Effects of Dark- Light Cycle on the Histological and Immunohistochemical P73 Marker Expression of Cajal Cells in the Cerebral Cortex of Mice

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Summary

background:.The horizontal cells of Cajal are small, fusiform, horizontally oriented cells found in the most superficial layers of the cortex. A dendrite emerges from each end of the cell, and an axon runs parallel to the surface of the cortex, making contact with the dendrites of pyramidal cells. the express P73 on nuclear membrane. aim of study: To evaluate the morphometric histological analysis of Cajal cells in cerebral cortex and evaluate the thickness of gyri and sulci, according to stimulation of light and dark field. To evaluate the P73 marker expression of Cajal cells in the cerebral cortex according to stimulation of light and dark field of male Swiss Albino mice. materials and methods: Coronal sections were placed on standards glass slides for H&E staining, and on positively charged slides for immunohistochemical staining with P73, which was evaluated using Aperio Image Scope analysis software V9. There were significant difference in body weight between the experimental and Control groups. Light group had a higher body weight than the control and dark groups. The histological features in experimental group showed significant changes in the thickness of cerebral cortex especially the molecular layer, also change in the depth and width of gyri and sulci, these changes associated with cytoplasmic vacuolation, nuclear apoptotic.. Change in P73 expression in cerebral cortex, $P < 0.05$ revealed significant differences across groups. It showed the most expression in the control group, with a mean value of 0.43±0.25 , in light group it increased compared to other groups with mean value of 0.41± 0.13. results: The experimental animals of light group reveal high body weight than that of dark animals due to increase in food consumption. The experimental animals of light group reveal less sleep insomnia and overactive than that of dark animals. Although these animals became adapting to such daily disruption of light or dark field. they override the tendency of mice to sleep during the light phase. The experimental animals of light group reveal repellent, lonely, isolated living attitude and developed quarreling with aggressiveness behavior with each other in comparison to dark group mice that reveals more active and social behaviors. The thickness of whole layers and especially molecular layer of temporal lobe reveals a significant decrease in the light group in comparison to other animals this is due to indirect stressful effect of light on the nerves cells of cerebrum of experimental animals that induces more apoptosis and cells death than other groups. The study about the marker expression of P73 of the Cajal (CR) cells in the molecular layer of dark group mice reveals a significant increase in values who found that there were neuronal cells proliferation , migration and differentiation are more apparent in comparison to other groups. conclusions: this indicate that P73 has vital role in neuronal cells development in different parts of the brain.

1. Introduction

The Cajal cells population of layer I neurons that possessed a short axon. These cells were distributed equally at different levels within layer I – superficial, intermediate and deeper portions – in neonatal rabbit and rat (Verónica Martínez-Cerdeño and Stephen C. Noctor. 2014). Cajal-Retzius cell is the prominent neuron of layer I of the cortex, playing a crucial role in cellular development and neuronal circuit formation, by secretion of reelin. Because Cajal-Retzius cells and reelin are important factors for the synaptogenesis in the hippocampus and the brain isocortex, their loss may be implicated in the synaptic pathology and the multifactorious pathogenetic pathways of Alzheimer's disease Stavros J Baloyannis 2005. The p73 is a homolog of the tumor-suppressor gene (Kaghad et al., 1997). In the brain, it is expressed in Cajal-Retzius (CR) cells (Yang et al., 2000; Meyer et al., 2002). The most striking brain abnormalities of p73 knockout mice are loss of CR cells, hydrocephalus, and dysgenesis of the dentate gyrus (Yang et al., 2000). Neurons in various p73/ brain structures undergo extensive postnatal apoptosis (Pozniak et al., 2002). The light is important for vision and light is essential for many cognitive tasks. however, it have powerful modulator of non-visual functions on several cognitive tasks. The brain mechanisms involved in the non-visual effects of light were largely unknown. In this review we first provide an overview of the physiological basis of the non-visual effects of light as they

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emerged from circadian rhythm. (Brainard, G.C et al., 2005). At the cerebral level, 20 minutes of bright white light induced both thalamic and cortical modulations that started to decline after the end of the exposure. (Vandewalle, G. et al., 2006).

