



## Research report

# The effects of different basal levels of anxiety on the behavioral shift analyzed in the central platform of the elevated plus maze



Maurizio Casarrubea, Fabiana Faulisi, Filippina Sorbera, Giuseppe Crescimanno\*

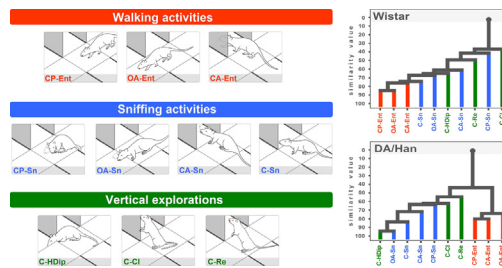
Dept. of Experimental Biomedicine and Clinical Neurosciences, Human Physiology Section, Laboratory of Behavioral Physiology, University of Palermo, Palermo, Italy

## HIGHLIGHTS

- Wistar and DA/Han rats were studied in EPM.
- The study was focused on rat activity in the central platform of the EPM.
- A complex structure of rat behavior in central platform was identified.
- Different basal levels of anxiety influence the behavioral architecture.
- Analysis of transition matrix allows the study of behavioral shift.

## GRAPHICAL ABSTRACT

Wistar and Dark Agouti (DA/Han) rats are two strains characterized by a different reactivity to anxiogenic stimuli. Such a different level of reactivity can be detected in terms of objective behavioral measurements in the central platform of the elevated plus maze test. Silhouettes on the left side of the image indicate the behavioral components included in the analysis. Dendrograms on the right side present the results obtained by means of hierarchical clustering analysis. A cascade-shaped dendrogram, branching from Walking activities, indicates that Wistar rat behavior is oriented to cross the central platform so to rapidly reach an arm; the dendrogram of DA/Han rats displays a behavior heavily oriented toward the permanence in the central platform.



## ARTICLE INFO

## Article history:

Received 16 October 2014  
 Received in revised form 5 December 2014  
 Accepted 7 December 2014  
 Available online 12 December 2014

## Keywords:

Anxiety  
 Behavioral shift  
 Decision making  
 DA/Han rat  
 Elevated plus maze  
 Multivariate analysis  
 Wistar rat

## ABSTRACT

The aim of the present research was to study the effects of different basal levels of anxiety on the behavioral shift studied in the central platform of the elevated plus maze. To this purpose, quantitative and multivariate analyses, the latter based on transition matrix elaboration, were carried out on Wistar and on DA/Han rats the latter belonging to a strain characterized by different reactivity to anxiogenic stimuli. Wistar rats spent  $74.11 \pm 5.11$  s in the central platform, whereas DA/Han significantly more:  $127.08 \pm 9.87$ . Per cent distributions evidenced a clear-cut difference in walking activities (46.25% in Wistar, 28.4% in DA/Han rats) and in the sniffing activities (45.82% in Wistar, 62.54% in DA/Han). Mean frequencies of each behavioral element showed in DA/Han strain a value significantly lower than in Wistar for central-platform entry, open arm-entry and closed-arm entry and a significant higher value for central-platform sniffing, open-arm sniffing and corner-rearing. Moreover, the ratio open-arm entry/open-arm sniffing and closed-arm entry/closed-arm-sniffing showed significant higher values in the Wistar strain. Finally, by means of hierarchical clustering analysis, strong differences between the two strains were observed in the behavioral architecture: a cascade-shaped dendrogram, branching from Walking activities, indicates that Wistar rat behavior is oriented to cross the central platform so to rapidly reach an arm; on the contrary, the dendrogram of DA/Han rats displays a behavior heavily oriented toward the permanence in the central platform.

\* Corresponding author at: Corso Tukory 129, 90134 Palermo, Italy. Tel.: +39 0916555847; fax: +39 0916555847.

E-mail addresses: [maurizio.casarrubea@unipa.it](mailto:maurizio.casarrubea@unipa.it) (M. Casarrubea), [fabiana.faulisi@unipa.it](mailto:fabiana.faulisi@unipa.it) (F. Faulisi), [filippina.sorbera@unipa.it](mailto:filippina.sorbera@unipa.it) (F. Sorbera), [giuseppe.crescimanno@unipa.it](mailto:giuseppe.crescimanno@unipa.it) (G. Crescimanno).

The results show that different basal levels of anxiety provoke significant differences in the behavioral shift studied in the central platform of the elevated plus maze. Such differences, evidenced by means of transition matrices elaboration, might represent the behavioral expression of anxiety-induced modifications of decision making process underlying behavioral shift activities.

