Free Exploration of an Elevated Plus-Maze in Mice

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ABSTRACT

In this research, we compared the behavior of mice allowed to freely explore an elevated plus-maze (EPM) with the behavior of mice exposed to the classical testing situation (i.e. forcible centre exposure). A detailed temporal analysis (min-by-min scoring) raises interesting results for practical and theoretical views on the elevated plus-maze task.

Author Keywords

Elevated plus-maze, free exposure, anxiety, emotional behavior, exploration, mice.

INTRODUCTION

The EPM is the most widely used model to evaluate rodent "anxiety-like behaviors". It is based on the a priori postulate that rodents exposed to the apparatus will respond to a conflict between safe parts of the maze that are protected (closed arms), and aversive parts of the maze that are unprotected (open arms). Several discrepancies emerge from the interpretation of an animal's behavior in the EPM. In particular, whether avoidance of open arms is really an unconditioned process, or whether it is rather something acquired during exposure to the test, is still very much an open question [1]. Indeed, rodent's aversion for open space is supposed to be natural; but the fact that open-arm entries actually decrease during exposure to the EPM argues in favor of some kind of open-arm avoidance learning. This is especially relevant with respect to some potential explanations of the one-trial tolerance effect, consisting of a high avoidance of open arms and a decreased effectiveness for anxiolytic drugs during re-exposure to the EPM.

In a recent study, a procedure of free exposure to the EPM was used to demonstrate that open arm avoidance is really a natural tendency in rats [3]. In order to generalize and extend these results, we conducted a follow-up experiment in mice using a detailed temporal analysis.

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MATERIALS AND METHODS

Animals

Male CD1, BALB/c and C56BL/6 mice (70 days-old) were tested in the EPM, either in the free exploration or in the forcible exposure situation.

Procedure

The mice were isolated for 24h in a compartment (20 x 20 x 20cm) with bedding, food and water access. On the testing day, animals from the "free exposure group" were given free access to the EPM thanks to a small removable door connected to the end of a closed arm (see Figure 1). Mice were thus allowed to freely explore the EPM with the possibility to return to the familiar compartment. Their behavior was recorded for a total of 5 minutes in the EPM. Control animals were handled from their familiar compartment and forcibly exposed to the EPM for 5 minutes. Control animals were placed at the centre of the maze, facing an open arm.

Maze and Video-Tracking

The elevated plus-maze (Intellibio, France) was made of ivory Perspex with a central zone area (5 x 5 cm) and facing closed and open arms (25 x 5 cm). It was elevated at a height of 50 cm, the closed arms were surrounded by 25 cm
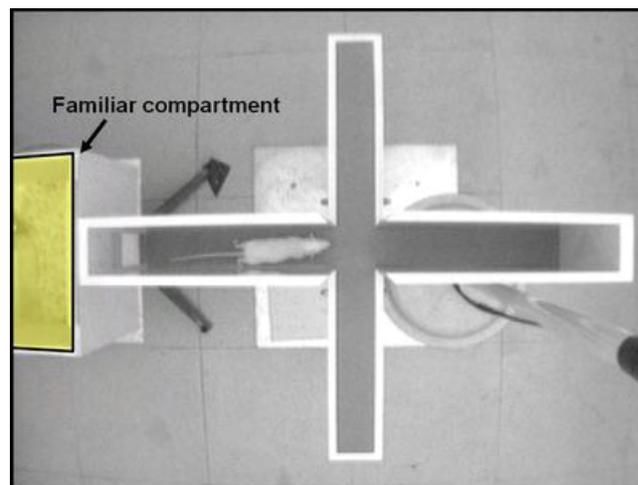

Figure 1. A mouse freely explores the EPM from a familiar compartment connected to a closed arm.

walls and the open arms had 0.25 cm edges. The testing room was illuminated with a dim white light that provided 100 lux for the open arms and 50 lux for the enclosed arms.

The ANY-maze® video-tracking system (Stoelting, version 4.60) was used to record the distances covered, the arm entries and the time spent in the open arms min-by-min. Entering an arm was counted when 98% of the animal's tracking area overlaid the arm whereas the animal was considered to be in the centre of the maze when 50% of the tracking area was out of the arm. Closed-arm returns were recorded when an animal exited a closed arm and re-entered the same arm. The ethological keyboard was used to record rears, head-scans and protected stretched attend postures (pSAPs).

Ethical Statement
The research was conducted in accordance with the guidelines for the care and use of laboratory animals established by the National Institute of Health of the United States of America [2].

Statistical Analysis
Data were analyzed with ANY-maze®. Two-way ANOVAs with condition and segment of test as factors were performed, followed by Tukey's tests for post-hoc comparisons when allowed.

RESULTS AND DISCUSSION
The results that will be presented in details at the meeting confirm that open-arms avoidance is more pronounced in the free exploration condition than in the forcible exposure situation. This difference is observed for the three mice strains and does not depend on locomotion (total distance covered) or exploration (head-scans) in the maze. The min-by-min analysis points out that this difference is evidenced during the first two minutes of the test (see Figure 2 which presents for instance the open-arm entries in CD1 mice).

First, these results confirm that open arm avoidance is also a natural tendency in mice and that it does not require some kind of learning during the first trial in the EPM. In addition, the present study also reinforces the hypothesis that initial open-arm entries during a forcible confrontation with the apparatus should possibly be interpreted as the attempts to escape the situation, rather than as the indications of a low anxiety level. This hypothesis is also consistent with the fact that pSAPs and closed-arm returns were more frequent in the free exploration condition. Indeed, the approach / avoidance conflict is more clearly
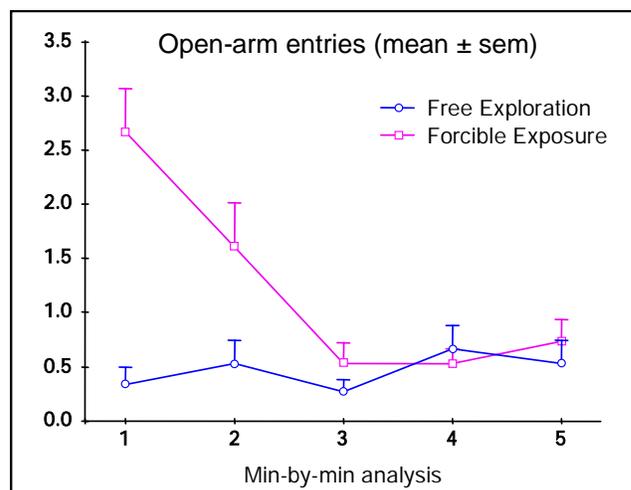

Figure 2. Open-arm entries in CD1 mice forcibly exposed or freely exposed to the elevated-plus maze.

defined in this situation since the familiar compartment stands really in contrast with the unknown elevated plus-maze and its anxiogenic open-arms.

CONCLUSION
Our work confirms that during initial exposure to the EPM, the rodents' behavior is not driven only by the approach / avoidance conflict towards the open arms, but also by some attempts to avoid the whole situation. These attempts can lead during the first minutes of the test to open-arm entries. Thus our work emphasizes the relevance of a detailed temporal analysis to avoid misinterpretations of the results in the EPM procedure.

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