Role of Neurotransmitter and Behavioral Changes in Mice due to Light-Dark Stress
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Abstract

Light-dark cycle has an evident role in maintaining the circadian rhythm of complex living organisms. Disturbance of light-dark cycle has an effective role in distressing the normal functions of living organisms. In this study we have analyzed the behavioral changes and role of neurotransmitter in mice due to light-dark cycle disturbances. Batch of animals were exposed to continuous light and dark conditions for 1, 2, 3, 4 and 5 days. The physiological behavioral analyses such as vertical, horizontal, ambulatory and response to light-dark exposures were recorded in the disturbed and control animals. Also the role of neurotransmitter such as Dopamine, 5-HT, 5-HIAA and Homovanillic acid were estimated in the disturbed and control animal nervous tissues such as cerebrum, cerebellum and brain stem. Based on the behavioral analysis it was found that continuous light exposures have increased the horizontal and ambulatory movement of mice more significantly than the continuous dark exposed animal upon comparison with normal 12-12 dark-light exposed animals. Also the continuous light exposed animals showed an aversion to light and liking to dark upon light-dark visit but the vice versa was not found with the dark exposed animals. Dopamines and 5-HT were found down regulated in both the light and dark exposed animals significantly when compared to the normally housed animals. The identified changes in behavioral and neurotransmitter level in mice due to light-dark disturbances are impacting the fact of association of stress, sleep and circadian rhythm in mice.

Keywords: Light-dark cycle; Stress; Sleep; Neurotransmitter; Behaviour

Introduction

The behavior and physiology of most of the animals existing on the earth are reliant on the 24-hour cyclic clock called light-dark cycle (LD). Disturbance of LD cycle affects the normal functions of living organisms by disturbing their circadian rhythm. The inbuilt endogenous oscillators command the circadian rhythm such as blood pressure, heart rate, sleep-wake cycle, hormonal secretion and metabolism [1]. The central circadian clock of mammals is maintained by the suprachiasmatic nucleus (SCN) of the anterior hypothalamus [2,3]. Mammals have the most complex and well-formulated circadian rhythm centered with light-dark cycle and sleep. Mice, the nocturnal animal does most of its active work during the dark phase, which is in contrary to the diurnal animals, which does majority of the activity in light phase. Sleep is regulated by homeostatic interaction of circadian processes, which controls the duration and optimal time for sleep [4]. Similarly the behavioral rhythms of rats were found deregulated with arrhythmic behavior when their circadian rhythms were disturbed by short light-dark and continuous light-dark exposures [5]. From our earlier study it has been shown that disturbances to LD cycle by exposing to continuous light or dark conditions causes adverse effect on zebra fish by deregulating large number of genes and proteins [6].

It is well understood that neurotransmitter plays prominent role in maintenance of circadian cycle by exhibiting its function in SCN. Fluctuations in the level of neurotransmitter in rat were observed when they were exposed to disturbed light-dark cycles [7]. In this study we aimed to understand the effective role of disturbance of LD cycle in mice by exposing the animals to continuous light and dark conditions.

Materials and Methods

Light/dark experiments in mice

Healthy Adult C57BL/6j mice of 3 months old maintained at the Animal house facility, Research Center, PSMMC, under standard 12-12 light-dark conditions were selected for the study. The animals were fed with continuous water and food for the entire period of the experiment. Batches of animals (6 animals per batch) were exposed to continuous light, continuous dark and the 12-12 light-dark conditions for 1, 2, 3, 4 and 5 days. For continuous dark experiment the animals were housed in the standard laboratory conditions in dark environment and similarly for the light experiment the animals were exposed to continuous light of 600 luminous flux intensity.

Behavioral analysis

The behavior of the animals housed at standard, continuous light and continuous dark conditions were monitored for its horizontal movement, vertical movement, ambulatory movement and grooming using Animal activity meter (Opto-Varimex, USA). The animals were monitored for 2 minutes per activity after acclimatizing the animal in the activity meter in their respective light-dark conditions. The time and light–dark conditions for the measurement of activity were maintained same throughout the experiment. The dark condition experiments were performed under a dim red light, whose illumination does not have any role in disturbing the dark conditions. Obtained readings were tabulated for statistical analysis.

Biochemical analysis of neurotransmitter

After behavioral analysis in their respective light-dark conditions the animals were sacrificed using cervical disc

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dislocation. Cerebrum, cerebellum and brain stem tissues were dissected carefully from the animals using stereoscopic microscope. The collected tissues were washed twice in PBS solution and frozen in liquid nitrogen. Analysis of Dopamine, 5-HT, 5HIAA and Homovanillic Acid neurotransmitters were performed on the collected brain tissue [8]. Brain tissues were weighed separately and homogenized using Teflon homogenizer in 0.1 M perchloric acid and 0.05% EDTA buffer for 10 seconds. The homogenate was centrifuged for 10 minutes and supernatant was carefully collected and filtered using 0.45 µm pore filters. High performance liquid chromatographic (HPLC) analysis for different neurotransmitters was performed on the supernatant in Waters HPLC-ECD (Model No. 2465, Waters Associate Inc. USA) system. The sample was HPLC analyzed using C18 µ-Bondapak Column with a 1.2 ml/min flow rate and 10-µl-injection volume. The relative level of neurotransmitter was compared for Light and dark conditions against normal.

