Official Journal of TESMA
Regenerative Research
www.regres.tesma.org.my
E-ISSN 2232-0822
Tissue Engineering and Regenerative Medicine Society of Malaysia

Regenerative Research 4(2) 2016 11-14

THE EFFECTS OF OPEN FIELD EXPOSURE ON THE ANXIETY AND LOCOMOTIVE BEHAVIOR OF ADULT MALE *WISTAR* RATS IN ELEVATED PLUS MAZE

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ARTICLE INFO

Published online: 29th February, 2016
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KEYWORDS

Open field,
Elevated plus maze,
Anxiety

ABSTRACT

Anxiety is a multifaceted emotional disorder which requires multiple research models for effective assessment of the condition. Usually, a large number of animals will be used for a single study which will not be reused in other studies. The use of different groups of animals for different aspects of any study may create inter-subject variations that can confound the observed results. Objective of this study is to investigate the effect of pre-exposure to open field (OF) on the anxiety and locomotor behaviors of male adult Wistar rats in elevated plus maze (EPM). We evaluated the effect of pre-exposure in OF on the anxiety and locomotor behaviors of rats at 3 different time intervals. The control group consisted of rats which underwent single exposure in EPM, and the other three groups consisted of rats which underwent a pre-exposure in OF immediately before the EPM session, 2 days before the EPM session, and a week before the EPM session. Our results show that there was no significant effect of OF pre-exposure on the anxiety and the locomotive behavior of rats in EPM at these 3 different intervals. In conclusions, these tests can be conducted successively with minimum time duration in the gap between these two tests.

1.0 Introduction

Anxiety is an apprehensive uneasiness of mind that can create feelings of fear, worry, and dread [1]. Anxiety has been studied in animals using various models [2, 3]. The use of various anxiety models to validate the anxiolytic property of a drug has been debated by many researchers, due to factors such as: (i) different tests measuring different aspects of anxiety [4, 5] (ii) the possible confounding effects of the locomotor activities of the animals being tested. These factors can significantly influence the interpretation of the results from an anxiety model [4].

Researchers have addressed this issue in many ways. In some cases, different groups of animals were used in each behavioral test requiring a large number of animals in a single study which were not reused in other studies [6]. In other cases, the same group of animals was used in a battery of tests, but with certain gaps in duration between the tests [7]. This however, gave rise to the concern regarding the order effects of the tests or the influence of pre-exposure of one test on another [8, 9]. Thus, although this gap in duration between

tests works in certain studies, nevertheless it cannot be applied in all anxiety models.

Among the various anxiety models available, open field (OF) and elevated plus maze (EPM) are the most commonly used [10, 11]. Mourao-Junior and colleagues have demonstrated that previous exposure of mice to OF did not interfere with the results of EPM at different time intervals between both tests of 6, 24, and 72 hours. However the results of this study were only confined to open arm behaviors [7]. This current study was undertaken to investigate the anxiety and locomotor behaviors of adult rats in the automated EPM following a pre-exposure in OF at different time intervals ranging from an immediate pre-exposure before the EPM session, pre-exposure 2 days before the EPM session and pre-exposure a week before the EPM session.

2.0 Materials and methods

2.1 Animal Preparation

All experiments were performed using male Wistar rats (8 weeks) weighing 200-220g obtained from the Laboratory Animal Research Unit, University Sains Malaysia (LARUSM) and maintained in a 12h light-dark cycle: lights on 19H-7H. Animals were housed individually in cages with dimension of 24x40x20cm (WxDxH) and kept at a constant room temperature of (24° C) and were allowed to adapt to their surroundings for at least 7 days prior to the experiment. All animal procedures in this study were approved by the Animal Ethics Committee of University Sains Malaysia.

2.2 Behavioral Assessment

2.2.1 OF Test

The OF consisted of a square box that measured 60x60cm with 35cm walls. Lines were drawn on the floor into 15x15cm squares and were visible through the clear plexiglass floor. The test arena was divided into central and peripheral zones. Each rat was placed in the central area and allowed to explore for 5 minutes. After the 5 minute test, the rats were returned in their home cages and the OF was cleaned thoroughly and allowed to dry between tests. The apparatus was placed under a homogenous illumination (14-20 lx) [12].

