Temporal Patterns of Rodent Behavior in the Elevated Plus Maze Test

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Abstract

The aim of the present study was to evaluate, by means of a temporal pattern analysis, rat's behavior in the elevated plus maze test. A specific software called THEME has been used. On the basis of an ethogram encompassing 24 behavioral elements, results demonstrated that 14 components represented the 98% of the displayed activities. 145 different temporal patterns have been detected; length distribution of such patterns showed that three-, four-, and five-elements patterns were more represented than two-elements ones. Finally, a complex temporal pattern encompassing 8 behavioral elements has been discussed. Present article demonstrated the presence of complex temporal patterns characterizing the behavior of rats in the elevated plus maze test.

Keywords. t-pattern analysis, elevated plus maze, anxiety, rat

Introduction

Introduced by Handley and Mithani [7], the elevated plus maze (EPM) apparatus is a well-known and widely used model to assess anxiety-related behavior in rodents. The rationale underlying the utilization of the EPM is based on the assumption that naïve subjects, exposed to the apparatus, will respond to an approach/avoidance conflict between safe parts of the apparatus that are closed and protected, and aversive parts that are open, unprotected and more brightly lit [12]. Surprisingly, despite the large utilization of the apparatus and the large amount of important articles published, scanty data are available concerning the temporal organization of rat behavior in this experimental assay. Several questions still remain unsolved. For instance: do the behavioral events occurring on the EPM show a specific temporal arrangement? If so, would it be possible to identify specific and/or characteristic sequences of recurring behavioral activities? Last but not least, do such sequences, when/if manifest, encompass an underlying ethological meaning useful to better understand rodents' anxiety related behavior? The aim of this preliminary study was to shed light on above questions performing a detailed temporal analysis of rat behavior in the EPM by means of a specific software. This software, called THEME (PatternVision Ltd, Iceland; Noldus Information Technology, The Netherlands), utilizes a complex algorithm able to detect statistically significant relationships among the behavioral events in the course of time [10][11]. In recent years THEME has been used in different experimental approaches, for instance, to study route-tracing stereotypy in mice [1], behavior in neuro-psychiatric diseases [9], behavioral patterns associated with emesis [8] or, in our laboratories, to investigate exploration and anxiety-related behavior in rodents [4][5][6].

Method

Subjects and housing

Five 90 days old male Wistar rats, specific pathogen free, 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). Subjects were group-housed (3/4) in a room maintained at the constant temperature of 21 ± 2 °C, under a partially reversed light/dark cycle (light on: 12 noon – light off: 12 midnight). Food and water were freely available.

Experimental apparatus

The EPM (Intellibio, France) used in the present study was made of ivory Perspex, the arms were 50 cm long and 10 cm wide, and the apparatus was elevated at a height of 45 cm. The closed arms were surrounded by a 50 cm wall while open arms presented 0.5 cm edges in order to maximize open-arm entries [12]. The floor of the maze was covered with grey plastic. Environmental temperature was maintained equal to the temperature measured in the housing room. 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.

Procedure

Rats were transported from housing room to testing room inside their home-cages to minimize transfer effect. To avoid possible visual and/or olfactive influences, animals were allowed to acclimate for 30 minutes far from observational apparatus. Each subject, experimentally naïve, was placed in central platform of the EPM and allowed to freely explore for 5 min. After each observation, the EPM was cleaned with ethyl alcohol (10%) to remove scent cues left from the preceding subject. Experiments were recorded through a digital videocamera and video files stored in a personal computer for following analyses.

Ethogram and coding

The first step in a behavioral analysis is normally represented by the construction of a formal list, namely an ethogram, containing descriptions of behavioral elements. It is important to note that establishing an ethogram is always a quite critical aspect because an error (for instance, a behavioral element not described or, worst, misinterpreted) is able to negatively influence the following analysis. This is even more important if data will be studied by means of t-pattern analysis: an event can be quite uncommon, e.g. occurring only few times for each subject and/or not in all subjects, nonetheless the temporal relationships it establishes can be extremely important for the behavioral architecture. The ethogram used in the present study encompasses 24 behavioral elements and is presented in Table 1.

