Effects of testing moment in the circadian sleep-wake rhythm on learning, memory and behavioural flexibility

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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 of physiology and behaviour such as activity, maintenance behaviours, alertness, body temperature, hormonal regulation or long-term potentiation (i.e. a process that plays a key role in memory consolidation)[1,3]. 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, under the standard laboratory lightning condition). Convenient for the experimenter, manipulations during day can generate some stress to the animal and produce few reliable data[2,3]. Moreover, several cognition studies about memory, learning, cognitive flexibility or attention have shown that mice performed better when tested during their active phase (i.e. the night)[2,3]. Thereby, the testing moment might be a predominant variable affecting animal behaviour and therefore all the inferences we make about cognitive processes. Nevertheless, a lack of data related to the effect of testing moment on behaviour was recently highlighted[2].

In this study, we focused on a memory test (the Object Recognition Test, ORT) and on a test of learning and behavioural flexibility (the operant Attentional Set-Shifting Task, ASST). Due to their ease of use, both tasks are widely used but rarely with the testing moment as an independent variable. Moreover, particularly for the ORT, those studies have led to conflicting results. Some authors underlined a better performance in mice in their active period whereas others highlighted a better performance in mice in their rest period. Theses discrepancies can be due to several methodological differences such as the nature of the tasks, the stimulation rate by direct or indirect manipulation, the difficulty of the task, the strain or specie used as well as the light testing condition. We hypothesize that mice tested during their active period will perform at least as good as mice tested in their rest period, arguing a change in laboratory practice.

METHOD

Animals and housing conditions : 24 female C57/BL6 mice were singly-housed under a standard light-dark cycle (lights on at 08.00 and off at 20.00, N=12) or under a reversed light-dark cycle (lights on at 20.00 and off at 08.00, N=12). They were maintained around 85% of their reference bodyweight during the operant task.

1. Object recognition task

Apparatus : Behavioural training occurred in a circle arena made of opaque plastic (Ø 45 cm). The light level was 50 lux at the center of the arena. For objects, we used glass bottles and plastic colored build blocks.

Procedures :
(1) Habituation phase to the empty arena (2x10 minutes). (2) Familiarisation phase to the identical objects (3-minute session). (3) Test phase (3-minute session) ; retention interval: 1 hour. We used a classical memory index (d2) based on the exploration time of each object[4] reflecting through the preferential exploration of the new object that a memory trace was kept of the familiar object.

2. Attentional set-shifting task

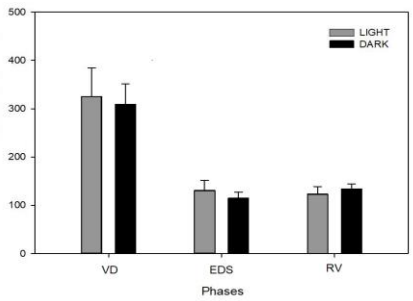
Apparatus : Behavioural training occurred in classic mouse modular operant chambers (MED Associates; St. Albans, VT) equipped with two nose poke devices for the operant response, stimulus lights and the reward dispenser. The only sources of light were the visual stimuli above each nose poke. The behavioural procedure used was inspired by Ortega et al. (2013)[5].

Procedures :
(1) Visual discrimination (VD). (2) Extradimensional shift (EDS). (3) Reversal learning (RV). Mice moved to the next stage when they reached the performance criterion of 10 consecutive trials over two consecutive sessions (the number of trials to reach the performance criterion was the dependent variable).

Statistical analysis :
- ASST: 2 (Testing moment: LIGHT vs DARK) X 3 (Phase: VD, EDS, RV) ANOVA with repeated measures on the phase factor.
- ORT: t-test for between subjects comparison and one-sample t-test to compare d2 to the chance level for each group.

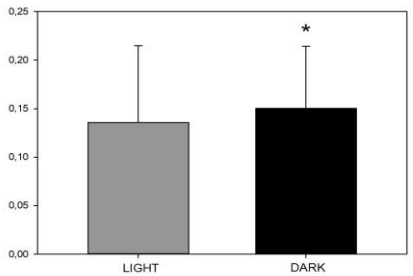
RESULTS

Fig.1. Number of trials to reach criterion for three successive phases of the ASST



According to the two-way ANOVA, there was no difference between both testing moment conditions on the number of trials needed to reach the performance criterion ($F(1,21)=0.088$; $p=0.76$). A principal effect of the phase was revealed ($F(2,42)=27.97$; $p=0.000$). No interaction was found between both factors ($F(2,42)=0.35$; $p= 0.70$).

Fig.2. Discrimination index for the ORT



No between groups difference was revealed by the t-test ($t(21)= -0.14$, $p = 0.88$). One sample t-test comparing the mean of each group to the chance level suggests that the discrimination index for the DARK group only is significantly greater than 0 (LIGHT: $t(11)=1.71$; $p=0.11$, DARK: $t(11)= 2.26$; $p= 0.037$).

CONCLUSIONS

During the ASST, mice tested during their active phase showed an equivalent performance compared to those tested during their rest phase (whichever the considered ability: ability to learn an initial visual discrimination or ability to show flexibility when the rules leading to the food reward were changed). During the ORT, mice tested during their active phase discriminated better the new object compared to those tested during their rest phase.

As cognitive indexes are better for the DARK group or equivalent between both groups, testing rodents during their active period should be privileged not only from an ethical perspective but also to improve the data quality (indeed, with some behavioural tests, testing the animals during their active period might help to obtain performances better reflecting their true abilities). Moreover, testing the animals during their active period does not require much changes for the experimenter (especially with operant equipment).Given the contradictory results found in the literature, further research should be conducted in this area in order to better understand the real effect of the testing moment on cognition.

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