2.2.2 Automated EPM

The automated EPM (Kinder Scientific, Poway, CA) consisted of two open arms (width, 10.8cm, length, 50.17cm) and two closed arms (width, 10.8cm, length, 50.17cm, walls, 40.01cm) with a central platform (10.8cmx10. 8cm). The maze was elevated 85.09cm from the floor and rat movements were tracked by infrared photobeams embedded along the entire length of the base of each arm and subsequently analyzed by Motor Monitor computer software. The assessment of locomotion of rats in the EPM is as per described in [13, 14]. Experiments were carried out during the dark phase of a light-dark cycle in a quiet room with homogenous illumination (2-4 lx) directed towards the apparatus [12]. The experiment was initiated by placing the rat in the center of the maze platform facing an open arm and followed by recording the activity of the rat in the maze for 5 minutes of a single session for each rat. The maze was wiped clean after each test session. Anxiety was measured as the time spent in open arms as a percent of the total time spent exploring both the open and closed arms (Open Arm Total Time Percentage) and the number of entries into the open arms as a percentage of the total number of entries into both open and closed arms (Open Arm Entries Percentage).

2.2.3 Behavioral testing: Experimental Procedure

A total of 32 animals were divided into 4 groups of 8 animals. Group 1 (Control) animals were assessed in the automated EPM only. Group 2 (EPM1) animals were pre-exposed to an OF and followed by an immediate submission to automated EPM. Group 3 (EPM2) animals were submitted to the automated EPM 2 days following a pre-exposure to the OF. Group 4 (EPM3) animals were submitted to the automated EPM one week following a pre-exposure to the OF.

2.3 Statistical Analysis

Data for EPM study were analyzed by One Way ANOVA. $p<0.05$ was considered to be statistically significant.

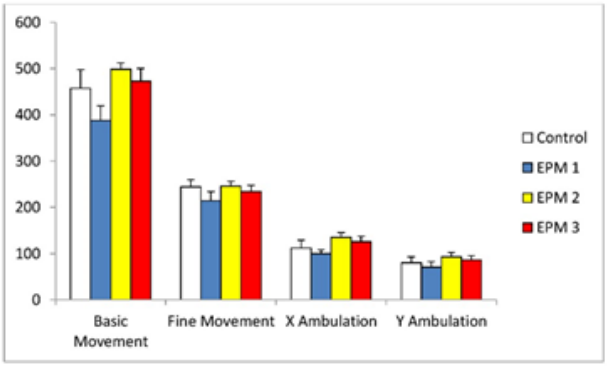


Fig.1: The effects of pre-exposure to open field on the locomotion of rats in the EPM.

The effects of pre-exposure to open field on the basic movement, fine movement, X ambulation, and Y ambulation of rats in the EPM. Each column represents mean ± S.E.M [n=8 for each group, Control= Group underwent single exposure in EPM only; EPM 1= Group underwent pre-exposure in OF followed by an immediate subsequent exposure in the EPM; EPM 2= Group underwent pre-exposure in OF followed by subsequent exposure in the EPM after 2 days; EPM 3= Group underwent pre-exposure in OF followed by subsequent exposure in the EPM after one week; *p<0.05 vs EPM 2, One Way Analysis of Variance and Post Hoc Tukey's test]