The horizontal cells of Cajal are small, fusiform, horizontally oriented cells found in the most superficial layers of the cerebral cortex. A dendrite emerges from each end of the cell, while an axon runs parallel to the surface of the cortex, making contact with the dendrites of pyramidal cells(Martinez-Cerdefio & Noctor, 2014).

There were dualistic (two) model in which cortical projection neurons were thought to migrate radially, and cortical interneurons were thought to migrate initially tangential to the cortical surface before following a radial route (Anderson et al., 2002).

The sleep-wake cycle, are organized by the circadian timing system (CTS) (Moore-Ede et al., 1982). The circadian clock in the SCN of the anterior hypothalamus is essential for starting the sleep-wake cycle in mammals that is demonstrated by the SCN, as the sleep-wake cycle occurs on a daily basis (Mistlberger, 2005). The circadian rhythm is a mechanism that allows living organisms to successfully coordinate their biological functions with the light and dark phases during the day. (Aschoff , 1965). The sleep, metabolism and hormones production are regulated by the circadian rhythm that acts as a master clock. The disturbance of the circadian rhythm has been shown to have significant health consequences, ranging from lethargy to an increased risk of cancer (Rea et al, 2008). The bright light exposure during the day is beneficial to sleep quality and circadian regulation in contrast to the effects of evening and/or night-time light exposure. the circadian system's phase response to bright light may be dependent on the duration of exposure, and that longer exposure at lower intensities may have a greater effect on delaying or phase-shifting sleep. The repeated intermittent exposure to intense light (in the morning or evening) generates a phase-shift response ,according to evidence (St Hilaire et al, 2012).

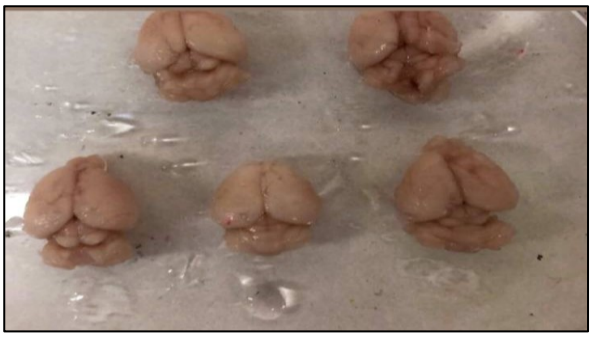
2. Materials and Methods

samples of experimental animals

Thirty male healthy Swiss albino mice weighing 25-30 g will be purchased from center for drug control and research. These animals were housed in 3 groups in the animal care facility of Al-Nahrain University College of medicine in a 12-hour light/dark cycle for control group. Regular rodent food and water will be provided and Grouping as group A (control) 10 male mice will be kept at a 12-hour light/dark cycle. The group B 10 male mice will be kept at a 24-hour light daily for 45 days from 1 November_15December, stimulation by applying light emitted diode (LED) (white with 500 lux level) light. Group C 10 male mice will be kept at a 24-hour in darkness daily for 45 days.

Brain harvested as whole mass Blocks from the cranial cavity then coronal section of the brain were done at mid temporal region. Temporal lobes were embedded in 10 % formalin so as to performed paraffin tissue block that were ready to make tissue slides for evaluation the morphometry, histologically and immunohistochemically for P73 expression of CR cells that had been purchased from ABCAM.

the euthanized animal were apply fixative solution as 10% formalin solution was injected intra-cardiac through left ventricle into aorta (so that tissues fixation was done by modified perfusion method) as done by certain researchers (H.A. Jaafer. et al; 2019)The specimens of brain were preserved in 10% formalin for (24-48) hours in order to obtain paraffin tissue blocks.

