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Multivariate temporal pattern analysis applied to the study of rat behavior in the elevated plus maze: Methodological and conceptual highlights

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HIGHLIGHTS

- The assessment of the temporal characteristics of behavior is a fundamental aspect in the study of rodent's activity in the elevated plus maze.
- The behavior of 15 male naïve SPF Wistar rats has been observed for 5 min in the elevated plus maze following a trial-1 trial-2 testing procedure.
- The behavior has been analyzed using both quantitative and multivariate t-pattern analysis.
- Conceptual, methodological and illustrative aspects concerning the application of t-pattern analysis in the study of rat's behavior in the EPM have been discussed.
- T-pattern analysis could represent a useful tool to study the intimate temporal features of rat's behavior in the elevated plus maze.

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ABSTRACT

Aim of this article is to illustrate the application of a multivariate approach known as t-pattern analysis in the study of rat behavior in elevated plus maze. By means of this multivariate approach, significant relationships among behavioral events in the course of time can be described. Both quantitative and t-pattern analyses were utilized to analyze data obtained from fifteen male Wistar rats following a trial 1–trial 2 protocol. In trial 2, in comparison with the initial exposure, mean occurrences of behavioral elements performed in protected zones of the maze showed a significant increase counterbalanced by a significant decrease of mean occurrences of behavioral elements in unprotected zones. Multivariate t-pattern analysis, in trial 1, revealed the presence of 134 t-patterns of different composition. In trial 2, the temporal structure of behavior become more simple, being present only 32 different t-patterns. Behavioral strings and stripes (i.e. graphical representation of each t-pattern onset) of all t-patterns were presented both for trial 1 and trial 2 as well. Finally, percent distributions in the three zones of the maze show a clear-cut increase of t-patterns in closed arm and a significant reduction in the remaining zones. Results show that previous experience deeply modifies the temporal structure of rat behavior in the elevated plus maze. In addition, this article, by highlighting several conceptual, methodological and illustrative aspects on the utilization of t-pattern analysis, could represent a useful background to employ such a refined approach in the study of rat behavior in elevated plus maze.

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1. Introduction

The construction of an ethogram, namely a list containing descriptions of individual behavioral components represents the first step in the experimental study of animal behavior. By means of the ethogram, the behavior of the subject is "dissected" into discrete components that, in turn, can be characterized by means of quantitative assessments such as latencies, durations, frequencies, percent distributions, and so on. However, as suggested by Spruijt and Colleagues, structural changes in behavior are not necessarily expressed by changes of purely quantitative parameters such as durations or frequencies: the meaning of the behavior lies in the relationships among its constitutive components (Spruijt and Gispen, 1984). As a consequence, a 360° approach to the study of behavior should take into consideration not only quantitative appraisements of each behavioral element but, importantly, also suitable analytical approaches able to assess the above-mentioned relationships. On this subject, multivariate analyses, namely a set of techniques aimed at the evaluation of data sets with more than one variable, represent useful tools for investigating human or animal behavior. Albeit the conceptual bases for these analytical techniques have been available for many decades, medium to high requirements in terms of computational capabilities and the lack of suitable software programs have greatly dampened the diffusion of multivariate data handling for a long time. This problem has been gradually overcome during the last three decades, as a result of the increasing development and diffusion of personal computers. Multivariate analyses are now considered important tools to study the structure of animal behavior in several experimental assays such as the hot plate (Espejo and Mir, 1993, 1994; Casarrubea et al., 2006, 2011b, 2012), the open field (Casarrubea et al., 2008), the hole board (Casarrubea et al., 2009a, 2009b, 2010b, 2010c) or the forced swimming test (Lino-de-Oliveira et al., 2005). Concerning the elevated plus maze, the application of multivariate analyses to study rodent behavior in this experimental assay has a long history. In particular, a specific multivariate approach, namely Factor Analysis, has been widely used (Lister, 1987; File et al., 1993; Trullas and Skolnick, 1993; Cruz et al., 1994; Rodgers and Johnson, 1995; Rodgers and Dalvi, 1997; Wall and Messier, 2000, 2001; Violle et al., 2009). In addition, interesting computational approaches have been proposed to study the behavior of rats in the elevated plus maze (Salum et al., 2000; Tejada et al., 2009; Costa et al., 2012). Even so, as recently underlined by Arantes and Colleagues (Arantes et al., 2013), the lack of detailed information concerning the temporal characteristics of the observed behavior is a common aspect of studies in the elevated plus maze. To fill this gap the t-pattern analysis can be used. Such an analysis is a multivariate technique developed to determine whether two or more behavioral events occur sequentially and with statistically significant time intervals (Magnusson, 2000, 2004). T-pattern analysis has been successfully used to study behavioral modifications in neuro-psychiatric diseases (Kemp et al., 2008), route-tracing stereotypy in mice (Bonasera et al., 2008), interaction between human subjects and animals or artificial agents (Kerepesi et al., 2006), hormonal–behavioral interactions (Hirschenhauser et al., 2002), feeding behavior in broilers (Hocking et al., 2007), patterns of behavior associated with emesis (Horn et al., 2011, 2013) and, in our laboratories, to investigate exploration and anxiety-related behaviors in rodents (Casarrubea et al., 2009c, 2010a, 2011a, 2013a,b).

