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Research report

Higher detection sensitivity of anxiolytic effects of diazepam by ledge-free open arm with opaque walled closed arm elevated plus maze in male rats

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HIGHLIGHTS

- Arm structure of the elevated plus maze alters detection sensitivity for anxiety.
- Open arm ledges increase open arm exploratory behavior.
- Closed arm wall opaqueness partially alters open arm exploratory behavior.
- No-Ledges/Opaque EPM shows high detection sensitivity for anxiolytic drug diazepam.

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ABSTRACT

The elevated plus maze (EPM) is an established method for testing animal anxiety. However, EPM apparatuses and their features can differ among laboratories, most notably in the presence/absence of ledges on the open arm and/or the transparency/opaqueness of walls on the closed arm. The combined effects of these variable arm features on EPM behavior are not yet fully understood. In the present study, we prepared four types of EPM apparatus – open arms with (0.5 cm) or without (0 cm) ledges × closed arms with transparent or opaque walls – and compared the maze-exploration behavior of male Sprague–Dawley rats. We found that the presence of open arm ledges significantly increased the incidence of open arm exploration. Furthermore, time spent in the distal segment of the open arm was shortest in the apparatus that had open arms with no ledges and opaque closed arms (No-Ledges/Opaque), and was longest in the apparatus that had open arms with ledges and transparent closed arms (Ledges/Transparent). Additionally, the No-Ledges/Opaque apparatus could detect the effect of 0.5 mg/kg diazepam, an anxiolytic drug, whereas the Ledges/Transparent apparatus could not. These results indicate that arm structure (features of both open and closed arms) significantly influences maze-exploratory behavior in rats, and that No-Ledges/Opaque apparatuses have higher detection sensitivity for anxiolytic effects of diazepam than that of Ledges/Transparent apparatuses.

1. Introduction

The number of patients suffering from mood disorder, including anxiety-disorder and depression, is increasing. According to the 2005 National Comorbidity Survey-Replication study, around 20.9 million American adults, or 9.5% of the population ages 18 and older, have mood disorders. Although the development of these disorders involves both genetic and environmental factors, the detailed pathogenic mechanisms that underlie mood disorders are not yet fully understood [1]. Therefore, further research needs to focus on uncovering the underlying mechanisms of mood disorders, as well as the development of effective drugs that have fewer side effects. Researchers also need to understand basic features of the experimental devices that have been adopted to evaluate mood disorders in order to provide reliable, precise outcomes in the laboratory.

The elevated plus maze (EPM), which was originally established to examine emotional reactivity in rodents [2,3], is widely used in laboratory research to investigate anxiety. In this maze, animals are free to explore the apparatus, which consists of two open and

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two closed arms, and is usually placed 50 cm from the floor. Because mice and rats prefer enclosed spaces, animals experiencing anxiety tend to avoid open arm exploration [3]. The incidence of staying in closed arms can be increased and reduced by anxiogenic and anxiolytic drugs, respectively. It is also known that the EPM induces an increase in freezing behavior, fecal boli, and plasma glucocorticoid levels [3]. Thus, the EPM can be applied to test animal anxiety and to perform preclinical drug screening of potential treatments for human mental diseases, such as anxiety-disorder. The EPM also offers a way to measure the effects of anxiety-inducing and anxiety-relieving drugs.

There are several structural differences between EPM apparatuses used in different laboratories [4,5]. For example, there are two types of open arms, namely those with or without ledges along the side. Similarly, there are two types of closed arms, namely those with transparent or opaque walls. The use of open arms with ledges prevents animals from falling off the apparatus [6] and the use of transparent walled closed arms facilitates the observation of behavior [7,8]. Although there are a few studies that have examined how these arm differences may differ in their ability to detect animal anxiety [4,7–10], the combined effects of open (with/without a ledge) and closed (transparent/opaque walls) arm features on maze-exploratory behaviors have not yet been investigated. Therefore, in the present study, we studied the combined effects of open/closed arm features on rat EPM behaviors in the first experiment. In the second experiment, we compared the detection sensitivity of selected pairs of open/closed arm combinations for the anxiolytic effects of diazepam. We discuss the combined effects of the arm features on rat EPM behaviors, sensitivity of the EPM apparatus to detect anxiolytic drug, and other findings from these experiments. We believe that our findings may enhance the basic understanding of EPM features and ultimately contribute to the broader field of anxiety research.

