The Impact of Cumulative Exposure to Blue Light during The Developmental Period of Melanopsin ipRGCs

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Abstract. Previous study revealed that exposure to blue light during postnatal period decrease the function of NIF in mice. However, duration effect of blue light exposure during the developmental period of melanopsin ipRGCs is still unknown. Twenty four Swiss-Webster pregnant mice exposure to 470 nm blue light spectrum of the LED lights and sunlight. As a control group pregnant mice was keep in no light condition. The length of exposure was varies based on the stage of development: (1) from gestation day 9 (E9) up to 18 days of gestation (E18), (2) newborn to 6 days old, (3) from gestation day 9 until aged 6 days old. At 6 days old, selected mice pups were performed for negative phototaxis using cylindrical bore assay. The sunlight mice groups showed that the fastest response occur on longer exposure. However, on blue light mice groups, the longer the exposure actually decreases the activity more compare to only postnatal exposure. These conditions maybe due to interference on synaptogenesis during developmental period of melanopsin ipRGCs. Thus it can be concluded that exposure to blue light since in utero until neonatal is harmful especially to NIF response behavior.

Keywords: blue light, melanopsin ipRGCs, mice, negative phototaxis

1. Introduction

Light plays an important role in our life. In mammals known to have two visual function, (i) play a role in vision, known as image forming function (IF) and (ii) controlling physiological conditions, behavior and cognitive function, known as non-image forming (NIF) [1,2]. Both functions are mediated by different photoreceptors located on the retina. IF functions mediated by rod and cone cells, while the NIF functions mediated by melanopsin ipRGC (intrinsically photosensitive retinal ganglion cells) [3-6]. Various studies show that not all of the light spectrum can effectively influence the activity of melanopsin. Melanopsin ipRGC reported to have activity peak at 470-480 nm light spectrum which is in the range of blue light [2,7-9].

Normal development of melanopsin ipRGC is important to form NIF function. Melanopsin ipRGC began to emerge since the 9.5 days of gestation in mice [10-12]. Parallel with it, melanopsin began to be expressed and increasing to a peak level at age 6 days post birth [11,13]. NIF function triggered by melanopsin phototransduction activity in melanopsin ipRGCs [3-5,7]. Phototransduction in melanopsin is unique, whereas it has bistable system to recover its chromophore. It occurs through isomeration of 11-cis-retinal into all-trans-retinal by short wavelength light spectrum and its recovery by long wavelength spectrum. [5,14-16]

In everyday life, people are exposed to light not only from natural light such as the sun, but also from artificial lighting. The most widely used artificial lighting is a cool white light LED (Light Emitting Diode) which is known to be an efficient artificial light sources of energy. White LEDs
commonly used in lighting was found to have major composition blue light with a value of about 35% [17]. This value is higher than the blue light spectrum emitted by the sun during the day which is about 25-30% [17]. Blue spectrum with a high intensity can also be obtained from any other electrical appliance devices that generate light such as mobile phones, computers and notebooks. This condition indicates that current lighting is dominated by blue light spectrum.

Based on the optical properties of tissue, blue light can pass through the various layers of the eye and reaching the retina approximately 65% [18]. Though the blue light spectrum is needed to activate the NIF, it has been reported that blue light can also induce retinal phototoxicity. In adults, retinal phototoxicity is known to induce damage to the retina and changes in the activity of the melanopsin-ipRGC against NIF functions such as modulation of sleep and circadian rhythm-related activities [1,19,20].

Exposure to blue light spectrum during development of melanopsin ipRGC from pre birth to post birth, allegedly can disrupt the development of melanopsin ipRGC which further lead to disturbances on the system that regulates NIF phototransduction signals to brain. Such conditions might cause disturbances throughout the life of exposed child especially in the NIF response associated with physiological, behavioral and cognitive.

Previous study (not reported) found that exposure to blue light during the development of the melanopsin ipRGCs especially the postnatal period can significantly decrease the function of NIF in mice. However, the effect of the duration of blue light exposure during the developmental period of melanopsin ipRGCs is still unknown. Cumulative exposure of blue light during pregnancy and newborn feared give more significant impact on behaviour. Therefore, research must be done to prove that blue light plays a role during development of ipRGC melanopsin response against NIF. In this research, light exposure given to the three duration of ipRGC melanopsin development, in the pre birth, after birth and from pre birth which continued until after birth. NIF measured response is negative phototaxis behavior include initial response and reverses direction latency on young mice. Negative phototaxis response is one of NIF function in mice that can be observed since newborn and arose due to induction of light. Its response mediated only by phototransduction of melanopsin ipRGC [21].

This research is expected to provide basic information about the effect of light on the development related to NIF function. Light which is considered safe in everyday life should be addressed and getting noticed as one of toxicans so we can attempt and prevent the behavior disorders which may appear on the next generation.

