How to Improve and Refine the Elevated Plus Maze for Laboratory Mice

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BACKGROUND

The daily light-dark cycle synchronizes numerous physiological and behavioral aspects. Mice are among the main animals used in behavioral neuroscience laboratories. Although nocturnal, they are generally tested during their resting phase, under light conditions convenient for the experimenter. This practice leads to a double concern: one about the quality of the scientific data collected and the other about ethics in animal research[1,3]. Insofar as the animal is tested at an inappropriate moment of the circadian sleep-wake rhythm, its performance may not truly reflect its abilities. In some cases, this practice could generate stress and anxiety, disrupt behavior and cognition and even cause health issues[2,3]. The time of day could be a critical variable affecting animal behavior and contribute to the reproducibility issue affecting behavioral neuroscience[1].To date, data on the influence of moment of the test on anxiety are lacking and existing data seem contradictory[5,6].

In this study, we focused on an anxiety test, the elevated plus maze (EPM), and tried to replicate our previous results[7] with a higher power, which partially suggested a circadian effect on anxiety. Comparatively to our first attempt, we used a more refined observation protocol which includes a larger set of classics and ethological behavioral measures[4].We tested mice at 4 different moments to determine if there was a more appropriate testing moment where mice show the less anxiety.

METHODS

Animals and housing conditions: 88[#] female mice C57/BL6 were pair-housed under a standard light-dark cycle (lights on at 09:30 and off at 21:30, N=44) or under a reversed light-dark cycle (lights on at 21:30 and off at 09:30, N=44). Mice were tested in the beginning or in the middle of the light/dark phase (ZT01, N=20, and ZT05, N=24, during the light phase and ZT13, N=20, and ZT17, N=24, in the dark phase).

[#] The number of subjects per group is unequal because the EPM sessions of several mice were not recorded correctly for technical reasons.

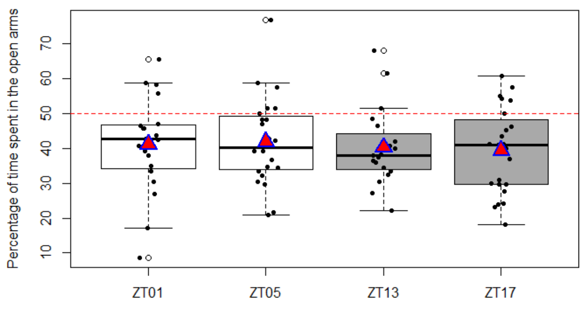
Apparatus and procedure: anxiety-related behaviors were collected in an elevated plus maze, under 75 lux for 5 min.

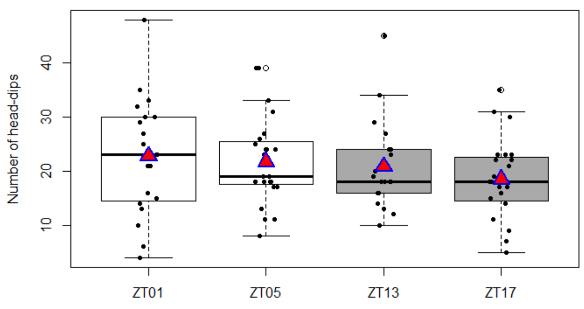
Behavioral measures: the percentage of time spent in the open arms and the total number of entries in both closed and open arms were used as conventional measures. The number of head-dips, rearing, stretching and enclosed arm returns were used as ethological measures. Two observers (one blind to mice condition) scored the behaviors, and the interrater reliability was calculated for each behavioral measure.

Statistical analyses: the conditions of application of the statistical tests have been verified. Two-way ANOVA with *phase* (light or dark) and *moment* in the phase (beginning or middle) as between-subjects factor were used on all behavioral measures. Three-way ANOVA with *phase* (light or dark) and *moment* in the phase (beginning or middle) as between-subjects factor and *time bin* as within-subjects factor were used to assess the minute-by-minute analysis on all behavioral measures[5]. Only the percentage of time spent in the open arms (ICC = 0.94) and the number of head-dips (ICC=0.92) are presented here (behavioral measures also assessed during our first experiment).