However, once video files have been collected and the ethogram is ready/available, the following step is normally represented by a coding process, that is, the utilization of specific software that allows the researcher to note the occurrences of all the behavioral elements carried out by the actor in the observed video file. The result of such a coding process is the so called *event log file*, that is, a sequence of behavioral events occurring at specific time points (namely, milliseconds, seconds or, even, video frames). In the present study all video files have been coded using The Observer (Noldus Information Technology, The Netherlands).

Data analysis

THEME software processes time stamped event log files searching for the particular but wide T-pattern class of hierarchical, sequential and significantly timed real-time patterns [10][11]. The search advances following a bottom-up process. In brief, being A, B, X three hypothetical events occurring in a given time window, the algorithm compares the distributions of each pair of the behavioral elements A and B searching for a time interval after occurrences of A such that, more occurrences of A are followed by at least one B within that window. In this case A and B are indicated as (A B) and form a t-pattern. After that, such first level t-patterns are marked and considered as potential A or B terms in higher patterns, e.g., ((A B) X), which are then also marked (added to the data) and may become an A or B part in still higher patterns, and so on up to any level of pattern complexity that may be found in the data. To avoid multiple detection of the same underlying patterns an evolution algorithm compares all detected patterns and selects only the most complete while deleting partial and redundant detections. Thus, more complex patterns may be created, step by step, following this bottom-up detection process. Before a t-pattern search is performed the software requires specific search parameters. In the present study t-pattern analysis was carried out by using the following search parameters: "*significance level*" (maximum accepted probability of any critical interval relationship to occur by chance) = 0.0001; "*lumping factor*" (forward and backward transition probability above which A and B of a t-pattern (A B) are lumped, that is, A and B are not considered separately but only as the (A B) pattern) = 0.90; "*minimum samples*" (minimum

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	the rat puts only head and forepaws in the central platform, then rapidly re-enters in the closed arm
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

percent of subjects in which a t-pattern must occur to be detected) = 100; "*minimum occurrences*" (minimum number of times a t-pattern must occur to be detected) = 5

Ethical statement

The experiment was conducted in accordance with the European Communities Council Directive 86/609/EEC concerning the protection of animals used for experimental scientific purposes.

Results

Coding process produced event log files containing a total of 1165 behavioral elements. Their percent distribution is presented in Figure 1. Concerning t-pattern analysis, 145 different t-patterns were detected. Figure 2 illustrates the distribution of such patterns on the basis of their length (that is, how many different t-patterns of two, three or *n* elements were detected). Figure 3 illustrates one of the most complex t-patterns detected, occurring twelve times and containing a sequence of eight behavioral events.

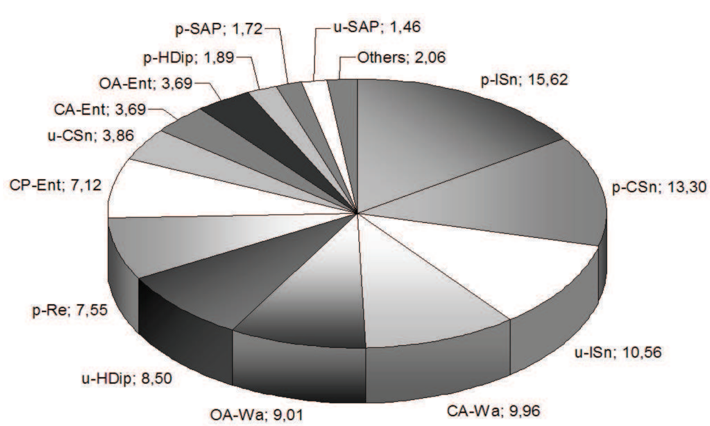

Figure 1. Per cent distribution of behavioral elements in the EPM. Others = p-Gr, u-Gr, CA-Ret, u-Def, p-Def, p-Imm, u-Imm, p-PL, u-PL, u-Re. Data obtained from the analysis of five subjects. See Table 1 for abbreviations.

