Effects of Testing Moment on Behaviour and Cognition in Mice

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BACKGROUND

The daily light-dark cycles allow the synchronization of behavioural and physiological processes to the external environment. Light is the most important environmental cue or *zeitgeber* that coordinates many aspects such as activity, maintenance behaviours, alertness, body temperature, hormonal regulation or long-term potentiation[1,2]. Mice are among the main animals used in behavioural neuroscience and preclinical research laboratories. Although nocturnal, they are generally tested during day (i.e. during their resting phase). Even if convenient for the experimenter, any perturbation of the sleep-wake cycle (such as manipulations during day), may generate some stress to the animal, produce few reliable data and lead to negative consequences for health, physiology, behaviour and cognition [2,3]. Thereby, the testing moment might be a predominant variable affecting animal behaviour and therefore all the inferences we make about cognitive processes. Recently, a lack of data related to the effect of testing moment on behaviour has been reported [2,3]. Moreover, several studies evaluating the effects of testing moment on learning, memory, attention or cognitive flexibility have shown conflicting results; these discrepancies can be explained by various methodological parameters such as light conditions during test, the strains, the species or the material used.

In this study, we focused on an anxiety test (the Elevated plus Maze, EPM) and a memory test (the Object Recognition Test, ORT). We compared subjects' performance at 4 different testing moments (two in the active phase and two in the resting phase) to determine if there was a more appropriate testing moment for animal experimentation where mice show reduced anxiety and the best performance. Concerning the ORT, we showed the way to analyse the behaviour may also affect the conclusion about the influence of the testing moment on the performance.

METHOD

Animals and housing conditions : 72 female C57/BL6 mice were pair-housed under a standard light-dark cycle (lights on at 09.30 and off at 21.30, N=36) or under a reversed light-dark cycle (lights on at 21.30 and off at 09.30, N=36). Mice were tested at the beginning or the middle of the light/dark phase (respectively, ZT01 and ZT05 in the light phase and ZT13 and ZT17 in the dark phase).

1. Elevated plus Maze

Apparatus and procedures : the investigation of the anxiety-related behaviours occurred in an elevated plus maze test with an illumination of 60 lux for 5 min.

Behavioural measures : Percentage of time spent in the open arms, percentage of entries into the open arms, number of head dips and the total number of entries in both arms.

2. Object Recognition test

Apparatus and procedure : behavioural training occurred in a circle arena made of opaque plastic (Ø 45 cm). The light level was 10 lux at the centre of the arena. Usual objects were used (glass bottles and plastic coloured build blocks). The test was divided in 3 phases : (1) habituation phase to the empty arena (1 hour), (2) familiarization phase with two identical objects (3-minute session), (3) test phase (3-minute session with replacement of a familiar object by a new and different one) with a retention interval of 1 hour.

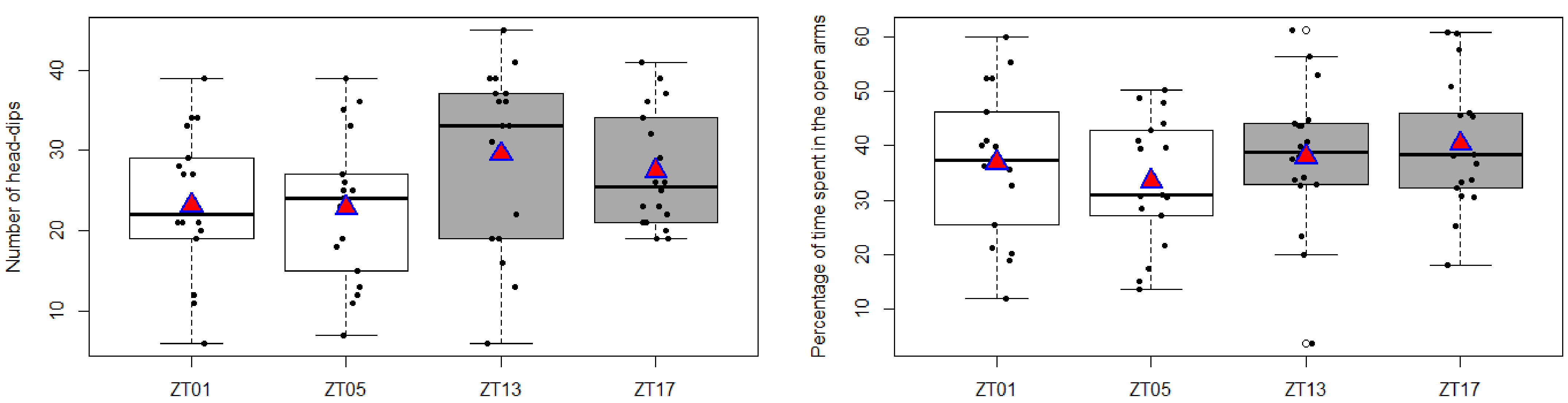
Behavioural measures : we used a classical memory index (d2) based on the exploration time of each object reflecting, through the preferential exploration of the new object, that a memory trace was kept of the familiar object. This index was computed from the exploratory behaviour in the first minute and in the three minutes of the test.

Statistical analysis : the conditions of application of the statistical tests have been verified.