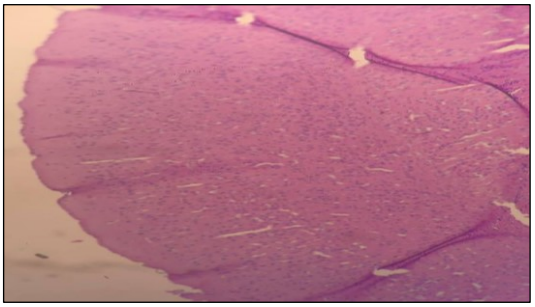


Figure(3-1) The dissection of brain coronally of the temporal lobes. Brain of control group.

The specimens were histologically prepared for paraffin section. The paraffin tissue sections for each group were stained with Hematoxylin (Harris Alum – hematoxylin) and Eosin stain for general histological tissue examination.

This study shows marked histological and morphometric changes in the molecular layer of temporal lobe of cerebral cortex structures in experimental animals of the light and dark groups, in comparison to control group. The study includes the changes in the weight of animals, weight of brain, as well as the thickness of molecular layer with the immunohistochemical expression of P73 in the temporal lobe of cerebral cortex of mice.

3. Results



(Figure 4.1): Cross section view of cortex of control group shows the six major layers of cerebral cortex. (10x; H&E).

Effect of light and dark on behavior and moods of experimental animal

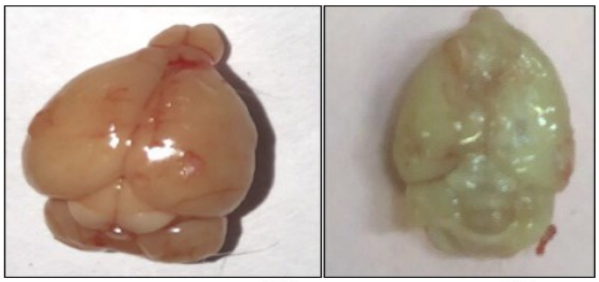
The experimental mice of light group revealed significant more requirements for food and water

with changes in behavior and moods were they become more irritable and aggressive.
The dark group mice which they reveal less requirements for food and water also show less aggressive behaviors to other and they gathered into one place.

Gross morphology

Effects of light and dark on brain size and weight of experimental animals

The brains from experimental animals at the end of the experiment were harvested. They showed a difference in size and weight of brain between light and dark groups, where increased brain size and weight in dark group animals in comparison to that of light group animals which shows a decrease in brain size and weight (Figure 4.2 A&B) in comparison to that of control group animals.

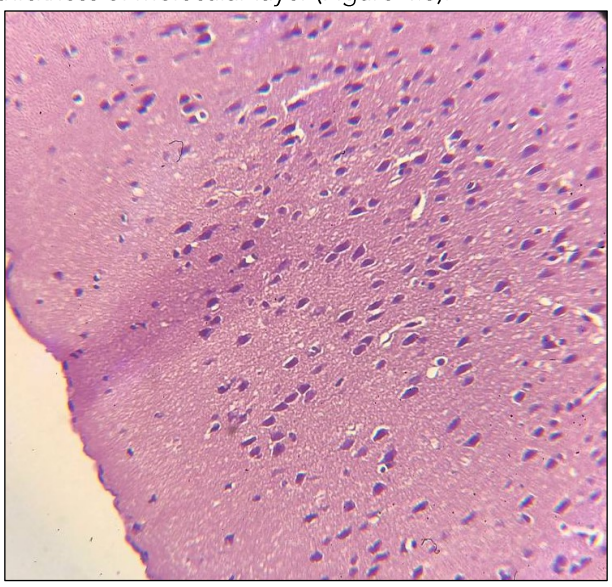


(Figure 4.2): at the end of the experiment the brains of mice were harvested. A- Brain of light group, B-Brain of dark group.