Statistical analysis

The data obtained from the behavior and neurotransmitter analysis were plotted and analyzed for its mean, SD and SEM using Microsoft Excel. The significance of the data were then calculated using PRISM software for One way ANOVA and post t-test, if overall value of p is more than 0.05 level of significance using Dunnett compare test data against control data.

Ethics

All the experimental procedures executed in this work were performed as per the norms of the Institutional Animal care and Ethics committee, Prince Sultan Military Medical City, Riyadh, Saudi Arabia (Research Protocol No.17/2015).

Results

Behavioral analysis

Mice exposed to continuous light and dark conditions showed discrete changes in their behavior upon analysis. The horizontal movements of the animals were found disturbed more significantly in continuous light exposed conditions for all five days in comparison against the control and dark exposed animals (Figure 1a and Table 1). Likewise dark exposed also showed non-significant increase in the horizontal movement (Figure 1a). The vertical movements of the animals were found decreased in dark exposed animals non-significantly (Figure 1b) without much change among the light exposed animals. Similar to horizontal movement, the ambulatory movements were found significantly increased among mice for all five days of continuous light exposures without much change among the dark exposed animals (Figure 1c). No significant changes were observed for grooming behavior among the light and dark exposure conditions (Table 1).

Analysis for response to light-dark test by the light and dark exposed animals showed significant aversion to light conditions by the light exposed animals. Based on the comparative analysis it was found that continuous light exposed animals showed less time spent in Light side of the box than rest of the conditions (Figure 2a). Whereas dark exposed animal did not show any aversion for dark conditions (Figure 2a). No significant association was observed with the number of light-dark visit by the light and dark exposed animals (Figure 2b).

Biochemical analysis of neurotransmitter

Based on HPLC analysis it was found that the level of Dopamine and 5-HT (Serotonin) were significantly decreased in the cerebrum of mice brain. The down regulation of Dopamine and 5-HT were

![Figure 1:](image-url)
observed both in the light and dark stress conditions when compared against the control levels (Figure 3a). Similarly, a non-significant down regulation of 5-HIAA was observed in the cerebrum for both the light-dark disturbances (Figure 3a). The level of Homovanillic acid was not found altered for the stressors in the cerebral tissue. The level of 5-HT and 5-HIAA were not found significantly associated in the other nervous tissues like cerebellum and brain stem (Figure 3b and 3c).

Discussion

In spite of mice being a nocturnal animal it is very interesting to understand from this study that disturbance to light-dark cycle has a major role in disturbing their sleep-wake cycle and circadian rhythm.

| S No | Behavioural conditions/Level of Neuropterans | Light environment | Dark Environment |
|------|---------------------------------------------|-------------------|-----------------|
| 1    | Horizontal Movement                          | **                | -               |
| 2    | Vertical Movement                            | -                 | -               |
| 3    | Ambulatory Movement                          | **                | -               |
| 4    | Grooming                                     | -                 | -               |
| 5    | Time spent in Light                          | **                | -               |
| 6    | Time spent in Dark                           | -                 | -               |

Table 1: Significance of different behavior conditions associated with light-dark cycle disturbances. Table represents the different behavior study associated with the light-dark cycle study. **: Levels of significance.

Figure 2: Response of mice to Light-dark exposures. The time spent by mice exposed to different light-dark conditions in dark/Light chamber were recorded and plotted against control animals (X-axis – Light or dark conditions; Y-axis – Time spent in seconds). The observation were reported as the mean ± SEM. Data were represented as the mean ± SEM. **p<0.01 vs. control group (student t-test, n=3 per group).

Figure 3: Relative expression of Neurotransmitter in Mice nervous tissue. The expressions were shown in 2 log fold relative to control level. a. Relative expression of Dopamine, 5HT, 5-HIAA and Homovanillic Acid in cerebrum. b. Relative expression of SHT and 5-HIAA in cerebellum and c. Relative expression of 5-HT and 5-HIAA in brain stem of mice tissue. The levels of transmitters were represented as the mean ± SEM. *P<0.05 versus control level, **P<0.01 versus control group (Student t-test, n=3 per group).
The associations of increased horizontal and ambulatory movement of the animal due to light exposures sway that the animals were totally stressed and were on constant movement due to disturbances. It is also well understood that constant light affects the locomotor activity in mice [9]. Light exposed animal’s aversion to light environment indicates that the animal is stressed with continuous light and is trying to avoid the same. But it is also interesting to observe that the dark exposed animals did not show aversion to the same environment in spite of its stress associated with continuous exposures. It is also very interesting to know that the level of neurotransmitters goes down in light and dark exposed animals, which confirms that the continuous exposure reduces dopamine and its metabolite in rodent nervous tissue [7,10]. Dopamine the reward-motivated neurotransmitter undergoes down regulation during stress condition in both light and dark exposures, revealing its direct association with sleep and stress. The observation also emphasizes its direct role in circadian cycle associated disturbances, as it was known that dopamine modulates diurnal and circadian rhythms in photoreceptor cells of mouse retina [11]. Serotonin, the other major neurotransmitter involved in cognitive functions was also found decreased in the disturbed cerebrum due to stress as it has been shown that serotonin has direct association with sleep as serotonin knockout mice exhibit more wakefulness [12]. This study has provided a new way of association of neurotransmitter along with varied movement behavior when mice were exposed to light and dark stress leading to disturbed circadian rhythm and sleep.

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