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## 1. Introduction

Anxiety, in humans, is defined as a long-lasting feeling of distress and/or apprehension for no evident reason [1]. Such a condition can be extremely disruptive to everyday life as it generates a decline of: life quality, inter-personal relations, normal everyday actions and job-related activities [1]. Thus, along with its neuropsychiatric correlates, significant consequences of anxiety disorder are of behavioral magnitude. Several animal models of anxiety have been proposed, all involving the exposure of the subject to anxiogenic stimuli resembling the ones capable of causing anxiety in humans [2].

The elevated plus maze (EPM), developed as a test able to screen benzodiazepine-like compounds, is probably the most applied model to assess anxiety-related behaviors in rodents [2]. The test evokes curiosity and fear at the same time thereby causing a typical approach/avoidance conflict [2–5]. There is substantial evidence that, during the conventional 5 min test, the rodent has a clear preference for the closed arms and only subjects with a reduced anxiety level do increase their activity in the open arms [3]. However, as suggested by various Authors such as Rodgers and Dalvi [2] or Carobrez and Bertoglio [3], cautiousness is always advised in the assumption that open arm avoidance means anxiety in the tested subject. Concerning the central platform (CP) of the EPM, it has been reported that the time spent in this zone of the maze is linked to decision making processes triggered by the approach/avoidance conflict [6–8]. In addition, previous studies have demonstrated that Wistar and Dark Agouti (DA/Han) rats (the latter belonging to a strain characterized by different reactivity to anxiogenic stimuli) present profound differences in the temporal structure of the behavior in the Elevated Plus Maze. These differences have been suggested to express different behavioral strategies in response to anxiogenic stimuli [9,10]. In particular, DA/Han rats have been shown to stay longer in the CP so suggesting a close relationship between the rodent's basal level of anxiety and the time spent in the CP [10]. However, despite the evidences on the central role of CP in addressing rodent's activity, there is relatively little known about the interpretation of the behavior in this zone of the maze. Therefore, our study was aimed at investigating the fine organization of rat behavior in CP and its relationships with the behavioral shift in subjects with different reactivity to anxiogenic stimuli. To this purpose, an experiment was carried out where the behavior in the CP was deeply analyzed in Wistar and DA/Han strain of rats. Since in the CP the animal carries out specific behavioral shifts (e.g., from the sniffing of a corner to the entry in the arm; from the entry in the central platform to the dipping of the head toward the floor etc.) a multivariate analysis, based on transition matrices elaboration, has been applied. This kind of analysis has been demonstrated to be able to reveal the structure of a behavior and its underlying dynamics [11–22].

## 2. Method

### 2.1. Subjects

Ten male Wistar and ten male DA/Han SPF rats were used. The DA/Han strain has been well characterized, in particular as to emotional reactivity tests [23–26]. The peculiar higher anxiety level

of the DA/Han rat could depend on an alteration of the enzymes belonging to the CYP2D- subfamily (member of the cytochrome P450 system) [27], whose activity has been demonstrated to be markedly reduced in the DA/Han rat, in comparison with the Wistar one [28]. Interestingly, also in humans, a correlation has been shown between the activity of specific enzymes belonging to this family and anxiety disorders [29]. 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  $21 \pm 2$  °C, under the following light/dark cycle: light on = 12 noon; light off = 12 midnight. Food and water were available "ad libitum".

### 2.2. Experimental apparatus

EPM was made of ivory Perspex, 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 [30]. The maze floor was covered with gray plastic. Environmental temperature was maintained equal to the temperature measured in the housing room. The testing room was illuminated with a dim white light providing 100 lx in the open arms, 85 lx in the central platform and 50 lx in the closed arms.

### 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. The rodents' behavior was recorded by means of a video camera, and video files were stored in a personal computer for subsequent analyses.

### 2.4. Data analysis

The behavioral elements characterizing the activity of the rat in the CP were selected and described by means of an ethogram. Video files were frame-by-frame analyzed using a personal computer equipped with a software coder (The Observer, Noldus IT, The Netherlands). Raw data were analyzed by means of quantitative approaches and by means of multivariate approaches based on the elaboration of transition matrices.

#### 2.4.1. Quantitative analysis

Min-by-min and overall average time spent in the CP, the mean number of all the behavioral components, the mean number of each behavioral component and their per cent distribution were calculated for both strains. Moreover, the ratios between the entrances in the arms and the preceding sniffings, i.e., open arm entries/open

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