Prior test experience produces changes of t-patterns spatial distribution in the Elevated Plus Maze test

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Abstract

Aim of present research was to investigate in male Wistar rats whether a prior elevated plus maze experience modifies the temporal structure of the behavioral response following a retest applied after 24h. Video files were coded by means of a software coder and event log files generated for each subject were analyzed by means of a specific software for temporal pattern analysis (Theme). Present research shows a clear reduction of the number of t-patterns from trial one to trial two. This reduction is provoked by the disappearance of t-patterns consisting of behavioral elements occurring in the unprotected zones of the maze. The results suggest that the previous experience in the maze causes learning-dependent behavioral changes inducing a more clear-cut response to environmental anxiogenic conditions.

Introduction

The elevated plus maze (EPM) is a behavioral model widely used to assess anxiety-related behavior in rodents. The utilization of EPMs is based on the premise that the simultaneous presence of the enclosed arms (scantly illuminated and surrounded by walls) and of the open arms (brightly illuminated and without walls) evokes a typical approach/avoidance conflict [2][9]. Today, the EPM is also one of the most commonly used assays to investigate the biological basis of emotionality. In addition, thanks to the test/retest protocol, researchers have extended its employment to the study of learning and memory [5][8]. By means of a multivariate approach, known as t-pattern analysis [7], we have already demonstrated the existence of a behavioral temporal structure characterizing the anxiety-related activity of Wistar rats tested in EPM [3]. Aim of present research was to determine in Wistar rats tested in EPM whether a prior maze experience was able to induce meaningful changes in the temporal structure of behavior analyzed following a re-test applied after 24h.

Method

Twenty, three months old, pathogen free, male Wistar rats were used. Animals were born in the animal facility of the University of Rouen (France) and breeders originated from Janvier (Le Genest-St-Isle, France). Rats were housed in groups of three in a room maintained at the constant temperature of 21 ± 2 °C, under the following light/dark cycle: light on = 12 noon; light off = 12 midnight. Food and water were available ad libitum. Each rat, experimentally naïve, was exposed to EPM for two times according to a test/retest protocol [2]. Retest was scheduled after an interval of 24 hours from the prior test. Each subject was placed in the central platform of EPM and allowed to freely explore for 5 min [10]. Experiments were recorded through a video camera and video files stored in a personal computer for following analyses. In present study we have employed the ethogram described in Table 1. As a result of the coding process, an event log file was obtained. This is a sequence of behavioral events chronologically ordered on the basis of their onset time. In this study, each video was coded following a frame-by-frame examination using the software The Observer (Noldus Information Technology, The Netherlands). To assess the temporal relationships among behavioral events, log files were processed by means of the software program Theme (Noldus Information Technology bv, The Netherlands; Patternvision Ltd, Iceland).

Table 1. Ethogram of rat behavior in the elevated plus maze test. (*) = the behavioral element is considered protected (p-) when performed in the central platform or in a closed arm, unprotected (u-) when performed in an open arm; (**) = head dip is considered protected (p-) only in the central platform and unprotected (u-) in an open arm.

Behavioral element	Abbreviation	Description
Closed Arm Entry	CA-Ent	rat moves from the Central Platform to a Closed arm (all four paws in)
Open Arm Entry	OA-Ent	rat moves from the Central Platform to an Open Arm (all four paws in)
Closed Arm Return	CA-Ret	rat puts only head and forepaws in the central platform, then rapidly re-enters in the closed
Closed Arm Walk	CA-Wa	rat walks in a Closed Arm
Open Arm Walk	OA-Wa	rat walks in an Open Arm
Central Platform Entry	CP-Ent	rat moves from an Open or Closed Arm to the Central Platform
Immobile Sniffing (*)	p-ISn; u-ISn	rat sniffs the surrounding area without walking activity
Corner Sniffing (*)	p-CSn; u-CSn	rat sniffs the entrance border of a Closed Arm
Stretched Attend Posture (*)	p-SAP; u-SAP	rat stretches its head and shoulders forward and then returns to the original position
Head Dip (**)	p-HDip; u-HDip	scanning over the sides of the maze towards the floor
Rearing (*)	p-Re; u-Re	rat maintains an erect posture
Defecation (*)	p-Def; u-Def	excrements are produced
Grooming (*)	p-Gr; u-Gr	rat licks/rubs its face and/or body
Paw Licking (*)	p-PL; u-PL	rat licks its paws
Immobility (*)	p-Imm; u-Imm	an immobile posture is maintained

Results

113 t-patterns of different composition were detected in trial-1, only 24 in the retest (Figure 1). Of the 113 t-patterns, 61 consisted of behavioral elements carried out in open arms and central platform (OA-CP), 28 consisted exclusively of behavioral elements carried out in the closed arms and central platform (CA-CP), and the remaining 24 showed a mixed composition (OA-CP and CA-CP). The t-patterns of trial-1 were composed by a total amount of 11 elements of which 6 protected (CA-Ent, Ca-Wa, CP-Ent, p-Csn, p-Isn, p-Re) and 5 unprotected (OA-Ent, OA-Wa, u-Csn, u-HDip, u-Isn). In trial-2, the 24 t-patterns observed were composed only by 6 behavioral elements carried out in the protected zones of the EPM (CA-Ent, Ca-Wa, CP-Ent, p-Csn, p-Isn, p-Re). Finally, the 113 different t-patterns of trial-1 occurred (mean ± SE) 482.65 ± 24.85 times (Figure 2); on the other hand the 24 different t-patterns of trial-2 occurred 227.6 ± 13.79 (Figure 2). Student's *t*-test revealed a highly significant ($p < 0.0001$) difference between these mean values.

