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Temporal patterns of rat behaviour in the central platform of the elevated plus maze. Comparative analysis between male subjects of strains with different basal levels of emotionality

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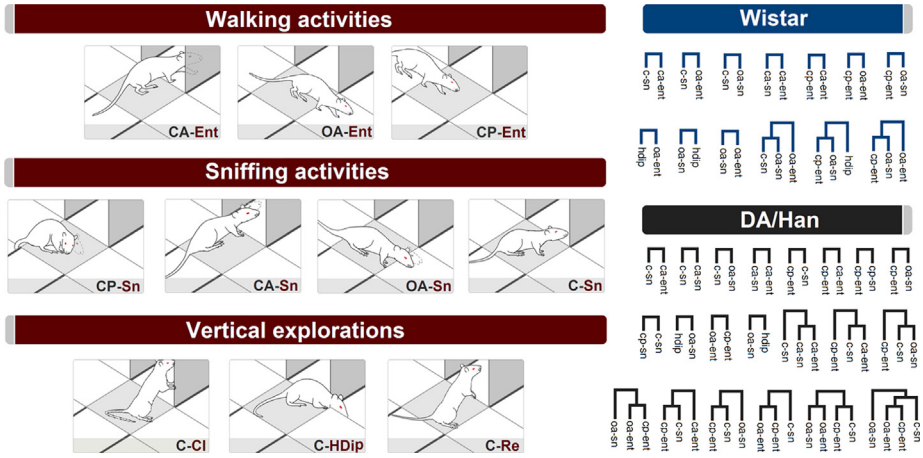
HIGHLIGHTS

- The central platform is the zone of the EPM where the rat selects the arm to explore.
- The behaviour of two strains of rats has been studied in the central platform.
- The temporal structure of rat behaviour has been analyzed through T-pattern analysis.
- Results indicate two different approaches in the interaction with the environment.

GRAPHICAL ABSTRACT

Wistar and DA/Han rats are characterized by a different basal level of emotionality. The application of multivariate T-pattern analysis to study their behaviour in the central platform revealed two completely different behavioural strategies. The illustration shows the ethogram and, on the right, all the different temporal patterns detected in the two strains.



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ABSTRACT

We have analyzed the temporal patterns of behaviour of male rats of the Wistar and DA/Han strains on the central platform of the elevated plus maze. The ethogram encompassed 10 behavioural elements. Durations, frequencies and latencies showed quantitative differences as to walking and sniffing activities. Wistar rats displayed significantly lower latency and significantly higher durations and frequencies of walking activities. DA/Han rats showed a significant increase of sniffing duration. In addition, DA/Han rats showed a significantly higher amount of time spent in the central platform. Multivariate T-pattern

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analysis revealed differences in the temporal organization of behaviour of the two rat strains. DA/Han rats showed (a) higher behavioural complexity and variability and (b) a significantly higher mean number of T-patterns than Wistar rats.
Taken together, T-pattern analysis of behaviour in the centre of the elevated plus maze can noticeably improve the detection of subtle features of anxiety related behaviour. We suggest that T-pattern analysis could be used as sensitive tool to test the action of anxiolytic and anxiogenic manipulations.

