., 2007). Stressful events have been found to enhance anxiety in rodents, and plasma corticosterone is reported to be a hormonal marker of anxiety (Mitra and Sapolsky, 2008; Zhao et al., 2014). Therefore, in the present hypothesis, if the present model facilitates the expression of a significant change in plasma nitrite and plasma corticosterone in mice, it will show the biochemical validation of animal model.

2. Materials and methods

2.1. *Animals*

Swiss albino mice (male; 20–25 g) were used in this study. Animals were housed under standard laboratory conditions,