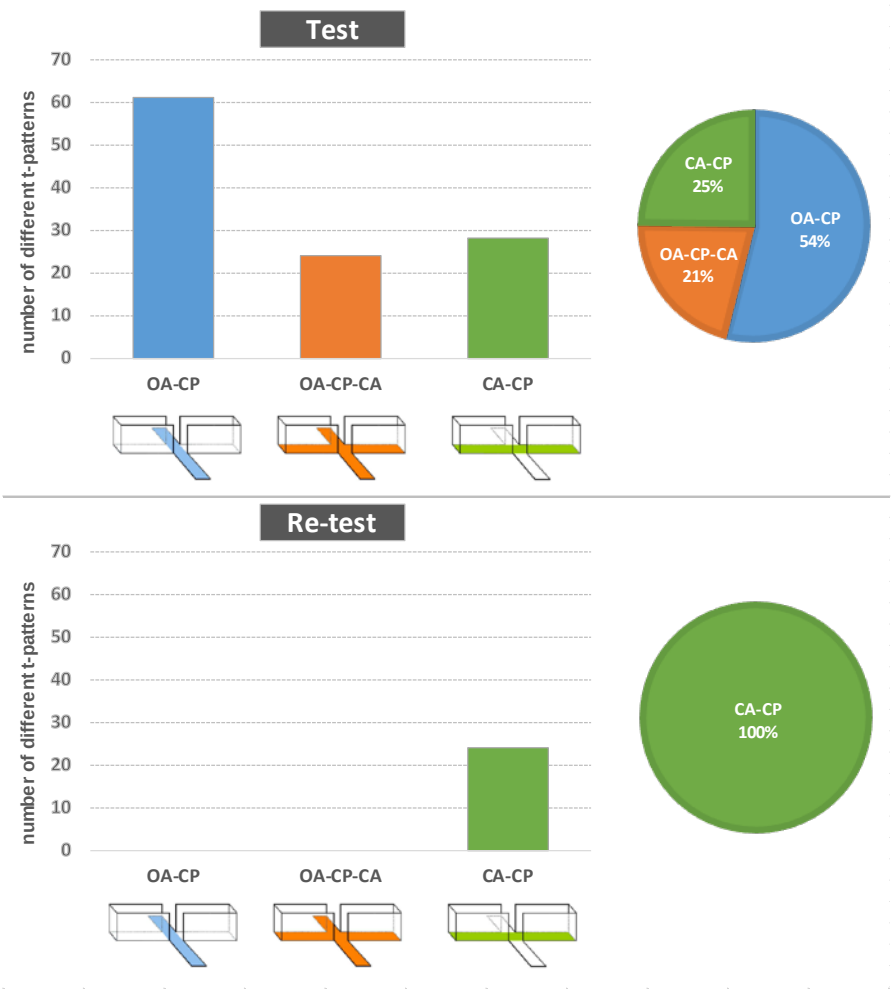

Figure 1. Number of different t-patterns and percent distributions (pie charts) in OA-CP, OA-CP-CA and in CA-CP.

Discussion

Present data show that in the Wistar rat tested in EPM the number of t-patterns undergoes a clear reduction from trial-1 to trial-2 (Figure 1 and Figure 2). This reduction is represented by the disappearance of t-patterns carried out in OA-CP and in OA-CP-CA (Figure 1). A new environment such as the EPM provokes a strong stress, inducing an acute fear condition. Fear triggers an active behavioral strategy that, as proposed by Walter Cannon [1], is aimed at removing the source of danger through the escape. During test one, when the impossibility of the escape is ascertained, escape attempts are progressively abandoned. In the second half of test one, when the fear changes into anxiety and the animal has collected the information on the risk, a strong approach-avoidance conflict occurs. The conflict is solved through the choice of the closed arms where the animal finds refuge and shows an activity characterized by t-patterns consisting of exploratory behavioral elements (CA-Ent, CA-Wa, CP-Ent; p-Csn, p-Isn, p-Re). In the retest, as primary purpose, the rat moves toward the protected zones (CA and CP) of the EPM, while a clear aversion is displayed toward the unprotected ones (OA). Indeed, the number of t-patterns decreases (Figure 2) and they consist of behavioral elements occurring only in CA and CP (Figure 1). These data can be interpreted as the effort of the animal to organize behavioral patterns aimed at a more prompt solution of the approach-avoidance conflict. In conclusion, according to the literature [2][4][6] our results suggest that the previous experience in the maze causes learning-dependent behavioral changes inducing a more clear-cut response to environmental anxiogenic conditions.

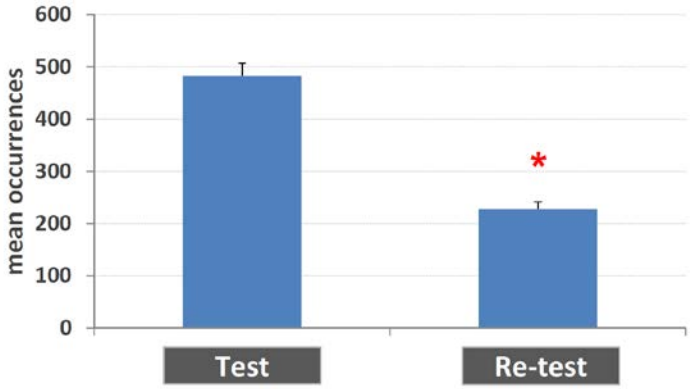

Figure 2. Mean number of t-patterns for each subject. * significant ($p < 0.0001$) difference (Student's *t*-test)

Ethical Statement

All efforts were made to minimize the number of animals used and their suffering. All the experimental procedures were conducted in accordance with the European Communities Council Directive (86/609/EEC) and approved by the Veterinary Committee officially appointed by the University of Palermo.

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