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Short Communication

Behavioural study of Nymphs of Periplaneta americana in a Light-Dark Box Subjected to Different Environmental conditions

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***Abstract*:** Nymphs of Periplaneta americana were used as an insect model to test behavioral changes in light-dark box experimental setup in relation to circadian rhythm disruption. The present study is focused mainly on light and dark preferences based on the three parameters of responses by a nymph of P. Americana in different environmental conditions by using light-dark box. The nymphs under constant light (LL) and constant darkness (DD) exposure were found to exhibit higher behavioral response in light-dark box test (Mean±SEM) when compared to 12 hours light-12 hours darkness LD condition.

***Keywords*:** Periplaneta americana, Circadian rhythm, Light-dark box.

1. Introduction

Periplaneta americana (L.) is an important peridomestic pests found throughout the United States and other part of the world (Mampe 1972, Wright et al. 1986) and also a common laboratory model. They are known to resides both outdoors and indoors and mostly harbour in dark and humid locations (Cornwell 1968).

Circadian rhythm is the overt expression of an underlying self-sustaining oscillator referred to as master clock (Aschoff *et. al.,* 1965). Synchronization of internal master clock with the external changing light dark cycle is referred as entrainment (Aschoff *et. al.,* 1965). Circadian rhythms are fairly stable and will not entrain to environmental cycles differing by more than a few hours from 24 hours (Pittendrigh and Minis, 1972). It is with the endogenicity of insect circadian rhythms and their coupling to the implied underlying physiological oscillators that it is mainly concerned. Insect circadian rhythms are divided into four major categories: Cellular metabolism, general physiology, developmental gating, and behaviour (Brady 1974). The circadian function of an animal is the overall outcome of all these rhythms in integrated form. Rhythms in general physiology are mainly not of the kind that suggests an origin in specific driving oscillators. The driving oscillator, which mediates circadian locomotor rhythms in cockroaches, appears to reside in the protocerebrum of the brain. The evidence indicates that the optic lobes are crucial elements in this circadian system, and that control of rhythmicity is mediated through electrical, rather than hormonal, channels (Roberts, 1974), Nevertheless of abundant long-standing evidence that the extent and directive behaviour of laboratory models can be influenced

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by a range of environmental condition such as rearing conditions. Measures of tendencies for rodents to leave black or darken environments to enter white or illuminated spaces exploit a conflict between their natural curiosity about a novel environment, and their fear of bright light (Hascoët *et al*., 2001; Sanchez, 1996). Consequently, tests involving such measures have been extensively used in the evaluation of anxiolytic and anxiogenic drugs (Flausino et al., 2002; Hascoët and Bourin, 1998). However, there is inconsistency amongst many of the outcomes, possibly because of the influence of non-pharmacological variables (Hascoët and Bourin, 1998). Many insects are known to exhibit clear circadian rest-activity rhythms (Tobler, 1983). Attention has focussed more on active behaviours such as locomotion than on rest. Therefore, the purpose of this study was to determine the consequences of disturbances of the rest period on activity states of the cockroach Periplaneta americana using light-dark box in laboratory setup.

2. Materials and methods

2.1 Insect

Male and female nymphal stage of *Periplaneta americana* were used (Fig. 1). They were maintained in 12:12 hours LD (light-dark) condition at room temperature in polyvinyl mice cage (30cm×15cm×14cm) covered using fine nylon net (Mira *et al.*, 2002; Brumeister,1838).

(a) Female (b) Male

Fig. 1

2.2 Apparatus

Light-Dark box measuring 16cm light,16cm dark and diameter of 6cm were used for experiment (Fig.2)

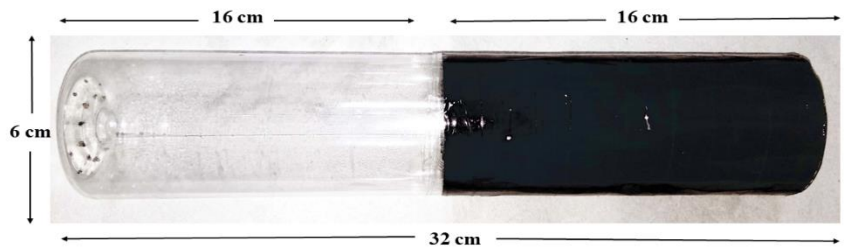

Fig. 2

2.3 Study system

P. americana (male and female nymph) were selected for the experiment. Male nymphs (n=5 each) were kept for 7 days entrainment. First set of experiment were exposed to 12:12 hours LD (light-dark) condition (Light=80-100 lux and Dark=0 lux); next set were exposed 24 hours to constant light (LL) and the last set were exposed 24 hours to constant darkness (DD), all for 14 days each. Same method was done for female nymphs. Behavioural analysis was done in Light-Dark box while emphasizing on the following parameters:

a) Latency to enter dark region of LD box (in seconds)

b) Time spent in light region of LD box (in seconds)

c) Number of entries in light region of LD box

3. Results

3.1 Latency to enter dark region of light-dark box (in seconds)

The One-way ANOVA analysis showed significant increase in the latency to enter in dark region in both the male and female nymphs in DD (constant darkness) and LL (constant light) than in LD (Light-dark) condition ($F12,59= 4.907$; $p<0.05$), and there were significant differences in mean behaviour (LSD *post hoc*, $P<0.05$).

