Combined effects of illumination, closed wall type and extramaze space size on the anxiety-related behavioral baseline of rats submitted to the elevated plus-maze

N. Violle, F. Balandras, Y. Le Roux, D. Desor, and H. Schroeder

URAFPA, INRA UC340, Nancy-Université, Vandœuvre-lès-Nancy, France, Henri.schroeder@scbiol.uhp-nancy.fr

Introduction

The elevated plus-maze (EPM) is currently used to study the neural basis of anxiety as well as to assess the anxiety-modulating activity of various pharmacological agents. Although the anxiogenic stimulus is supposed to be the open space in this test, the anxiety-related behavioral baseline of rats is sensitive to numerous methodological-linked parameters [1]. In order to improve the quality of behavioral measurements, some modifications have been performed on both original apparatus or procedures initiated by Pellow et al. [2]. Two modifications are often reported: 1) rats are tested under a red light in order to mimic the dark phase of the cycle, and 2) some laboratories used a modified EPM with translucent closed walls to increase the accuracy of the behavioral scoring. At present, the combined effects of these two modifications on spontaneous rat behavior in the EPM have not been sufficiently investigated. Besides, the effect of extramaze space size (i.e. size of the experimental room) on this baseline remains untested. However, an inadequate behavioral baseline may reduce the efficacy of the EPM for measuring anxiety variations in animals. Thus, this study aimed to clarify the respective effects of illumination (ILL), closed-wall type (CWT) and extramaze space size (ESS) on the anxiety-related behavioral baseline of rat in the EPM.

Material and methods

The experiment was carried out on 80 naïve Wistar male rats (Harlan, Gannat, France) maintained in a regulated environment (20±1°C; humidity 50±5%; free access to food and water) under a reversed light/dark cycle (light on at 7 p.m.). Two EPMs were used: a Plexiglas one with translucent walls and a wooden one with opaque walls. They presented identical dimensions and configuration: two opposed open arms (40 x 10 cm) and two opposed closed arms with walls (40 x 10 x 40 cm) linked by a central square (10 x 10 cm) and elevated at 70 cm of height. The EPMs were placed at the centre of a small (2 x 1.7 m) or a spacious experimental room (3.9 x 3.5 m). These two rooms presented identical floors, ceilings, and uniformly white-painted walls. They were empty except for the EPM and the recording facilities. The illumination of the rooms was provided by either 2 white bulbs or 4 red bulbs. The light was homogeneously fitted at 40 lux of intensity by using a luxmeter in such way as the maximal difference observed between the different parts of maze never exceeds 5 lux. Thus, animals were randomly allocated to the 8 experimental groups (10 rat/group) defined by the combination of the 3 tested factors (see table 1). For the testing, each rat was placed at the centre of the maze, head turned toward an open arm, and let freely explore for 5 min. Testing was recorded with a video camera. The number of closed arm entries, the number of open arm entries and the time spent in the open arms were scored from videotapes. The percentage of entries in the open arms and the total number of entries were also calculated. The rat was considered in a part of the maze when is 4 paws were.

Table 1. *Allocation of the 80 rats in the 8 experimental groups defined by the combination of the three tested variables: closed wall type, illumination and extramaze space size.*

		Extramaze space size	
Closed wall type	Illumination	Small	Spacious
Opaque	Red	n =10	n =10
	White	n =10	n =10
Translucent	Red	n =10	n =10
	White	n =10	n =10

Results

The three-way ANOVA detected significant main effects of ILL, CWT and an interaction between the ESS and the CWT, on behavioral items related to anxiety, i.e., the number open arm entries, the time spent in the open arms (see Figure 1) and the percentage of entries in the open arms. Data analysis indicates that exposure to the white light induced decreases of these 3 behavioral items referred to the red light. Rat behavior presented the same variations when tested in the EPM with opaque closed walls compared to the one with translucent walls. *Post-hocs* also indicates that this closed wall effect was higher in the spacious experimental room than in the small one. Regarding the number of closed arm entries, the statistical analysis showed only a main effect of the ESS which resulted in a significant reduction of this variable in animals tested in the spacious room. Finally, ANOVA indicates main effects of the three factors with interactions between CWT and ESS and between ILL and ESS on the total number of entries.

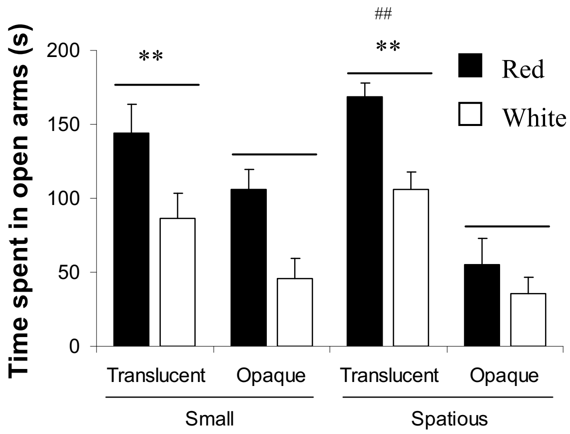

Figure 1. *Effects of illumination (red vs white), closed wall type (translucent vs opaque) and extramaze space size (small vs spacious) on the time spent in the open arms. Data are presented as mean ± S.E.M. Three-way ANOVA detected main effects of illumination and closed wall type (not showed). Tukey's multiple comparisons test was used to study the interaction between closed wall type and extramaze space size: ** p<0.01 different from rats tested in the EPM with translucent walls in the spacious extramaze space. ## p<0.01 different from rats tested in the EPM with opaque walls in the small extramaze space.*

Discussion

Our findings clearly indicates that environmental parameters can strongly modify the animal behavior in the EPM. Concerning ILL, the use of a white light instead of a red one increased the open space avoidance whatever the CWT and ESS, probably due to a less perception of the light intensity when a red one is used [3]. Whatever the type of light used (red or white), the EPM with opaque walls has been perceived as more protective than the translucent one whereas the light intensity was the same everywhere in the maze. Moreover, we founded that the spacious ESS enhanced the effect of the CWT. This result suggests that a spacious ESS may increase the anxiogenic feature of the open arms, but this effect would be more efficient when the closed arms are well discriminated (i.e. opaque walls). Thus, the rat anxiety-related behavioral baseline in the EPM seems to be the result of the perception and the integration of multiple environmental stimuli and to depend on the level of discrimination between the aversive feature of the open space and the relative protection offered by the closed arms. In conclusion, the choice of adequate ILL, CWT and ESS for EPM testing is critical to obtain a behavioral baseline in rats that allowed the detection of an anxiolytic drug. These conditions of testing were applied to the assessment of the activity of the well-known anxiolytic agent, diazepam.

References

1. Rodgers, R.J., Dalvi, A. (1997) Anxiety, defence and the elevated plus-maze. *Neurosciences and Biobehavioral Reviews* **21**, 801-810.
2. Pellow, S., Chopin, P., File, S.E., Briley, M. (1985) Validation of open/closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods* **14**, 149-67.
3. Jacobs, G.H., Neltz, J., Deegan, J.N. (1991) Retinal receptors in rodents maximally sensitive to ultraviolet light. *Nature* **353**, 655-656.