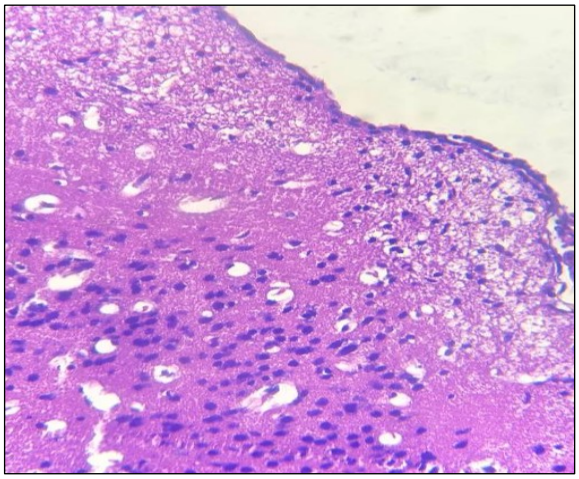
Histological feature

Effects of light and dark on the thickness of molecular layer of cerebral cortex

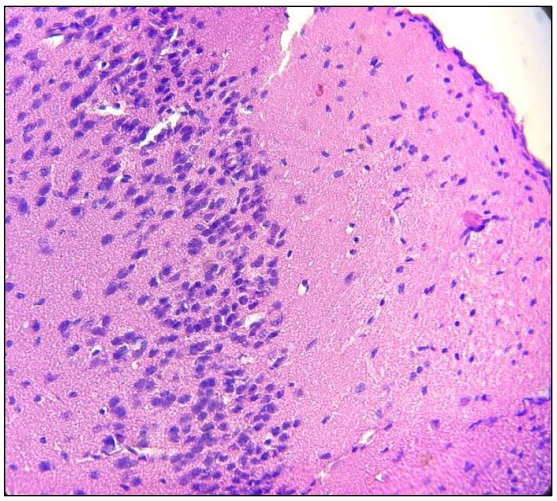
After the end of the experiment and the application of the histological routine stain (Hematotoxyline and Eosin) of the samples used in the experiment (brain), they showed marked differences in the morphometric analysis of the samples including changes in the thickness of molecular layer (Figure 4.3).



(Figure 4.3): Cross section view of cortex of control group, shows the molecular layer in the temporal lobe of cerebral cortex. (40x; H&E).



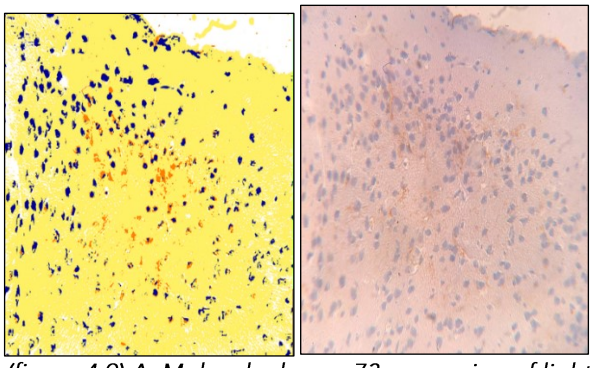
(Figure 4.4): Cross section view of cortex of light group, shows the molecular layer in the temporal lobe of cerebral cortex. (40x; H&E).



(Figure 4.5): Cross section view of cortex of dark group, shows the molecular layer in the temporal lobe of cerebral cortex. (40x; H&E).