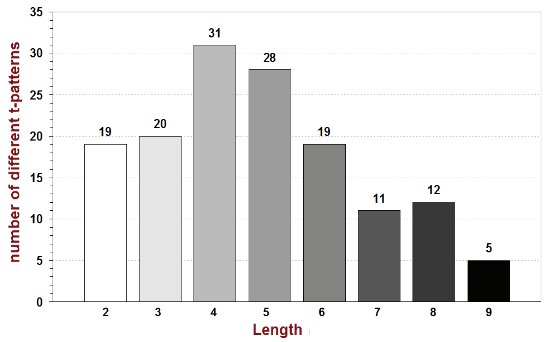

Figure 2. Number of different t-patterns on the basis of their length. X-axis= number of behavioral elements encompassed in the t-pattern's structure. Y-axis= number of t-patterns of different composition. Data obtained from the analysis of five subjects.

Discussion

Present research demonstrates the presence of temporal patterns in the behavior of Wistar rats observed in the elevated plus maze test. Pie chart shows that, among the 24 behavioral elements of the behavioral repertoire (see Table 1), 14 components represent the 98% of the behavior in the EPM (see Figure 1). On the contrary, groomings, immobilities and paw lickings are much more seldomly observed. Such behavioral elements share a common aspect: they require, to be performed, an immobile position. Hence it is possible to conclude that naïve rats in the EPM show a behavioral repertoire heavily oriented toward locomotion and exploration. Such a result appears to be quite noticeable if a comparison with different assays to study anxiety, such as the open-field and the hole-board, is carried out. Indeed both in open field and in hole-board groomings, immobilities and paw lickings are, by far, much more represented [2][3][4]. We hypothesize that such an important difference could be the result of the heavier impact of the EPM in terms of approach-avoidance conflict. T-patterns length distribution (see Figure 2) clearly shows that rodent's behavior in the EPM is well structured from a temporal point of view. Taking into consideration the peculiar bottom-up process carried out by THEME's search algorithm, it is quite common the detection of a large basis of two-elements t-patterns and a lower number of higher-order and more complex patterns [4][5][6] as the search proceeds. The condition in the EPM is very different since three-, four-, and five-elements t-patterns are more represented than two-elements ones. Such an evidence suggests that rat's behavior in EPM has more complex and structured temporal characteristics in comparison with open field and/or hole-board [4][5][6].

The t-pattern in Figure 3 well depicts rodent behavior in the EPM: the animal starts exploring by entering in an open arm (OA-Ent) and explores the unprotected zone (u-ISn, OA-WA); after that, the sniffing of central

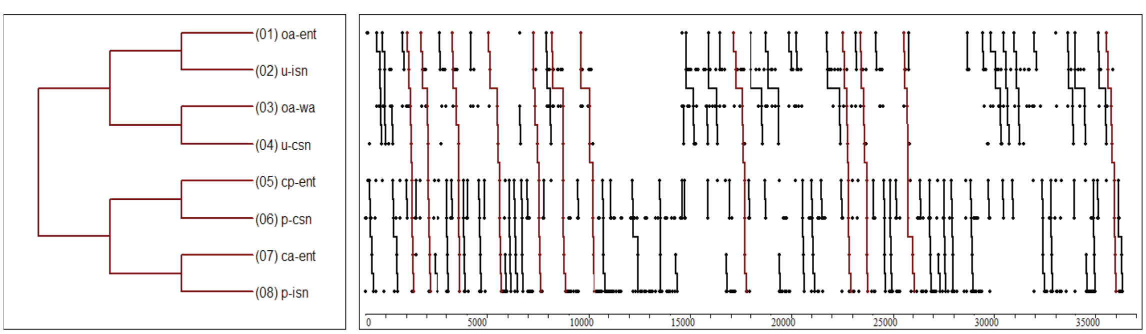

Figure 3. T-pattern of 8 behavioral events occurring 12 times. T-pattern tree structure is illustrated in the left box; numbers in brackets indicate the order of appearance of behavioral events. Onset and occurrences of the detected t-pattern along with incomplete sub-patterns are indicated in the right box. Data obtained from the analysis of five subjects. See Table 1 for abbreviations.