2. Materials and methods

2.1. Experimental design

Two experiments were performed. In Experiment 1, we prepared four types of EPM apparatus: open arms with ledges and closed arms with transparent walls (Ledges/Transparent), open arms with ledges and closed arms with opaque walls (Ledges/Opaque), open arms without ledges and closed arms with transparent walls (No-Ledges/Transparent), and open arms without ledges and closed arms with opaque walls (No-Ledges/Opaque). Rat behaviors in each of the four EPM apparatuses were then compared. In Experiment 2, we tested the effect of diazepam, an anxiolytic drug, on the expression of anxiety-related behaviors in the two apparatuses that were found to be the most and least anxiety-inducing in the first experiment. This allowed us to compare the detection sensitivity between the apparatuses.

2.2. Animals

In Experiment 1, 64 male Sprague–Dawley rats at 4 weeks-old were purchased from Charles River Japan (Shiga, Japan) and randomly assigned to four experimental groups (see experimental design). In Experiment 2, 63 male Sprague–Dawley rats at 4 weeks-old were purchased, and randomly divided into two apparatus groups (see experimental design). In these two apparatus groups, animals were further subdivided into three treatment groups (vehicle, low-dose, or high-dose diazepam treatments). All animals underwent at least 1 week of habituation to the animal housing conditions at Meiji University before the experiment. The animal housing room was maintained at standard ambient conditions of light (12:12 light/dark schedule with lights on at 10:00 h), room temperature ($25.0 \pm 0.5\,^{\circ}C$), and humidity ($55 \pm 10\%$), and animals had *ad libitum* access to food and water. All experimental procedures performed in this study were approved by the Institutional Animal Care and Use Committee of Meiji University.

2.3. Drug preparation

In order to determine the detection sensitivity of the EPM apparatus to animal anxiety in Experiment 2, low-dose diazepam (0.25 mg/kg, intraperitoneal (i.p.), Taiyo Pharmaceutical Industry, Aichi, Japan), high-dose diazepam (0.50 mg/kg, i.p.) dissolved in 0.9% saline with 10% Tween 80 (Wako Pure Chemical Industries, Osaka, Japan), or vehicle was administered 30 min prior to the EPM test.

2.4. EPM test

The EPM apparatus was located in a handmade container (W150 × D150 × H200 cm), which was elevated to a height of 50 cm from the floor, and its two open and two closed arms merged at a central square (10 × 10 cm) to form a plus shape. Illumination on the surface of the apparatus was adjusted to approximately 100 lux. For the present study, we prepared four types of EPM apparatus with the following arm structure combinations: open arms (W50 × D10 cm) with (H0.5 cm) or without (H0 cm) short ledges, and closed arms (W50 × D10 × H45 cm) with black opaque or transparent walls (W50 × H45 cm). Each rat was placed on the central square facing an open arm and was allowed to freely explore the maze for 5 min. Behavior was recorded by a digital video camera, and the time rats spent within each arm, as well as the number of entries into these arms, was measured by three independent observers using stopwatches. An 'entry' into one of the four arms was defined as instances in which all four paws of the animal were on an arm [11]. With these data, the percentage of time spent in open arms (time spent in open arms/(time spent in open arms + time spent in closed arms) × 100) and the percentage of entries into open arms (number of entries into open arms/(number of entries into open arms + number of entries into closed arms) × 100) were calculated. Furthermore, risk-assessment behavior, defined as the frequency of stretched-back posture toward an open arm while being backed into a closed arm on the central square was also manually observed. The traveling distance, time spent in the three equal segments of the open arm (proximal, middle, and distal segments from the central square), and a minute-by-minute analysis for time spent in open and closed arms were measured by EthoVision XT version 8.0 software (Noldus, Wagenigen, Netherlands). This software allows the animal to be tracked via its contrast with the background field; the animal, which is assigned a fixed size, is identified as the brighter object and locomotion of its center point can thus be used for animal tracing. The relative value of time spent in proximal and distal segments over the time spent in the middle segment was calculated in order to gain a more detailed understanding of the open arm exploratory pattern for each of the four apparatuses. The apparatus was cleaned with 70% ethanol solution and wiped with distilled water before each test. During the EPM tests in Experiment 2, two animals (one from the vehicle- injection group and one from the low-dose diazepam injection group) from the No-Ledges/Opaque apparatus, and three animals (from the high-dose diazepam injection group) from the Ledges/Transparent apparatus fell off of the apparatus. Thus, their data were not included in the final analysis.