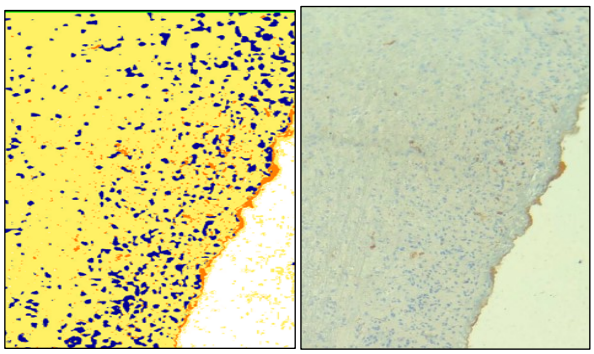
Effect of dark and light on the marker expression of the P73 of cerebral cortex of experimental animals by using Aperio program

The samples were prepared for each group to perform immunohistochemistry evaluation by apply Anti-P73 antibody for detection presence of this P73 protein in cells of temporal lobe of cerebral cortex. The detecting secondary antibody is labelled by peroxidase enzyme that eventually oxidized the colorless staining material di amino benzidine (DAB) leading to oxidized brown colored DAB (figure4.9)



(figure 4.9) A: Molecular layer p73 expression of light group in analyze view.

B: Molecular layer p73 expression of light group



(Figure 4.10) A: Molecular layer p73 expression of dark group in analyze view

B: Molecular layer p73 expression of dark group .

The marker expression of P73 in the molecular layer of light group mice reveals a significant decrease in values, which was very little in comparison to dark group mice and this was recorded as (0.41±0.13) for light group mice and (0.63±0.28) for the dark group mice, respectively. This is achieved in regarding to control group animals.

General histological changes in the cerebral cortex in the experimental groups

The most common histological changes in the cerebral cortex of experimental groups showed gross changes(with low magnification) in cortical thickness, and identification in the cerebral cortex layers. Also these changes in high magnification were characterized by presences of advanced cellular degeneration. These findings involve cytoplasmic vacuolation and wide range of apoptotic nuclear changes that are normal programed cell death (apoptosis).

4. Discussion

The role of light dark cycle on the regulation of the sleep in the mice

The experimental animals of light group reveal less sleep (insomnia) and overactive than that of dark animals and this agree with others who found that mice are naturally nocturnal animals (Refinetti, 2004) which tend to be active after dark and rest and sleep during the day (light time) (Arakawa et al., 2007). The dark and light day time is sometimes referred to as "active phase" and "inactive phase", respectively, reflecting the nocturnal nature of the mouse (Arakawa et al., 2007). Numerous studies have described circadian variations in certain physiological processes (female, male reproductive system and on retina of the eye) in the body by apply histological and immunohistochemical methods for this variance (H. A. Jaafar et al.,2019), while other researchers who found that there is electrical activity in neurons of suprachiasmatic nucleus in a brain region that regulates circadian rhythms in the mice that normally made the life patterns of these animals differ between day and night. While animals that were exposed to light largely would lost the diurnal variations in physiological and biological processes in the body of these animals. So that light made these nerve cells of the suprachiasmatic nucleus of the hypothalamus were stimulated that lead in disturbance in the circadian rhythms with consequent disturbace in certain biological and physiological processes (endocrinal-hormonal, neural and limbic system) in the body. Eventually these variations lead to disturbance in sleep, mood, animals' behaviors and other daily activities.

The nighttime light had a greater effect on the nerve cells of the suprachiasmatic nucleus. They found that the blue wavelengths light of electronics(high energy light), can increase attention and energy of animals which is good during the active period but particularly harmful for bedtime (which is sleep and rest time). They also found that disrupted sleep can cause brain activation and inflammation (Wang H.B. et al. 2020). Furthermore, other authors found that light would affect the sleep time in mice and other animals. They concluded that non artificial (environmental) light has a strong impact on human physiology and behavioral activities. These studies had shown that short light–dark (LD) cycles would influence the sleep in the albino rat. The recorded responses show that sleep in mice is affected and interrupted by photic stimulation. They demonstrated that pigmented animals can show sleep induction after dark onset and this indicate that light has significant effects on the regulation of sleep.(Tom D ., et al 2007). The rodents despite their nocturnally life that are active during night period, that prefer dark fields for their sleep time. The sleep patterns of albino rats is constant during light and dark daily cycle, although light is stimulus that disrupts sleep nonspecifically. (Tobler et al., 1994). Howerver, Mistlberger and Skene, 2004 found that mice are conducted during the light phase. It is probable that these animals are better at adapting to such daily disruption. These mice override and has the tendency to sleep during the light phase by seeking the dark fields for their sleep time.