RESULTS

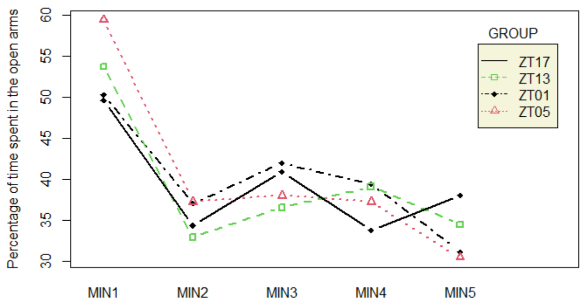
Full-session analysis

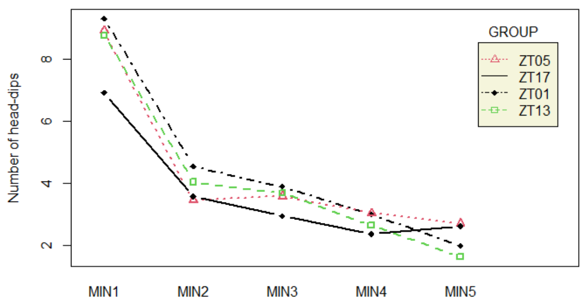




There was no main effect on percentage of time spent in the open arms (*phase*: $F(1,84)=0.785$, $p=0.378$, $\eta^2<0.01$; *moment*: $F(1,84)=0.006$, $p=0.94$, $\eta^2=0.02$), or on head-dips (*phase*: $F(1,84)=2.016$, $p=0.159$, $\eta^2=0.02$; *moment*: $F(1,84)=0.453$, $p=0.503$, $\eta^2<0.01$). No interactions were found between both factors.

Minute-by-minute analysis





A main effect of the time-bin is found in the time spent in the open arms ($F(4,336)=86.879$, $p<0.01$, $\eta^2=0.36$) and in the head-dips ($F(4,366)=26.116$, $p<0.01$, $\eta^2=0.16$). No interaction was found between the factors.

CONCLUSIONS

In our previous experiment, the animals tested during their active phase (or dark phase) did significantly more head-dips; this may suggest less anxiety for this group. However, a minute-by-minute analysis underlined that this significant effect was present only during the first two minutes of the EPM test. If the phase effect really exists, it is a very short-term effect. In the present experiment, we did not replicate that effect despite increasing the sample size and doing a more refined analysis of behaviors. Moreover, no main effect on other anxiety-related behavior was found. Thus, a proposal to refine the analysis of anxiety-related behaviors during EPM would be to consider their intra-session evolution already noted in the literature[5]. Moreover, when significant effects are found, one can still question the strength of the evidence[8] (too rarely the power and size of the effects are mentioned). To conclude, recent literature still discusses the validity of this test[9], which has been used for several decades; it also shows that the evaluation of anxiety remains complex since the measures obtained from anxiety-related behaviors in different anxiety tests (such as the EPM, the open-field and the white and black box) are not necessarily correlated.

REFERENCES

[1]Nelson, R. J., Bumgarner, J. R., Walker II, W. H., & DeVries, A. C. (2021). doi : j.neubiorev.2021.05.017

[2]Peirson, S. N., Brown, L. A., Pothecary, C. A., Benson, L. A., & Fisk, A. S. (2018). doi : 10.1016/j.jneumeth.2017.04.007

[3]Hawkins, P., & Golledge, H. D. R. (2018). doi : 10.1016/j.jneumeth.2017.05.014

[4]Espejo, E. (1997). doi : 10.1016/S0166-4328(96)02245-0

[5]Bertoglio, L.J., & Carobrez, A.P. (2002). doi : 10.1016/s0166-4328(01)00396-5

[6]Bilu, C., & Kronfeld-Schor, N. (2013). doi : 10.3109/07420528.2013.773439

[7]Zaros, A., & Ferrara, A. (2021). https://hdl.handle.net/2268/264467

[8]Bespalov, A., & Steckler, T. (2018). doi : 10.1016/j.jneumeth.2017.10.018

[9]Carola, V., D'Olimpio, F., Brunamonti, E., Mangia, F. & Renzi, P., T. (2002). doi : 10.1016/S0166-4328(01)00452-1

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