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Previous exposure of mice to open Field doesn't interfere in the results of elevated plus maze

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Abstract. With the growth of research on experimental models, has also been increasing concern about animal welfare and has been suggested that every effort should be made to avoid excessive waste and sacrifice of animals. The open field (OF) and elevated plus maze (EPM) apparatuses are widely used in animal behavior, in addition EPM is the most validated test to evaluate anxiety, which is considered a strong confounding variable in this type of study. Thus, our study was conducted to determine whether prior exposure to the OF could produce anxiety, affecting the parameters in the EPM. We intend with this experiment to want whether one group of mice could be used in both tests, without producing any bias and avoiding the waste of animals.

Keywords: Animal waste, anxiety.

Resumo. Com o crescimento das pesquisas com modelos experimentais, também vem aumentando a preocupação com o bem estar animal, bem como tem sido sugerido que todos os esforços possíveis devem ser feitos no sentido de evitar desperdício e sacrifício exagerado de animais. O campo aberto (OF) e o labirinto em cruz elevado (EPM) são aparatos amplamente utilizados em comportamento animal, além disso o EPM é o teste mais validado para avaliar ansiedade, a qual é considerada uma forte variável de confusão nessa modalidade de estudo. Sendo assim, nosso estudo foi desenvolvido a fim de verificar se uma exposição prévia ao OF poderia produzir ansiedade, afetando os parâmetros analisados no EPM. Pretendemos com isso testar se um mesmo grupo de camundongos poderia ser utilizado nos dois testes, sem produzir nenhum viés e evitando o desperdício de animais.

Palavras-chave: Desperdício animal, ansiedade.

Introduction

Currently several experimental animal models, such as rodents, are widely used in scientific experimentation in behavioral studies. Several test equipments have been sufficiently validated to be able in measuring various types of behavior in animals (Martin & Bateson, 2007).

With the growth of research on experimental models, has been also increased the concern with the animal welfare and has been suggested that every effort should be made to avoid excessive waste and sacrifice of animals (National Research Council, 1996).

In studies involving behavior in rodents, are often made several behavioral tests, and, in general, for each test is used a group of animals. Thus, sometimes just a single study uses a very large number of animals, which are euthanized immediately after use, not being reused in other tests (Balls, 1994).

Among the most commonly used in research on animal behavior are the open field test (OF), which

is often the first test to be conducted to evaluate behavior, to have the ability to measure various parameters such as locomotor activity, patterns of anxiety and stress level of animals (Walsh & Cummins, 1996). And test the elevated plus maze (EPM), which is the most validated test to evaluate the anxiety behavior, which often acts as a confounding variable in studies with a memory, learning, sleep, eating, etc. (Lister, 1987).

Therefore, the objective of this study was to determine whether previous exposure to the OF could affect the parameters examined in the EPM to assess whether the same group of mice could be used in both tests, without producing any bias and avoiding the waste of animals.

Material and methods

1. Animals

Were used Swiss mice adult males (mean age 90 days) and average weight of 40 g, from the Center for Reproductive Biology (CBR / UFJF). The animals were kept in a vivarium of the Laboratory of Neurophysiology, Federal University of Juiz de Fora, accommodated in polypropylene cages with dimensions of 41 x 34 x 16 cm, lined with pine shavings. Each cage had accommodated ten animals.

To ensure maximum welfare of animals and to avoid environmental influences in behavioral tests, the cages were placed in a ventilated rack with controlled temperature (22 ± 1 º C) and humidity (50 ± 5%) and light-dark cycle of 12 hours (light from 07:00 h). Before the experiment the animals were in ambiance for 2 weeks, with free access to water and food and were only handled during cleaning of the cages, which was made by the same person and at the same time. All the experimental protocol followed the Guide for the care and use of laboratory animals (USA, National Academy Press) and was approved by the local ethics committee.

2. Apparatus

2.1. Open Field Test (OF)

The open field used in this experiment consists in an apparatus of a wooden square with 45 cm side, enclosed by side walls 15 cm high (Carobrez & Bertoglio, 2005). The floor is made of rough glass to facilitate the locomotion of the animals. The apparatus is in a room lit by dim light (22 ± 1 lux) and the tests were performed during the light cycle between 10:00 and 16:00 h. After each test the apparatus was cleaned with isopropyl alcohol 70%.

2.2. Elevated Pluz Maze (EPM)

The elevated plus-maze consists in an apparatus made of MDF, consisting of four arms of 30 x 8 cm shaping a cross, with two opposing open arms and two opposing closed arms connected by a central area of 8 x 8 cm. The closed arms are surrounded by a wall 20 cm high and open arms have a small edge of 0.5 cm to prevent the possible fall of the animal. The apparatus is positioned at a distance of 50 cm of ground. The test was observed by trained staff. The apparatus are in a room lit by dim light (22 ± 1 lux) and the tests were conducted during the light phase of the cycle, between 10:00 and 16:00 h. After each test the apparatuses were cleaned with isopropyl alcohol 70%.

