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Research report

Temporal structure of the rat's behavior in elevated plus maze test

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HIGHLIGHTS

► Aim of the research was to evaluate the temporal structure of rat's behavior in the elevated plus maze.
► The ethogram encompasses 24 behavioral elements originating 126 different temporal patterns.
► Rat's behavior shows important rearrangements of its temporal structure during the test progress.
► Behavioral stripes show that t-patterns are not homogeneously distributed during test progress.
► T-pattern analysis is useful to characterize the dynamics of anxiety-related rodent behavior.

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ABSTRACT

Aim of the research was to evaluate, by means of quantitative and multivariate temporal pattern analyses, the behavior of Wistar rat in elevated plus maze (EPM) test. On the basis of an ethogram encompassing 24 behavioral elements, quantitative results showed that 130.14 ± 8.01 behavioral elements occurred in central platform and in closed arms (protected zones), whereas 88.62 ± 6.04 occurred in open arms (unprotected zones). Percent distribution was characterized by a prevalence of sniffing, walking and vertical exploration. Analysis of minute-by-minute duration evidenced a decrease for time spent in open arms and central platform and an increase for time spent in closed arms. As to multivariate t-pattern analysis, 126 different temporal patterns were detected. Behavioral stripes, summarizing distribution of such t-patterns along time, showed that several t-patterns were not homogeneously distributed along the test observational period: t-patterns encompassing behavioral events occurring prevalently in central platform-open arms were observed during the first minutes, whereas t-patterns structured on the basis of events occurring mainly in central platform-closed arms were detected during the last minutes. Therefore, during the observation in elevated plus maze, rat's behavior undergoes significant rearrangements of its temporal features. Present research demonstrates, for the first time, the existence of complex and significantly timed behavioral sequences in the activity of Wistar rats tested in elevated plus maze. Application of t-pattern analysis can provide useful tools to characterize the behavioral dynamics of anxiety-related rodent behavior and differentiate the effect of various anxioselective substances.
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1. Introduction

Introduced by Handley and Mithani [1], the elevated plus maze (EPM) apparatus is a commonly employed behavioral model of animal anxiety predominantly used to study the anxiety-related behaviors and the effects of anxiety modulators [2,3]. Its usefulness has spread towards the understanding of the biological basis of emotionality related to learning and memory, hormones, addiction, and withdrawal [2]. The rationale of EPM in the study of anxiety is based on the strong approach/avoidance conflict induced in naïve subjects by the presence of safe parts of the apparatus that are closed and protected, and open ones, aversive and unprotected [2,4,5]. However, despite the large utilization of the apparatus and the large amount of results reported in literature, no data are available on the temporal structure of rat's behavior in this experimental assay. In particular, important questions need to be addressed: are the behavioral events occurring in EPM structured in recurring sequences? If they are, what is the ethological meaning of these behavioral sequences? Do they undergo specific changes during the test? Are such sequences linked to different zones of the maze?

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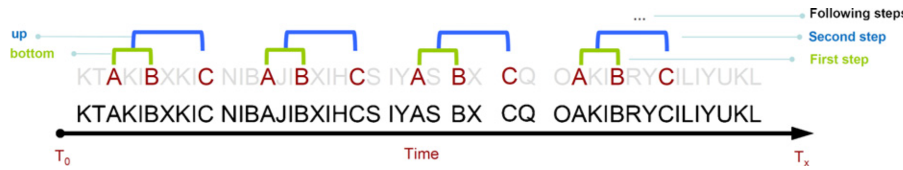

Fig. 1. T-pattern of three hypothetical events during a given observation $T_0 - T_x$ consisting of 45 events (black letters). The ((A B) C) t-pattern, occurring four times, becomes evident if all the other behavioral events are left out (grey letters). "First", "second", and "following steps" (. . .) indicate search runs carried out by Theme's software following a bottom-up detection process.

This study has been designed to shed light on the aforementioned questions. To this aim, a refined analysis of the temporal features of the behavior, known as t-pattern analysis, has been applied. This analysis is a multivariate approach developed to determine whether two or more behavioral events occur sequentially and with significant constraints on the interval lengths separating them [6,7]. In recent years, the t-pattern analysis has been successfully used in several and quite different experimental approaches and various interesting results have been presented [8–15].