Role of light dark cycle on modality of behavior aspect in the mice:

The present study with experimental animals of light group reveal repellent, lonely and isolated behavior pattern in their cages and this approve with other researchers who testing social approach behaviors in opposite circadian phases yield quantitatively different outcomes that these animals were sociability more in the light phase produced results highly comparable to that of dark phase experiments. (Yang et al., 2007). Furthermore, circadian variations in social behaviors of animals that had be studied the levels of sociability in mice in opposite phases, and the fact that many light phase studies have yielded meaningful results as field studies have shown that many species of nocturnal rodents(mice) are able to adjust their activities according to non-photic factors including hunger for food, seeking for water, predation and competition

(Daily and Ehrlich, 1996). The mice have active motility and behaviors that occur after dark period (nocturnal life pattern animals). Arakawa et al. (2007) provided a useful description of social behaviors of inbred mice in a laboratory environment across their circadian cycle. They found that these mice were built to resemble the burrow systems in which small rodent species dwell in the wild. They showed that these mice exhibit more social approach towards other animals in the dark phase and more away from each other during the light hours. In comparison to dark phase they confirmed that standard laboratory inbred mice like wild dwelling mice, prefer to involve in social activities with other animals in the dark field. So that the active approach of mice may be the major social behavior in the dark phase, while inactivity animals that were more away from each other as a reflection of unsociability in the light phase (U. H. Hadi and H. A. Jaafar., 2019 and A. A. Naji, H. A. Jaafar and H A jaralla 2019 and A. Abdulsattar, H.A. Jaafar and H.A jaralla 2019).

Role of light dark cycle on behavior pattern of mice that is correspond to autistic behavior

The present study with experimental animals of light group reveal isolated living pattern similar to autistic behavior that agree with certain researchers who found nighttime light pollution is linked to cognitive dysfunction of brain. In addition to that the autism spectrum disorders (ASD) had shown disturbances in their sleep/wake cycle, and that had been particularly vulnerable to the impact on circadian cycle. They found the effect of exposure to even dim light at night time in a model of ASD. They found that dim light was sufficient to disrupt locomotor activity rhythms, exacerbate the excessive grooming with diminish in the social preference of experimental mice. They found that sleep (dark phase) induction by daily treatment of melatonin would reduced the excessive grooming of the experimental mice (with ASD) to wild-type levels and improved activity rhythms. The common circadian disruptors such as light at night should be considered in the management of ASD.

Changes in the Thickness of molecular layer, Depth of sulci & width of gyri according to light dark phases

The present study reveals that thickness of whole and molecular layer of temporal lobe reveals a significant decrease in the light in comparison to other animals this is due to stress nous effect light on the histological features of experimental animals that agree with other researchers who found that thickness of the cortex is of great interest in evaluation the normal brain development as well as a wide variety of neurodegenerative and psychiatric mood disorders. They concluded that various changes in the gray matter that made up the cortical sheet were manifested in normal aging, Alzheimer's disease, other types of dementias, Huntington's disease, cortico-basal neurodegeneration, amyotrophic lateral sclerosis, as well as schizophrenia. They also found that the cortical thinning is often regionally specific, and the progress of the cortical brain atrophy that reveal about the progress and causative factors of a nervous disease. Moreover, these studies that measuring the cortical atrophy and its thickness were potentially of great value in assessing the progress and efficacy of a wide variety of managements. These certain methods about evaluating morphometric properties of the human cerebral cortex, with important applications in the study of the patterns of geometric changes that were associated with specific diseases, as well normal brain development and aging. (Bruce F and Anders M. D. 2000).