By applying trial 1–trial 2 protocol (Carobrez and Bertoglio, 2005), the article is aimed at providing methodological information on how the t-pattern analysis could be employed in the study of rat behavior in the elevated plus maze.

2. Method

2.1. Apparatus

The elevated plus maze, introduced by Handley and Mithani in 1984 (Handley and Mithani, 1984), needs little introduction being a widely accepted and used model to assess anxiety-related behaviors in rodents. As already underlined by Carobrez and Bertoglio in their review (Carobrez and Bertoglio, 2005), it is much likely that the popularity of this experimental apparatus, with thousands of published papers so far (Web of Knowledge, 2014), is due to its numerous advantages such as economy, rapidity and simplicity of design. The plus maze usefulness has spread toward the understanding of the biological basis of emotionality related to learning and memory, hormones, addiction and withdrawal (Carobrez and Bertoglio, 2005). Basically, the apparatus consists of an elevated plus-shaped platform with two open arms (OA) and two enclosed arms (CA), originating from a central platform (CP). The rationale underlying the utilization of the elevated plus maze in the study of anxiety-related behaviors is based on the assumption that rodents exposed to the apparatus will respond to a conflict elicited by the presence of safe parts of the maze that are closed and protected, and aversive parts of the maze that are open, unprotected and more brightly lit (Carobrez and Bertoglio, 2005; Arabo et al., 2014; Ohl, 2003). The apparatus we used was elevated at a height of 50 cm above the floor (Roy et al., 2009; Casarrubea et al., 2013a,b). 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 (Treit et al., 1993). The floor of the maze was covered with grey plastic. The testing room was illuminated with a dim white light that provided 100 lux for the open arms, 50 lux for the closed arms and 85 lux for the central platform.

2.2. Subjects

Fifteen, three months old, specific pathogen free, male Wistar rats, randomly selected from a sample of 21 subjects utilized in a recent study (Casarrubea et al., 2013a), were analyzed. 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 freely available.

2.3. Experimental procedure

To minimize transfer effects and avoid possible visual or olfactory influences, rats were transferred from housing room to testing room inside their own home cages and allowed to acclimate for 30 min far from the experimental apparatus. Environmental temperature in testing room was maintained equal to the temperature measured in the housing room. Each rat was placed in the central platform facing an open arm and allowed to explore the maze for 5 min.

All rats, experimentally naïve at the beginning of trial 1, were re-tested in the same apparatus, under the identical environmental conditions of illumination and temperature, after 24 h. After each observation, the apparatus was carefully cleaned with ethyl alcohol to remove possible scent cues left by the animal. Rodents' behavior was recorded through a digital camera placed above the maze, and video files were stored in a personal computer for following analyses.