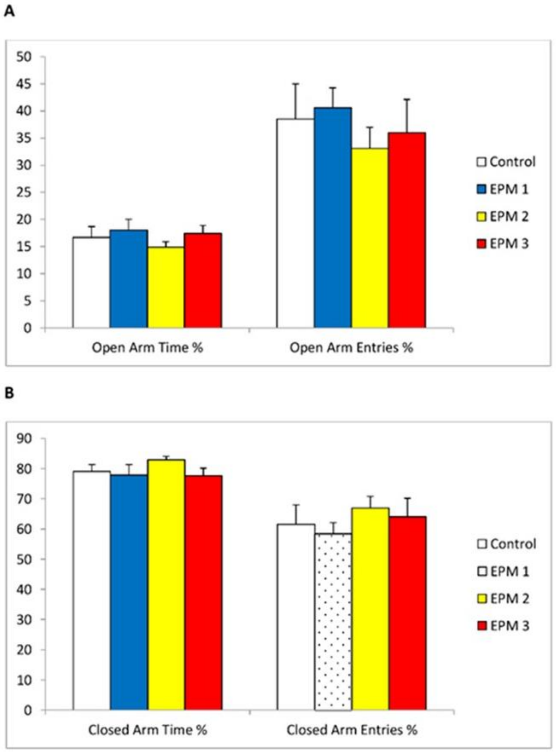
3.0 Results

3.1 Locomotion

There was no significant difference in basic movement [F (3,28) =2. 487; p>0.05], fine movement [F (3,28) =0. 892; p>0.05], X ambulation [F (3,28) =1. 501, p>0.05] and Y ambulation [F (3,28) =0. 730; p>0.05] between control and groups pre-exposed to the OF.

3.2 Anxiety

No significant difference was reported in the percent open arm time [F (3,28) =2. 392; p>0.05)], percent open arm entries [F (3,28) =2. 051; p>0.05)], percent closed arm time [F (3,28) =0. 973; p>0.05)], and percent closed arm entries [F (3,28) =2. 051; p>0.05)] between control and animals underwent pre-exposure in the OF (Figure 2).



The effects of pre-exposure to open field on the anxiety behaviors of rats in the EPM.
The effects of pre-exposure to open field on (A) percent open arm time, open arm entries, (B) closed arm time, and closed arm entries. Each column represents mean ± S.E.M [n=8 for each group, Control= Group underwent single exposure in EPM only; EPM 1= Group underwent pre-exposure in OF followed by an immediate subsequent exposure in the EPM; EPM 2= Group underwent pre-exposure in OF followed by subsequent exposure in the EPM after 2 days; EPM 3= Group underwent pre-exposure in OF followed by subsequent exposure in the EPM after one week, One Way Analysis of Variance].

4.0 Discussion

In the present study, we examined whether pre-exposure to an OF before the EPM session in three different time intervals elicited any significant changes in specific EPM parameters in adult male Wistar rats. Our study showed that there was no significant difference in both the open and closed arm parameters between control and groups pre- exposed to the OF. These findings were contrary to some previous studies carried out under different conditions where exposure to a novel environment immediately before testing in the EPM increased the likelihood of rats entering the open arms of the maze [15, 16]. This showed that the time interval between the OF and EPM session can be further reduced with little effect on the behavioral responses of the rats. However, there are a few factors must be taken into consideration prior to executing such experimental order, including the age, sex, and strain of the animals [17, 18, 19], the time of the tests and the set up of the behavioral test room [20, 21].

On the other hand, the locomotion (basic movement, fine movement, X ambulation, Y ambulation) of animals which underwent immediate pre-exposure in the OF was lower (statistically not significant) compared to the control group in this study. This is contradictory to certain previous findings where an increase in the motor activity was reported in the maze following a pre-exposure to a novel environment immediately before the EPM session [15, 16].

The multifaceted assessment of anxiety will be very beneficial for stem cell or gene therapy based animal models as there is a current need for the resemblance between human and animal behaviours for successful treatments [22]. Recently, scientists have employed gene therapy to treat anxiety using mouse model [23]. As in our study, the use of multiple tests to assess anxiety will provide a close resemblance to multi-dimensional state of anxiety among humans. This may not only mimic the intricate nature of human emotions, but also truly tests the efficacy of the treatments at the preclinical stage.

5.0 Conclusion

Overall, our study showed that pre-exposure in OF before EPM session had no significant effect on both the anxiety and locomotor behavior of adult male Wistar rats.

Acknowledgement

We are grateful to BRAINetwork, Laboratory Animal Research Unit, and Universiti Sains Malaysia (LARUSM) for their kind assistance throughout the study. This study was supported by the USM Research University Grant [1001/PSKBP/8630021] and USM Postgraduate Research Grant [1001/PPSK/8145003].

Competing Interests

There are no competing interests among authors.

Authors' Contributions

All authors have made substantial contributions to conception and design, acquisition, analysis and interpretation of data, drafting and revision of the manuscript (JK, HH, Y-TGB, ZI). All authors have read and approved the final manuscript.

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