The cerebral cortex has a high convoluted appearance with distinct morphologic features that known as gyri, sulci and fissures, which are related with the functional integration center in the human brain. So that the brain atrophy was accompanied with cognitive disabilities and weakening that is a well-accepted in aging process. The detailed configurations of cortical folding were be changed during aging, especially the changing age-dependencies of gyri and sulci, which is essential to effect on brain functioning. These researchers studied the morphology of gyral and sulcal regions from pial and white matter surfaces using MR imaging(MRI) data from adulthood to elderly (21–92 years). They evaluate their age-dependencies during normal aging by measuring the cortical thickness of gyri and sulci. With comparing to gyri and the sulcal thinning which is the most prominent pattern during the aging process, and the gyrification of pial and white matter surfaces were also affected differently, which implies the vulnerability of functional exclusion during aging. They concluded that the morphological model of aging may provide a framework for understanding the mechanisms underlying gray matter degeneration that mean more appoptosis of neurons with loss of their functions. This accomplished by calculating the shortest distance between the pial surface and the gray matter-white matter boundary of the tessellated surface, so that the vertex-wise cortical thickness was obtained. So that the relationship between thickness and age across the whole-brain vertices were confirmed. The effects of aging on the cortical features, was done by calculating the average thicknesses of the gyral and sulcal regions and the ratio of the gyral and sulcal thicknesses (Lin HY, et al., 2021).

Effect of light on induction the programmed cell death (apoptosis) of neurons in gray matter of temporal lobe of cerebral cortex

The present study reveals that light group animals were developed marked apoptotic histological features of nerve cells in the cortex of temporal lobe (neurons with foamy cytoplasm, multiple blebed cell membrane and nuclear fragmentation) and this agree with researchers who found that inappropriate light-at-night can have adverse long-term effects on the survival of nerve cells in the developing brain of chicks that persist reaching the adult life(Haraguchi et al., 2019). They applied chicks as an ideal model method for studying evaluate the effects of light on the developing brain. Chicks like human people, have diurnal life (they are active during the day and sleep at night). They concluded that exposing chicks

to constant light, or one hour of aberrant light per night, in the week after hatching would cause an increase in the number of death in the Purkinje nerve cells of cerebellum. Also, these degenerated nerve cells were observed predominantly in the part of the cerebellum that is closest to the pineal gland and persisted into young adulthood. The Purkinje nerve cells death due excessive light exposure were seen in several neurological disorders, including autism (**Jayabal and Watt, 2019**), and the pattern of Purkinje cell death following light at night exposure during development is similar to the patterns of cell death that was observed in several neurological disorders as cerebellar ataxia that affect motor coordination (**Langmade et al., 2006; Larivie`re et al., 2015**). However, certain researchers found that the disrupted sleep is a common component of many ataxia neulogical disorders (**Huebra et al., 2019**), their study suggested that the neurons survival in neurogenerative diseases may be linked to changes in neurosteroid production such as melatonin that caused by interrupted sleep. So that to get a good night's sleep, turning off screens and shutting blinds at night may help maintain the production of a neurosteroid that the neurons in the developing brain need to survive. This study concluded that light is stressful stimulus that potentiates apoptotsis of nerve cells in the cortex of temporal lobe wnd even other lobes in brain.

Role of light dark cycle on the P73 expression in neurons of the gray matter of temporal lobe of cerebral cortex

The present study found that p73 has role in brain development that is agree with other authors who found that p73 same role of p53 and family member that is essential for brain development. Global p73 deficiency reveals an overt marked cortical hypoplasia with resulting in hippocampal dysgenesis and hydrocephalus. The p73 isoform protein is known to function as a prosurvival factor of mature neurons after mitotic division. There is a novel essential role of p73 in the regulation the division of the neural stem cell of neuroblast and neuroglia cells. In neurogenesis, p73 has a vital role in maintaining an adequate neurogenic cells pool by promoting self-renewal and proliferation with preventing the premature senescence (aging) of neural stem cell and other early progenitor cells. they found that the p73 gene locus on its chromosome 4 that are essential maintenance factors in the central nervous system, whose wide action stretches across the entire differentiation arch from stem cells until become mature neurons with marked differentiation. (Talos F., et al 2010)