- Two-ways ANOVA with phase (light or dark) and testing moment (beginning or middle) as between factor on all behavioural measures.
- One-sample t-test to compare d2 to the chance level for each group in the ORT.

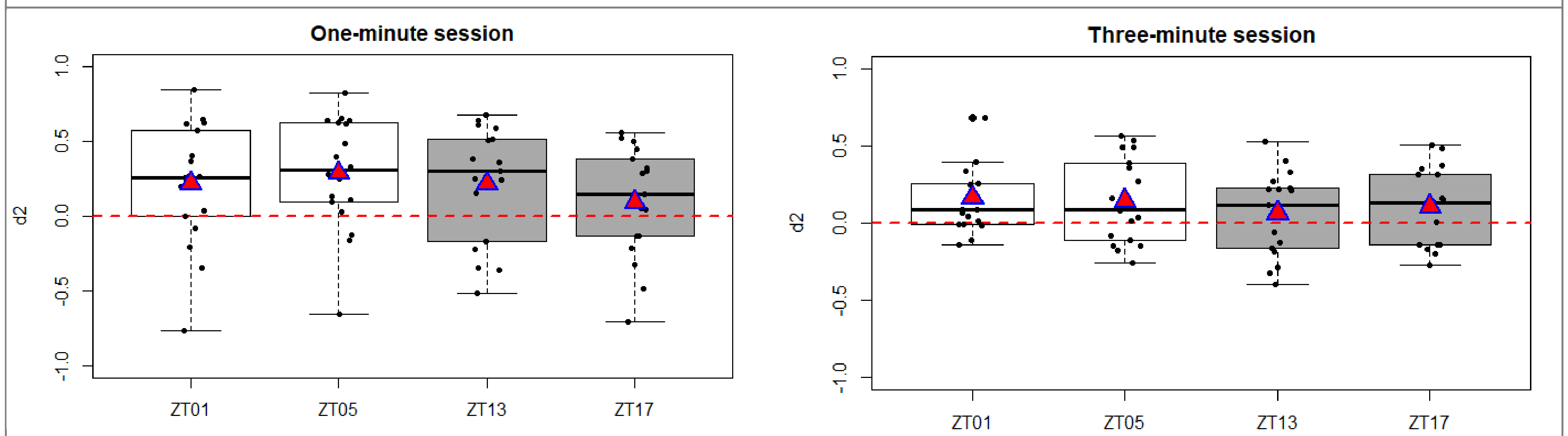
RESULTS

1. Elevated Plus Maze



There was no main effect on total entries (*phase* : F(1,67)=0.02, p=0.86 ; *testing moment* : F(1,67)>0.01, p=0.98), on percentage of entries in open arms (*phase* : F(1,67)=0.61, p=0.43 ; *testing moment* : F(1,67)=1.24, p=0.26), on percentage of time spent in the open arms (*phase* : F(1,67)=1.71, p=0.19 ; *testing moment* : F(1,67)=0.02, p=0.87) but there is an effect of phase on the head-dip (F(1,67)=6.12, p=0.01) but not of the testing moment (F(1,67)=0.29,p=0.58). No interactions were found.

2. Object Recognition test



There was no main effect of phase (F(1,66)=1.16, p=0.28) or testing moment (F(1,66)=0.07, p=0.78). No interaction were found.

One sample t-test comparing the mean of each group to the chance level suggests that the discrimination index for the ZT01, ZT05 and ZT13 group is significantly greater than 0 (ZT01: t(17)=2.27; p=0.01, ZT05: t(17)=3.25; p<0.01, ZT13: t(16)=2.23; p=0.02, ZT17: t(16)=0.99; p=0.16).

There was no main effect of phase (F(1,66)=1.16, p=0.20) or testing moment (F(1,66)=0.06, p=0.80). No interaction were found.

One sample t-test comparing the mean of each group to the chance level suggests that the discrimination index for the ZT01, ZT05 and ZT17 group is significantly greater than 0 (ZT01: t(17)=3.26; p<0.01, ZT05: t(17)=2.38; p=0.01, ZT13: t(16)=0.79; p=0.22, ZT17: t(16)=1.86; p=0.04).

CONCLUSIONS

In most of our measures in the EPM, we did not find any effects of testing moment except when analysing the number of head-dips. The animals tested during their active phase do more head-dips than mice tested during their resting phase regardless of the testing moment in the phase, which could translate less anxiety in those groups. However, considering the analyses of the other anxiety-like behaviours we could not conclude to between-group differences in anxiety level. The joint use of multiple tests measuring anxiety such as the open field and the light-dark box in addition to the EPM in the same study should improve the assessment the anxiety behaviour.

Regarding the ORT, we noticed some variability between studies concerning the test session duration which could also explain conflicting conclusions about the effect of the moment of the test on the performance. As mentioned earlier, the preference for the new object may only be expressed for a short time at the beginning of the test session[4]. We conducted our analysis of the ORT on the first minute and on the three minutes of the test session. When comparing these two analyses, not the same groups of animals discriminate the new object from the familiar one; so, differently defining the observation time leads to different conclusions. The duration of the test session is potentially an important parameter and might be further considered and justified when analysing the behaviour in such a test.

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