Behavioral analysis of male (Fig. 3a) and female (Fig. 3b) nymph in light-dark box test in LD, DD and LL showed that constant darkness and constant light causes significant increase in latency to enter in dark region (Mean±SEM) of light-dark box when compared to LD condition. Error bar represent SEM, n=5, *$p<0.05$, **$p<0.01$, ***$p<0.001$.

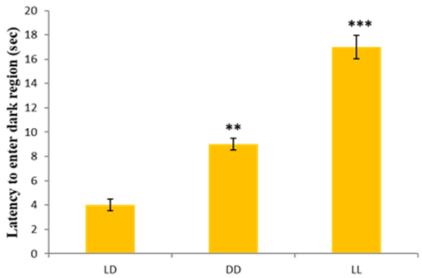

Fig. 3a

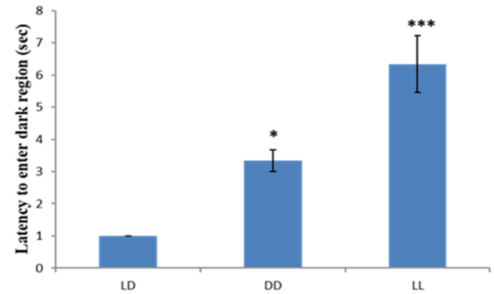

Fig. 3b

3.2 Time spent in light region of light-dark box (in seconds)

The One-way ANOVA analysis showed significant increase in the time spent in light region in both the male and female nymphs in DD (constant darkness) and LL(constant light) than in LD(Light-dark) condition ($F_{3,17}$= 21.07; $p<0.05$), and there were significant differences in mean behaviour (LSD *post hoc*, $P<0.05$).

Behavioural analysis of male (Fig. 4a) and female (Fig. 4b) nymph in light-dark box test in LD, DD and LL showed that constant darkness and constant light causes significant increase in time spent in light region (Mean±SEM) of light-dark box when compared to LD condition. Error bar represent SEM, n=5, *$p<0.001$.

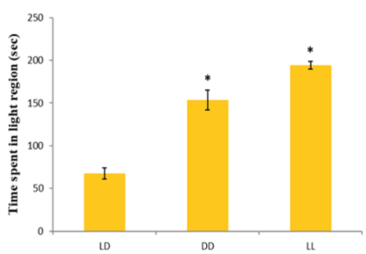

Fig. 4a

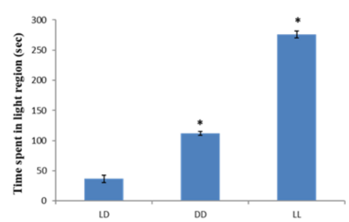

Fig. 4b

3.3 Number of entries in light region of light-dark box

The One-way ANOVA analysis showed significant increase in the number of entries in light region in both the male and female nymphs in DD (constant darkness) and LL (constant light) than in LD (Light-dark) condition ($F_{13,18}$= 9.07; $p<0.05$), and there were significant differences in mean behaviour (LSD *post hoc*, $P<0.05$).

Behavioural analysis of male (Fig. 5a) and female (Fig. 5b) nymph in light-dark box test in LD, DD and LL showed that constant darkness and constant light causes significant increase in number of entries in light region (Mean±SEM) of light-dark box when compared to LD condition. Error bar represent SEM, n=5, *$p<0.05$, **$p<0.01$, ***$p<0.001$.

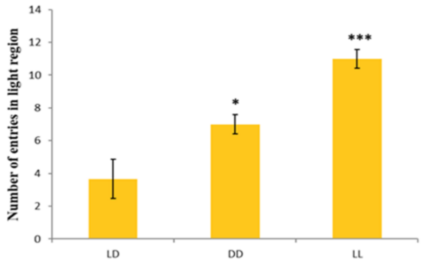

Fig. 5a

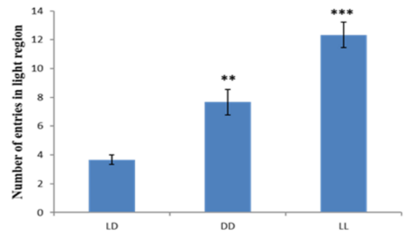

Fig. 5b

4. Discussion and conclusion

The results of the present study showed significant increase in all the parameters in constant darkness DD and constant light LL condition as compared to 12 hours light-12 hours darkness LD condition for both male and female nymph. This significant increase in parameters was observed more in LL condition in both male and female nymphs as compared to LD and DD condition. Constant dark (DD) exposed male and female nymph spent more time in light region of light-dark (LD) box as compared to LD condition nymphs. These significant changes might be due to disrupted circadian rhythm and adaptation to their changed environment. Change in normal light dark period leads to probable disruption of circadian rhythm in both male and female nymph of *P. americana.* Due to disrupted circadian rhythm and adaptation to constant light, both male and female nymphs are not being able to respond to altered environmental condition.

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