In the present study the marker expression of P73 on the CR cells in the molecular layer of dark group mice reveals a significant increase in P73 expression where there are neuronal cells proliferation, migration and differentiation that are more apparent in dark group mice in comparison to other groups and this indicate that darkness stimulates the P73 on neurons which has vital role in neuronal development, differentiation and act as a prosurvival factor for these neurons this agree with other researchers who found that neurons located within the marginal zone (layer I), there are the Cajal-Retzius (CR) cells that provide diffusible signals, pivotal for the proliferation, migrations of these cortical plate neurons (Frotscher. 1997), while the other found that CR cells secrete the another glycoprotein called REELIN, which is essential for neuronal glial cells mediated migration (De Rouvroit et al. 2001). They found that this glycoprotein REELIN induce other neurons to migrate preceding their precursors (predecessors) (D'arcangelo and Curran.1998). The present study found that the six layers of cerebral cortex were arranged according to functional localization of these nerve cells whether pyramidal or granular or multiform layers which indicate that REELIN has provide a stop signal to the migrating cells for making them detach into specific functional cortical layer from the already radial neurons migrating that accompanied the radial glial cells. This means that REELIN has been shown to inhibit neuronal migration via its interaction with α3-β1 integrin protein, a downstream component of its signaling pathway (Dulabon et al. 2000). This migration-inhibiting activity of REELIN should be relevant at the end of radial migration, when the neuronal leading process contacts the marginal zone and pyramidal neurons detach from the radial glial cells that act as a scaffold (Nadarajan et al. 2001).

This present study concluded that increment in P73 expression will indicate neuronal cells proliferation, migration and differentiation in dark phase so that darkness stimulates the P73 on neurons which has vital role in neuronal growth and development with differentiated active functioning neurons with less apoptosis that act as a prosurvival factor. This is reverse for light group as light acts as stimulant physical factor for apoptosis and nerve cells death with subsequently neurodegeneration brain atrophy and aging progress (senesence). This was agree with other researchers who found effect of light on the activity of the developing follicles in ovaries, or germinative epithelium of seminiferous tubules of testis or inner layer of eye ball, retina in mice (U. H. Hadi and H. A. Jaafar., 2019 and A. A. Naji, H. A. Jaafar and H A jaralla 2019 and A. Abdulsattar, H.A. Jaafar and H.A jaralla 2019).

5. Conclusions

The experimental animals of light group reveal high body weight than that of dark animals due to increase in food consumption. The experimental animals of light group reveal less sleep (insomnia) and overactive than that of dark group animals. Although these animals became adapting to such daily disruption of light or dark field. they override the tendency of mice to sleep during the light phase. The experimental animals of light group reveal repellent, lonely with isolated behavior and the reverse occur in dark field mice were close together with active social behaviors. The experimental animals of light group reveal quarreling with aggressiveness behavior in comparison to dark group mice. The study reveals that thickness of

whole layers and especially molecular layer of temporal lobe reveals a significant decrease in the light group in comparison to other animals this is due to stress nous effect light on the histological features of experimental animals that induces more apoptosis and cells death. the study reveals that light group animals showed marked less cellular than that of dark group mice due to more apoptosis of nerve cells in the cortex of temporal lobe. The P73 expression of the CR cells in the molecular layer of dark group mice was indicating neuronal cells proliferation, migration and differentiation. This means P73 on neurons which has vital role in neuronal growth and development with differentiated active functioning neurons with less apoptosis that act as a pro-survival factor. light acts as stimulant physical factor for apoptosis and nerve cells death with subsequently neurodegeneration brain atrophy and aging progress (senesence).

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