1. Introduction

With thousands of published papers so far, the elevated plus maze (EPM) is, probably, the most popular and used experimental apparatus to assess anxiety-related behaviour in rodents (Web of Knowledge, 2015). The EPM usefulness has spread towards the understanding of the basis of emotionality related to learning and memory, addiction and withdrawal, as well as phobia, generalized anxiety and post-traumatic stress (Carobrez and Bertoglio, 2005). The apparatus consists of an elevated plus-shaped platform comprehending four arms extended from a common central platform: two facing arms lack of enclosures and are more brightly lit (so called open arms), the remaining two facing arms are surrounded by opaque enclosures and, therefore, are less illuminated (so called closed arms). Basically, the rationale of the utilization of EPMs orbits around the evidence that naïve rodents, usually very cautious in novel and unfamiliar environments, show a clear-cut preference for the closed arms and only subjects with a reduced anxiety level increase their activity in the open arms (Carobrez and Bertoglio, 2005). In such a context, the central platform of the apparatus is the zone of the EPM where the animal selects "*what to do*" or, in other terms, the zone where decision making processes are carried out (Rodgers et al., 1992; Rodgers and Johnson, 1995; Cruz et al., 1994; Casarrubea et al., 2015a). Simply stated, the central platform of the EPM, on the basis of its position and structural characteristics, is crucial in moulding the actual behaviour of the rodent in the apparatus. Consistently, the importance of studies evaluating the behaviour of the rodent in the central platform has been underlined since two decades ago (Rodgers and Dalvi, 1997). Even so, surprisingly scanty data do take into consideration the behaviour of the rodent in the central platform of the maze. To shed light on this matter, we have recently focused on the behaviour of two strains of rats with different emotional reactivity, analyzed in the central platform of the EPM (Casarrubea et al., 2015a). By using a multivariate approach based on transition matrices elaboration we have demonstrated that rats with different reactivity to anxiogenic stimuli present profound differences in behavioural switching, that is, the ability to move from a behaviour to another one. However, beyond transitions among the elements of the behavioural repertoire, an interesting issue still represents matter of debate deserving supplementary experimental analyses: the real time structure of rat behaviour in the central platform. Actually, transition matrices and related elaborations (e.g. dendrograms, stochastic path diagrams or adjusted residuals) (Spruijt and Gispen, 1984; Espejo and Mir, 1993; Espejo, 1997; Lino-de-Oliveira et al., 2005; Casarrubea et al., 2009, 2010a, 2010b, 2011, 2012; Kalueff and Tuohimaa, 2004; Van Den Berg et al., 1999; Vanderschuren et al., 1996) do represent the behaviour resembling a snapshot of the entire period of observation: information is not provided on the real time structure of behaviour. On the contrary, by means of T-pattern analysis, the real time structure of behaviour can be minutely analyzed (Magnusson, 2000; Casarrubea et al., 2009, 2013a, 2013b, 2014, 2015b). In present paper, we use multivariate T-pattern analysis to investigate the temporal structure of behaviour in the central platform of the maze in Wistar and in DA/Han strain, the latter has been demonstrated to possess a behavioural profile compatible with a high anxiety level (King, 1999; Mechan et al., 2002; Chapillon et al., 2002; Roy and Chapillon, 2002, 2004; Roy et al., 2003, 2009; Casarrubea et al., 2013b, 2015a). By providing information on the temporal structure of the behaviour in the central platform, new insights on rat behavioural dynamics in the elevated plus maze can be provided. A particular attention will be dedicated to methodological aspects of the experiment.

2. Method

2.1. Subjects

Ten male Wistar and ten male DA/Han, SPF, rats were used. Both Wistar and DA/Han subjects were three months old. 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 available "ad libitum".

2.1.1. Ethical statement

All efforts were carried out to minimize the number of animals used and their suffering. The experiments were conducted in accordance with the European Communities Council Directive 86/609/EEC concerning the protection of animals used for experimental scientific purposes.

2.2. Experimental apparatus

The experimental apparatus was made of Perspex. Each arm was 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 opaque wall, the open ones presented 0.5 cm edges to maximize open-arm entries (Treit et al., 1993). The floor of the apparatus was covered with grey plastic. Environmental temperature in testing room was continuously measured and maintained equal to the temperature in housing room. The testing room was illuminated with a dim white light providing 100 lux in the open arms, 85 lux in the central platform and 50 lux in the closed arms.

2.3. Experimental procedure

Rats were transported from housing to testing room inside their home-cages to minimize transfer effect. Each rat was allowed to acclimate for 30 min far from the observational apparatus to avoid possible visual and/or olfactory influences. Each subject, experimentally naïve, was placed in the central platform of the maze, facing an open arm, 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. Rat behaviour