2. Materials and methods

2.1 Animals
Female Albino Swiss Webster mice were obtained from Rumah Hewan School of Life Sciences and Technology ITB (SITH ITB) and kept in the animal facilities of SITH. Twenty four eight-week old pregnant mice (27-30 g) were used in this study and the number of pups from each dams were eight. Pregnant mice were obtained by mating the eight weeks old female with ten weeks old male mice. Gestation was counted as gestation day zero (E0) if there was vaginal plug on the other day after mating. The mice housed in standard mouse cages under 12 h light/dark cycle before experiment. In all cases, animals were cared for in accordance with the guidelines defined by the OECD 426, 2007.

2.2 Lighting design
The experiment cages were equipped with lights and feeding tools set. The experiment lamps (blue Epistar 470 nm, 1 W) mounted on the top of the cage. In this study, the dose of light exposure was $9.33 \times 10^{-4}$ W/cm² and it was calculated to reach the fetus inside uterus and affect melanopsin. This value is derived from measurements at the base of the enclosure and obtained by calculating limits or irradiation power density (W/cm²) based on Rao et al. [12].
2.3 Experimental design
The design used in this study is a randomized design based on two factors, namely light source (A) and the duration of light exposure (P). Based on the factors of the light source (A), there are two treatment groups, namely (A1) groups of mice by exposure to the sunlight indoor and (A2) a group of mice by exposure to blue light. The second factor in the grouping is the light exposure time (P) were divided into four groups, namely (P0) without exposure to light during treatment; (P1) light exposure is only performed on day 9 of gestation (E9) up to 18 days of gestation (E18); (P2) exposure to light from newborn until the mice were 6 days old neonatal mice (PN6); (P3) light exposure from gestation day 9 until aged 6 days old. At 6 days old, selected mice pups were performed for negative phototaxis.

2.4 Phototaxis assay
The mice dams and pups were kept in darkness for at least one hour prior to the test. Pup movements in a cylindrical chamber were recorded. Light stimuli given to test was blue LED λmax 470 nm, position at 4.5 cm from the end of the test chamber. Each pups’ position was monitored for a 5-min period in a state after a light was directed at the pup’s face. Each pups was tested once. Offline video observed and analyze by three observers. Phototaxis behavior viewed offline using the application Kinovea. Parameters measured were the first latency response to light stimuli and latency reversed from original position.

2.5 Statistics
The main parameters were compared between treatment groups by randomized block design. phototaxis data calculation is processed with statistical tests in SPSS ver.16. A comparative analysis between variables have a normal distribution (Kolmogorov-Smirnov) and inhomogeneous (Levenne test), continued to post hoc test Games Howel with 95% confidence interval to see the significance of differences between groups.

3. Results
3.1 Effect of blue light exposure during the development ipRGC against NIF response of neonatal mice
The role of exposure to light during the development melanopsin ipRGCs against neonatal mice's ability to respond to light is observed through the initial and reverse response latency. Based on the data presented in figure. 1 A and 1 B, it can be seen that the group of mice with blue light exposure of all exposure time (P1, P2 and P3) showed no significant differences (p> 0.05) with the group without exposure to light (P0). This condition shows that the blue light spectrum is not optimal spectrum of light to induce normal development of melanopsin ipRGCs.

Figure. 1A shows that the initial response latency of neonatal mice against the first light stimuli is slower in blue light exposure (13.65 ± 0.87 second) mice group compared to sunlight exposure group (27.75 ± 1.38 second) postnataly (P2). The same pattern was seen on neonatal mice latency to turn around from original direction (figure. 1B). Turn around latency of neonatal mice on blue light exposure group at the time of light exposure after birth (P2) is significantly slower (205.4 ± 0.7 second) than the mice group of sunlight exposure (P <0.05). This suggests that exposure to blue light during postnatal development ipRGC melanopsin causes a decrease in neonatal mice's ability to respond to light.
Figure 1. Latency initial response (A) and latency reverse direction response (B) of mice on sunlight and blue light exposure. Significant differences between groups are indicated by small letters. **Description:** P0 = group without exposure to light; P1 = prenatally light exposure group; P2 = postnatally light exposure group; P3 = prenatal to postnatal light exposure group. Sunlight groups are (a), (b), (c) and (d), respectively. Blue light groups are (e), (f), (g), and (h), respectively.

Figure 2. Latency reverse direction response (B) of mice on sunlight and blue light exposure. Significant differences between groups are indicated by small letters. **Description:** P0 = group without exposure to light; P1 = prenatally light exposure group; P2 = postnatally light exposure group; P3 = prenatal to postnatal light exposure group. Sunlight groups are (a), (b), (c) and (d), respectively. Blue light groups are (e), (f), (g), and (h), respectively.
Figure. 2 shows that the duration of exposure during development melanopsin ipRGC could affect NIF response of neonatal mice. Exposure to sunlight exposure time from pre-birth and continued after birth (P3) of neonatal mice accelerates latency reverse direction in response to light. This indicates that sunlight is needed since the pre-birth to post-birth for normal neonatal mice's behavior in response to light as a negative phototaxis demonstrated by quick response.

