

The effect of test sequence in a battery of behavioral tests with C57BL/6J mice

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Abstract

The objective of this study was to determine whether the performance of mice (C57BL/6J) in a battery of three behavioral tests were affected by the sequence of the tests. The test battery included the open field test (OFT), elevated plus maze (EPM), and tail suspension test (TST). Young adult, female mice (aged 2 to 3 months) were randomly assigned to one of six groups (5-6 mice/group). The sequence of the tests varied for each group. The behavioral parameters measured in each test included those reported in the scientific literature, such as the time spent in the periphery of the OFT box, the time spent on the open arms of the EPM, and immobility time in the TST. Statistical analyses (ANOVA and Tukey's t-tests) were performed to detect any group effects. Our prediction was that administration of the TST early may induce anxiety-like behavior in later tests. However, the results revealed no significant differences among the groups for the behavioral parameters examined. Therefore, we conclude that the sequence of the tests in the test battery was not an important factor that affected the animals' behavior. These results will guide the design of future experiments on age-related changes in behavior.

Introduction

Mice are increasingly being used as subjects in behavioral neuroscience. Testing paradigms have been developed for mice that allow for objective measurements of behavioral correlates to depression, anxiety, sociality, learning and memory, and motor skills (e.g., Gould, 2009, p. 4). Thus, questions about pathophysiological mechanisms and disease treatment can be tested on mice and directly correlated to changes in behavior. In these types of experiments, it is common to inject mice with a drug (or control solution) and record their behavior on a number of tests. Many studies suggest to start with experiments that are more susceptible to the handling effect

and less invasive to the mice (Võikar et al., 2004, p. 1). In other words, the investigators order the sequence of tests based on what they assume the mice will perceive to be more or less invasive (i.e., unpleasant, startling, or noxious). The least invasive tests are presented first and the most invasive tests later. While there is a certain logic to this approach, it is imprudent to assume what an animal perceives or how it experiences a novel situation. In this study, we will examine the effect of test sequence in a battery of behavioral tests that include the open field test (OFT), elevated plus maze (EPM), and tail suspension test (TST). The results will guide the design of future

experiments on age-related changes in behavior.

Materials and Methods

Four week old female mice were purchased from Jackson Laboratories. For four weeks, the animals were regularly brought to the laboratory to be weighed and to acclimatize them to the testing environment and handling. After reaching 8-16 weeks of age, the mice were randomly assigned to one of six groups (5-6 mice per group). Each group of mice will experience the OFT, EPM, and TST in a different sequence as shown below:

Group ETO	EPM→TST→OFT
Group EOT	EPM→OFT→TST
Group TOE	TST→OFT→EPM
Group TEO	TST→EPM→OFT
Group OET	OFT→EPM→TST
Group OTE	OFT→TST→EPM

The mouse was placed on each test for 6 minutes and was allowed a 5-7 minute rest period between each test. A number of behavioral parameters were analyzed (see below). To control for the possible effects of learned responses, each animal will be tested only one time. Brief descriptions of the behavioral tests follow.

- a) The EPM (e.g., Komada, et al., 2008, p. 1) is a well-established measure of situational anxiety in rodents. The plus-shaped maze is constructed with two open arms (30cm x 5cm x 0.5cm) and two closed arms (30cm x 5cm x 15cm) mounted 50cm above the floor. The animal is placed in the central platform of the apparatus facing the same closed arm. The number of entries into open and closed arms and the time spent in open vs. closed arms during a 6-minute period is recorded.
- b) The TST (e.g., Can et al., 2012, p. 1) is commonly used to measure anti-depressant activity

in mice. The animal is suspended by its tail using adhesive tape placed approximately 1 cm from the tip of the tail and hung 30 cm above a table. The amount of time the animal is immobile during the 6 min testing period is measured. Immobility is defined as the absence of any limb or body movements, with the exception of those caused by respiration.

- c) The OFT (e.g., Siebenhener & Wooten, 2015, p. 1) is primarily used to measure locomotor activity, while aspects of the test (e.g., thigmotaxis) have been reported by some to reveal anxiety-like behavior. The animal is placed in the corner of a box (50cm x 50cm), the floor of which is divided into 10x10cm squares. During the test duration (6 min), as the mouse explores the box, the number of squares crossed, time spent in the periphery of the box, and the total number of rears are recorded.