platform's corners (u-CSn) is followed by the entrance in the central structure of the maze (CP-Ent); the t-pattern ends with the sniffing of a closed arm's border (p-CSn), followed by the entry in the closed arm (CA-Ent) and, finally, a further sniffing activity (p-ISn). Interestingly, such a t-pattern (see Figure 3), as several others detected in the same subjects, is structurally organized on the basis of sub-patterns related with the three main EPM sections, that is, open arm, central platform and closed arm. Further studies are in progress in our laboratories to deepen and better clarify the temporal features of rodent behavior in the EPM.

References

1. Bonasera, S.J., Schenk, A.K., Luxenberg, E.J., Tecott, L.H. (2008) A novel method for automatic quantification of psychostimulant-evoked route-tracing stereotypy: application to Mus musculus. *Psychopharmacology (Berl)* **196**, 591-602

2. Casarrubea, M., Sorbera, F., & Crescimanno, G. (2009a). Structure of rat behavior in hole-board: I. Multivariate analysis of response to anxiety. *Physiology & Behavior* **96**, 174-179.

3. Casarrubea, M., Sorbera, F., & Crescimanno, G. (2009b). Structure of rat behavior in hole-board: II. Multivariate analysis of modifica-tions induced by diazepam. *Physiology & Behavior* **96**, 683-692.

4. Casarrubea, M., Sorbera, F., Crescimanno G. (2009c). Multivariate data handling in the study of rat behavior: an integrated approach. *Behavior Research Methods* **41**, 772-781.

5. Casarrubea, M., Sorbera, F., Magnusson, M., Crescimanno, G. (2010). Temporal patterns analysis of rat behavior in hole-board. *Behavioural Brain Research* **208**, 124-131.

6. Casarrubea, M., Sorbera, F., Magnusson, M.S., Crescimanno, G. (2011). T-pattern analysis of diazepam-induced modifications on the temporal organization of rat behavioral response to anxiety in hole-board. *Psychopharmacology (Berl.)* **215**, 177-189.

7. Handley, S.L., Mithani, S. (1984). Effects of alpha-adrenoreceptor agonists and antagonists in a maze exploration model of "fear"-motivated behavior. *Naunyn-Schmiedeberg's Archives of Pharmacology* **327**, 1-5

8. Horn, C., Henry, S., Meyers, K., Magnusson, M.S. Behavioral patterns associated with chemotherapy-induced emesis: a potential signature for nausea in musk shrews. (2011). *Frontiers in Neuroscience* **5**, 1-17.

9. Kemp, A.S., Fillmore, P.T., Lenjavi, M.R., Lyon, M., Chicz-Demet, A., Touchette, P.E., et al. (2008) Temporal patterns of self-injurious behavior correlate with stress hormone levels in the developmentally disabled. *Psychiatry Research* **157**, 181-189.

10. Magnusson, M.S. (2000). Discovering hidden time patterns in behavior: t-patterns and their detection. *Behavior Research Methods Instruments and Computers* **32**, 93-110.

11. Magnusson, M.S. (2004). Repeated patterns in behavior and other biological phenomena. In: Oller DK, Griebel U, editors. *Evolution of Communication Systems: A Comparative Approach. Cambridge: The MIT Press*, 111-128

12. Roy, V., Chapillon, P., Jeljeli, M., Caston, J., Belzung, C. (2009). Free versus forced exposure to an elevated plus-maze: evidence for new behavioral interpretations during test and retest. *Psychopharmacology* **203**, 131-141.