Exposure to blue light turns out shows different results compared to the exposure of sunlight when the exposure light from pre-birth and continued after birth (P3). Based on the data shown in figure. 2, the exposure of blue light from pre-birth and continued after birth (P3) actually cause latency reversed behavior in mice neonatal much slower compared to all groups of exposure (P0, P1, P2) (P < 0.05). This suggests that long duration of exposure to blue light during the development melanopsin could decreased neonatal ability to respond to light as indicated by a slower response than normal. Based on the results, both exposure to sunlight or blue light, can affect the development of melanopsin ipRGC and further reflected in the behavior of neonatal mice.

4. Discussion

Results showed that mice NIF response to blue light exposure treatment is slower compared to treatment with sunlight exposure. The condition is thought to be caused by decreased activity in melanopsin ipRGC impulse, so phototransduction to the brain is disrupted and eventually NIF response delayed. The decline in impulse activity by exposure to blue light in melanopsin ipRGC allegedly because of an imbalance in the activation process chromophore melanopsin.

Melanopsin ipRGC phototransduction in normal condition occurs when the melanopsin absorb blue light through retinaldehyde chromophore 11-cis-retinal into all-trans-retinal. Activation of all-trans-retinal can induce changes in membrane potential through a cascade of signaling proteins Gq and phospholipase C to open the transient receptor potential (TRP) channel. Opened TRP channels can trigger depolarization signals along the axon melanopsin ipRGC towards the brain that processes the behavior of NIF [11,22-28]. Different from the rod and cone cells, melanopsin photopigment has bistable system. Melanopsin ipRGC have the ability to turn back the chromophore 11-cis-retinal which has been activated to all-trans-retinal by exposure to orange light, so that melanopsin ipRGC can restore the balance of the sensitivity of melanopsin to normal conditions [5,14-16]. However, the bistable systems in the group treated mice with exposure to blue light was not working. This is because the blue light exposure treatment, mice were treated isolated from exposure to other wavelengths.

On the other hand, all-trans-retinal is a photosensitive compound which can excite free electrons when the energy-absorbing blue light in melanopsin [29]. Those molecule can directly react with the substrate or transfer excitation energy to molecular oxygen and produce singlet oxygen which is included in the free radical compounds [30-33]. Singlet oxygen is electrophilic and can react with molecules containing double bonds to bind hydrogen to return to a stable condition. Photoreceptor membrane contains a lot of unsaturated fatty acids that have double bonds. When the unsaturated fatty acids in the membrane loses hydrogen atoms of the bond double, will produce hydrogen peroxide which can increase levels of free radicals and starts a chain reaction known as lipid peroxidation, especially at the cell membrane [34,35]. The condition can cause instability in the photoreceptor membrane integrity and consequently phototransduction signals to the brain would be disturbed.

The formation of free radicals caused by excited electrons of all-trans-retinal, under normal circumstances can be overcome by a bistable system melanopsin by an orange light or long wavelengths and the mechanism of natural antioxidant produced by the cells. However, the bistable systems in the group treated mice with exposure to blue light was not working. The result was a buildup of electron excitation results from the activation of all-trans-retinal. The condition is supported by the characteristics of neonatal retina that is still not fully developed. Changes in the environment of hypoxic conditions (in the womb) to normoxia (postnatal) and down regulate blood flow system to neonatal retina which is still not fully developed, making neonatal eye containing extremely high oxygen levels compared to the eyes of adults [33,36]. In addition, although the cells naturally have a
defense system that is an antioxidant compound to minimize the damage caused by free radicals, the levels of natural antioxidants neonatal eye was reportedly still low [36].

The combination of the eyes of neonatal containing high oxygen levels, low natural antioxidants, the buildup of free radicals, and decreased ability to change the all-trans-retinal into 11-cis-retinal due to unavailability of orange light on a group of mice with exposure to blue light, causing a decrease in the high impulse activity neonatal mice's ability to respond to light, as indicated by the negative phototaxis slow response.

In this study, it appears that the impact of blue light exposure is cumulative. This is apparently due to ipRGC actively synaptogenesis with other retinal tissue to form a layer of the normal retina postnatally. ipRGC impulse activity disorder caused by exposure to blue light, could be expected to interfere with the process of establishing synaptogenesis in the retina that causes the formation of abnormal retinal layers. This condition causes a decrease in dopamine cell activity amacrine also in regulating the expression of melanopsin in ipRGC resulting in decreased expression of ultimately impulse activity declined more drastically and provide a cumulative impact.

Therefore, when the impulse activity melanopsin ipRGC disrupted by exposure to blue light, especially in the early postnatal, it will cause disorder in the retina synaptogenesis and one of them is amacrine dopamine cell activity disrupted. Decreased amacrine dopamine cell activity would lead to decreases melanopsin expression, which in turn NIF signals will be disrupted and the response is experiencing a delay. In this study, it is proven through the reduction of negative phototaxis responses in neonatal, indicated by the slow response.

5. Conclusion
It can be concluded that: (1) blue light exposure during postnatal melanopsin ipRGC development causes disturbances in development with implications to NIF function decline; (2) NIF function decline represented by a decrease in the response speed negative phototaxis behavior.

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