Results

Test & Parameter	Group EOT	Group ETO	Group OET	Group OTE	Group TEO	Group TOE	P-values
<i>Elevated Plus Maze</i>							
Latency to first open arm entry, s	14.92 (3.85)	21.50 (8.92)	5.30 (2.22)	14.3 (11.4)	20.58 (8.76)	10.58 (7.12)	0.729
Closed entries, nr	16.33 (1.76)	13.83 (1.58)	16.80 (2.91)	13.00 (2.62)	15.17 (1.80)	11.50 (1.75)	0.468
Open entries, nr	8.50 (1.52)	8.67 (2.32)	7.40 (1.33)	3.67 (1.17)	5.50 (0.85)	3.83 (1.08)	0.061
Open entries, %	34.28 (5.91)	35.13 (8.01)	31.07 (5.29)	25.00 (6.54)	27.27 (3.94)	24.21 (4.82)	0.672
Time open, s	72.3 (13.2)	72.6 (20.1)	69.1 (14.7)	32.4 (11.7)	53.8 (11.1)	54.3 (20.9)	0.447
Time central, s	137.8 (12.1)	89.92 (8.20)	89.2 (13.4)	52.0 (12.6)	95.3 (11.8)	50.7 (11.3)	0
Rearings, nr	18.50 (1.15)	14.00 (2.02)	14.60 (1.91)	19.50 (3.39)	21.67 (2.03)	11.00 (2.16)	0.018
Head dips, nr	24.00 (2.70)	18.83 (2.88)	18.20 (3.47)	12.50 (3.13)	17.50 (3.94)	12.00 (2.89)	0.108
Stretches, nr	6.50 (0.99)	6.83 (1.25)	6.00 (1.70)	5.17 (1.47)	7.00 (1.750)	5.67 (0.76)	0.918
Fecal boli, nr	0 (0)	1.00 (0.52)	0.40 (0.40)	0.67 (0.49)	0 (0)	0 (0)	0.172
<i>Tail Suspension Test</i>							
Immobility Time, s	169.8 (18.6)	176.2 (14.9)	179.4 (27.5)	169.0 (32.5)	176.2 (11.9)	197.67 (9.17)	0.928
Fecal boli, nr	0.67 (0.21)	1.00 (0.37)	1.20 (0.80)	1.83 (0.48)	2.17 (0.70)	1.33 (0.62)	0.421
<i>Open Field Test</i>							
Box entries, nr	316.0 (18.2)	303.7 (24.1)	340.6 (33.1)	283.3 (33.4)	277.7 (30.6)	247.0 (21.8)	0.259
Time in periphery, s	263.2 (15.0)	264.0 (17.1)	275.5 (11.4)	286.3 (11.9)	268.3 (15.6)	262.67 (8.44)	0.797
Rearings, nr	43.83 (2.44)	42.00 (6.13)	40.40 (5.23)	48.33 (4.64)	42.17 (2.36)	36.83 (5.27)	0.618
Jumps, nr	2.33 (0.92)	0 (0)	4.60 (3.63)	0.83 (0.48)	1.33 (1.15)	0 (0)	0.247
Fecal boli, nr	2.50 (0.92)	0.33 (0.33)	0 (0)	1.33 (0.80)	0 (0)	0 (0)	0.01

Conclusion

We conclude that the sequence of a battery of tests does not play an important factor in the animals' behavior. The results revealed that there was no significant difference among the behavioral parameters examined; such as, the time spent in the periphery of the OFT box, the time spent on the open arms of the EPM, and immobility time in the TST. Therefore, the data does not support the prediction that administration of the TST may induce anxiety-like behavior in later tests.

References

- Can, A., Dao, D. T., Terrillion, C. E., Piantadosi, S. C., Bhat, S., & Gould, T. D. (2012) The Tail Suspension Test. *Journal of Visualized Experiments*, 59, 1-5.
- Gould, T. D., Dao, D. T., & Kovacsics, C. E. (2009) Mood and Anxiety Related Pheotypes in Mice. *Neuromethods*, 42, 1-20.
- Komada, M., Takao, K., & Miyakawa, T. (2008). Elevated Plus Maze for Mice. *Journal of Visualized Experiments*, 22, 1-4.
- Siebenhener, M.L., & Wooten, M.C. (2015). Use of the Open Field Maze to Measure Locomotor and Anxiety-like Behavior in Mice. *Journal of Visualized Experiments*, 96, 1-6.
- Võikar, V., Vasar, E., & Rauvala, H. (2004) Behavioral alterations induced by repeated testing in C57BL/6J and 129S2/Sv mice: implications for phenotyping screens. *Genes, Brain, and Behavior*, 3, 27-38.

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