Time to switch off lights? Effects of testing moment in the circadian sleep-wake rhythm

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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 test of learning and behavioural flexibility (the operant Attentional Set-Shifting Task, ASST) and on a motivational task (the Fixed Ratio task, FR). Both tasks are widely used in preclinical research but hardly ever with the testing moment as an independent variable. Several authors underlined in others types of tasks a better performance in mice during their active period. Then, 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 tasks.

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 ASST procedure used was inspired by Ortega et al. (2013)[4].

General procedures : The experimental protocol have been approved by the ethic committee of the University of Liège in accordance with recommendations of the European Community Council for the Ethical Treatment of Animals.

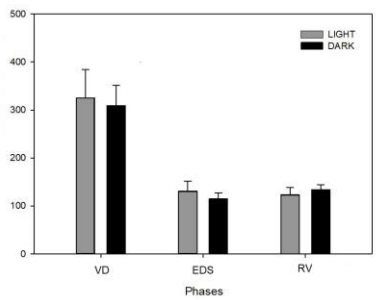
1. Attentional set-shifting task :
   (1) Visual discrimination (VD).
   (2) Extradimensional shift (EDS).
   (3) Reversal learning (RV).
   Mice moved to the next phase when they reached the performance criterion of 10 consecutive trials over two consecutive sessions.
   - Assessment of learning (VD) and cognitive flexibility (EDS and RV).

2. Fixed ratio task : mice needed to make 30 responses to obtain a reward during 30 trials per session.
   - Assessment of motivation.

Statistical analysis :
- ASST: 2 (testing moment: LIGHT vs DARK) X 3 (phase: VD, EDS, RV) ANOVA with repeated measures on the phase factor.
- FR: 2 (testing moment: LIGHT vs DARK) X 10 (sessions) ANOVA with repeated measures on the session factor.

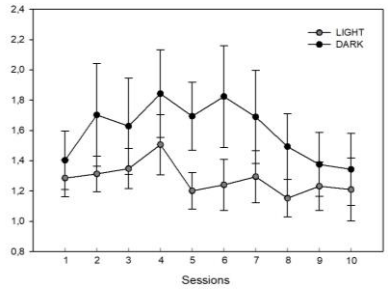
RESULTS

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



As expected, 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. Inter-responses time during the 10 sessions of the FR



As expected, 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 ($F(1,21)=1.55$; $p=0.62$). A principal effect of the session was revealed ($F(9,189)=2.03$; $p=0.03$). No interaction was found between both factors ($F(9,189)=0.78$; $p= 0.62$).

CONCLUSIONS

Operant tasks such as Attentional set-shifting and Fixed ratio do not seem to be influenced by testing moment. During the ASST, mice tested during their active phase showed an equivalent ability to learn an initial visual discrimination or ability to show cognitive flexibility compared to those tested during their rest phase. During the FR, both groups of mice show a similar level of motivation to obtain the reward. Despite the lack of effects, it should be noted that several factors such as the nature of the task, the presence of *zeitgeber*, the modulation of arousal, light pollution as well as light conditions during the test itself can explain this absence of differences between both groups.

Thus, as cognitive and behavioural indexes are equivalent among both groups, testing rodents during their active period should be privileged not only from an ethical perspective but also to improve the data quality. Testing the animals during their active period should help to obtain performances reflecting their true abilities. Moreover, testing the animals during their active period does not require much changes for the experimenter, especially with operant equipment. Furthermore, given the lack of results found in the literature about operant testing and testing moment effect, further research should be conducted in this area in order to better understand the real effect of the testing moment on cognition and behaviour.

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