3. Behavioural assessment

3.1. Open field test

The animals were placed in the center of OF, always oriented in the same direction and by the same person. The animals remained in the appara-

tus for 5 minutes. At the end of the exposure, the animals were put back in their cages to await the test in the EPM.

3.2. Plus-maze test

The test began with the animal placed on the center of the apparatus, facing the open arm, and lasted 5 minutes. Since the degree of anxiety of a mouse is directly related to the tendency of the animal to avoid the open arms of EPM, preferring the closed arms, were used as indicators of anxiety two percentage parameters. The first parameter evaluated was the percentage number of entries in open arms (% NOA), given by (number of entries in open arms divided by the number of entries in the open and closed) x 100. The second parameter was the percentage time the animal spent in the open arms (% TOA), given by (time spent in open arms divided by time spent in the open and closed) x 100. The use of % NOA and % TOA instead of a simple measure of the number of entries and time spent in open arms has the advantage of controlling an important intervening variable that is the locomotor activity of the animal, as when measuring the degree activity in the open arms in relation to the activity in both arms the effects of a more or less exploratory activity are corrected (Rodgers & Dalvi, 1997). The parameters of activity in the EPM were recorded by Hindsight software version 1.5 (Dr. Scott Weiss, 1995, University of Leeds, UK) for a behavioral analysis.

3.3. Experimental Procedure

Were used a total of 40 mice, which were identified and divided into two groups, each one with 10 animals. The first group (N = 10) was used as control (Group C), was only subjected to the EPM. The other 30 animals (experimental group) were divided into 3 groups (N = 10 in each group) and each group was exposed to the OF and then submitted to the EPM in a different time interval: 6, 24 or 72 hours after exposure the OF (Group 6, Group 24 and Group 72). Because of the exposure to OF (which can be used as a model of habituation) can produce memory traces due synaptic plasticity (Pedrazza *et al*., 2007), we chose to test the experimental rats in three different time intervals, since in 6 hours already established the biochemical changes related to short-term memory, and between 24 and 96 hours the traces of long-term memory are already formed (Izquierdo *et al.*, 1998). Thus we try to establish whether the formation of memories would affect the activity of animals in the EPM.

3.4. Statistical analysis

The results were submitted to a statistical descriptive and inferential analysis. Descriptive data were expressed as mean ± standard error. The means of groups were compared using analysis of variance of a factor, and the factor was the group to which each animal belonged. Then was performed the post-test of multiple comparisons by Dunnett to compare difference of the means between each group with the control group. The results of Dunnett's test were expressed by the confidence interval of 95% of the difference between the mean of each group and control group. In all tests was adopted a significance level of 5% and before the ANOVA procedure were verified the assumptions to perform parametric tests. We used the Prism software version 5.00 (GraphPad Software, San Diego, California - USA) in the data analysis.

Results

Data from descriptive statistics for the %NOA and %TOA in the four studied groups are shown in Table 1. An ANOVA of one factor performed to com-

pare the means of % NOA of the 4 groups produced the following results: F (3, 36) = 1.1 and P = 0.35. The same statistical procedure applied to %TOA produced the following results: F (3, 36) = 0.97 and P = 0.42. Soon there were not found statistically significant results in ANOVA for %NOA and %TOA. Still, it was done the Dunnett test to quantify the confidence intervals (95%) of the differences of the means for each group compared with the control group mean. The results produced by Dunnett's test are shown in Table 1, which shows that all confidence intervals contain the value zero, suggesting the possibility that any difference found between the means is due to chance.

Table 1: Descriptive and inferential statistical data of the groups in the plus-maze.

Group	n	% nOA		% tOA	
		mean ± SE	95% IC	mean ± SE	95% IC
Control	10	49 ± 3.3		62 ± 3.9	
6 hours	10	41 ± 3.1	-4.2 to 20	52 ± 3.4	-4.7 to 25
12 hours	10	48 ± 3.4	-12 to 12	57 ± 4.5	-10 to 19
24 hours	10	46 ± 4.0	-9.2 to 15	59 ± 4.9	-11 to 18

95% IC is the confidence interval for differences between means according to the Dunnett's post-test.

Discussion

The results of our study suggest that animals previously exposed to the OF can be reused in the EPM. As levels of anxiety measured in the EPM was not higher in animals previously exposed to OF, in our sample the exposure to the OF did not induce anxious behavior in animals. Our results differ from those of another study suggests (Kalueff *et al*., 2007), which mentions that exposure to OF could induce anxiety in rodents. Perhaps this difference in results is due to biological characteristics in different populations of animals studied (Ferrari *et al*., 1998).

It is possible that the fact that exposure to the OF do not produce behavioral change, as shown by our results, it should be the essential feature of the apparatus, which allows an assessment of the behavior of the animal through an innate exploratory behavior, without imposing any aversive stimulus to the same (Archer, 1973).

We couldn't find more studies evaluating the effect of re-use of animals in different behavioral apparatus, although there are reports of behavioral changes produced by re-exposure to the same apparatus, such as EPM (Carobrez & Bertoglio, 2005). Therefore, we suggest further studies to evaluate the effects of exposure to various apparatuses in behavioral tests, in order that to avoid the waste of animals in research.

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