For instance, Kerepesi et al. in the study of the interactions between human subjects and animals, have demonstrated that the dog–human interaction is much more regular and structured than yet has been thought [8]; Kemp et al., in the study of human behavior in neuro-psychiatric diseases, have shown that temporal patterns of self-injurious behavior are correlated with stress hormone levels in the developmentally disabled [10]; in addition, Bonasera et al. have applied t-pattern analysis to quantify psychostimulant-evoked route-tracing stereotypy in rodents [11].

2. Method

2.1. Subjects and housing

Twenty-one, 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 $21 \pm 2\,^{\circ}C$, under the following light/dark cycle: light on = 12 noon; light off = 12 midnight. Food and water were freely available.

2.2. Experimental apparatus

EPM was made of ivory Perspex with its arms 50 cm long and 10 cm wide. The apparatus was elevated at a height of 50 cm above the floor. The closed arms were surrounded by a 50 cm wall, the open ones presented 0.5 cm edges in order to maximize open-arm entries [16]. The maze floor 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 closed ones.

2.3. Experimental procedure

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

2.4. Ethogram and coding

Present study has employed an ethogram partially constructed on the basis of previous researches from different authors [17–20]. The result of the coding process is the 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 were coded using 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 (Patternvision Ltd, Iceland; Noldus Information Technology bv, The Netherlands).

2.5. Quantitative analysis

Frequencies and percent distributions were calculated to evaluate the occurrence of each behavioral element and its impact within the comprehensive behavioral repertoire in the maze. In addition, to assess possible modifications during the observational period, the time spent by animals in the open arms, in the central platform and in the closed arms was calculated minute-by-minute.

2.6. Multivariate t-pattern analysis

Theme's detection algorithm searches for relationships between events in behavioral data, by taking into account the order, timing, and frequency of these events [6,7]. Fig. 1 exemplifies four occurrences of a three-element t-pattern within a hypothetical data set encompassing 45 events. The algorithm compares the distributions of each pair of the behavioral elements A and B searching for a time window (interval) so that, more often than chance expectation, A is followed by B within that time window. In this case, A and B are by definition a t-pattern indicated as (A B). In a second step, such first level t-patterns are marked and considered as potential A or B terms in higher patterns, for example, ((A B) C). Thus, more complex t-patterns may be created following this hierarchical bottom-up detection process up to any level. When no more t-patterns are found, the search stops. The comprehensive procedure is called t-pattern analysis.

Before a t-pattern search is performed, the software requires specific search parameters which, in the present study, are: "*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*" percent of subjects in which a pattern must occur to be detected) = 100; "*minimum occurrences*" (minimum number of times a t-pattern must occur to be detected) = 21. Such parameters were selected to obtain the identification of behavioral sequences that were present in all the samples and were characterized by high level of significance. More exhaustive descriptions of concepts, theories and methodological approaches underlying t-pattern analysis, can be found in previous works [6,7,21].

2.7. Graphical representations

Frequencies, percent distributions and durations have been shown respectively, by means of a histogram, a pie chart and a time course diagram. T-patterns can be represented in different ways, one of them consists in the illustration of relationships among events by means of a tree structure [6,7], that is, the dendrogram-like connection diagram shown in Fig. 1. The drawback of such a graphical representation is the space required if a high number of t-patterns is detected. An idea of the drawback is shown through the exemplificative illustration of two t-patterns from only one subject (Fig. 9). An alternative method, we have developed to graphically illustrate t-pattern occurrences, is represented by behavioral stripes (Fig. 8) [14,15], that is, a number of vertical marks indicating the onset of each detected t-pattern along a given time window (*x*-axis).

2.8. Statistics

To assess possible differences in the duration of time spent in the open arms, in the closed ones and in the central platform, and to evaluate possible differences in the mean number of t-patterns detected minute-by-minute in the various zones of the maze, one-way ANOVA followed by Newman–Keuls post hoc test for multiple comparisons was carried out. In addition, as to detected t-patterns, albeit each time interval among events implies the existence of a statistical significance, the large amount of possibilities of such relationships, in data with numerous occurrences of behavioral events, might raise the question on whether the detected t-patterns were different by chance. Theme deals with such an important issue by performing repeated randomizations and analyses of the original data. Thus, the mean number of t-patterns (±1 S.D.) detected in randomized data is compared with the real data. T-patterns found in the real data can be considered not detected by chance when they result more numerous and/or longer than those obtained following randomization.

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