Title
Photo-environment affects disease progression in bacterial artificial chromosome (BAC) Huntington's disease mouse model

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Publication Date
2015

Peer reviewed|Thesis/dissertation
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A thesis submitted in partial satisfaction of the requirements for the degree Master of Science in Physiological Science

by

Huei-Bin Wang

2015
ABSTRACT OF THE THESIS

Photo-environment affects disease progression in bacterial artificial chromosome (BAC) Huntington’s disease mouse model

by

Huei-Bin Wang

Master of Science in Physiological Science

University of California, Los Angeles, 2015

Professor Christopher S. Colwell, Committee Co-Chair

Professor Gene D. Block, Committee Co-Chair

Circadian rhythms are generated by an intrinsic timing system which broadly regulates our body. Patients with Huntington’s disease (HD) exhibit disrupted circadian rhythms. By itself, disruption of the circadian system results in a broad range of symptoms and it is likely that the circadian dysfunction seen in HD contributes to the symptoms of the disease. Here we show that inappropriate photo-environment such as constant dim light can further disrupt sleep/wake rhythms and make HD symptoms worse. On the other hand, blue light treatment improved the sleep/wake cycle but did not alter motor symptoms. My results suggest that HD patients may be vulnerable to the negative impact of light pollution and may be able to improve their sleep cycle by careful attention to the photic environment.
The thesis of Huei-Bin Wang is approved.

Fernando Gomez-Pinilla

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University of California, Los Angeles

2015
# Table of Contents

1. Introduction  
   
1.1 Circadian System  
   1.1.1 The ipRGCs with the photopigment melanopsin directly send photic information to the central oscillator 1  
   1.1.2 The molecular clock is composed of three core feedback loops 2  
   1.1.3 SCN outputs maintain coherence between peripheral clocks 4  
   1.1.4 Inappropriate light cues result in physiological disruption and may trigger mood disorders. 6  

1.2 Huntington's Disease  
   1.2.1 HD is a neurodegenerative disease resulting from abnormal CAG repeats 8  
   1.2.2 Both HD patients and animal models exhibit circadian deficits 9  
      1.2.2.1 Movement Disorder 9  
      1.2.2.2 Psychiatric Disturbance 12  
      1.2.2.3 Cognitive Impairments 12  
   1.2.3 BACHD mouse model of HD was chosen for the study 13  

1.3 Links between the circadian system and HD  
   1.3.1 Circadian dysfunctions might interact with HD and makes it worse 14
1.3.2 Previous studies in BACHD mice support that the circadian rhythm in HD is broken.

1.3.3 Light therapy is useful for many disorders including neurodegenerative diseases.

2. Specific Aim and Hypothesis

2.1 Specific Aim

2.2 Thesis Hypothesis

3. Material and Methods

3.1 Animals

3.2 Housing Conditions

3.3 Cage Activity

3.4 Video Measurement of Immobility-defined Sleep

3.5 Motor tests

3.6 Novel Object Recognition Test

3.7 Open Field Test

3.8 Intruder Test

3.9 LPS Challenge and Multiplex Assay

3.10 Statistical methods

4. Results
4.1 Constant dim light significantly decreased the body weight of BACHD mice.

4.2 Constant dim light dampened whereas blue light treatment reinforced activity and sleep rhythms of BACHD mice.

4.3 Constant dim light but not blue light may have an effect on their motor performance.

4.4 No significant impairment or effect of lighting treatments on recognition memory on BACHD mice at 3 or 6 month of age.

4.5 Constant dim light resulted in declined locomotor activity but no effect on anxiety in BACHD mice

4.6 Constant dim light increased the percentage of BACHD mice showing aggressive behavior

4.7 Lower level of LPS-challenged IL4 in blue light-treated BACHD mice.

5. Discussion

5.1 Long-term exposure of constant dim light might diminish food intake of BACHD mice and contribute to their declined body weights at 6 month of age

5.2 Blue light treatment might prevent circadian rhythm from breakdown and benefit both general health and life quality of HD patients

5.3 The beneficial effect of blue light on motor performance may require longer treating duration and become more dominant at later age
5.4 The influence of lighting treatments on the recognition memory of BACHD mice might be more dominant after 9 month of age.

5.5 Constant dim light likely made BACHD mice more irritable to the surrounding events

5.6 The effect of lighting treatments on immune response could be masked by existed higher levels of cytokines and abnormal immune system in BACHD mice.

6. Conclusion

Reference
First and most importantly, I am grateful to my thesis committee, Dr. Gene Block and Dr. Fernando Gomez-Pinilla. Your advice and support are always invaluable and motivating.

A big thank you goes to Dr. Christopher Colwell, who always patiently guides me and helps me to become better. He is the best and kindest mentor I have known. I still remember that I was touched by his enthusiasm and kindness in his office at my very first day in his lab. Chris is always supportive and has faith in me irrespective of my limited English skills and minimal neuroscience background. I fall short of words to express my gratitude towards you.

It was grateful that I had the opportunity to meet many different people in UCLA and Colwell lab. Thank you for all the guidance, smiles, stories and encouragement. In fact, I would not be able to finish my master thesis without the help of the other lab members. Since my first day in the Colwell lab, Dr. Lol has kept on motivating me. I cannot thank enough to Dr. Lol for her help and guidance in everything that improved my research skills and thoughts. I also want to express my gratitude to other lab members, especially Daniel Whittaker, Collette Kokikian, and Laura Gad for their invaluable advice and help.

Thanks to all the people in UCLA for their friendly support. I will never forget all the cheerful moments I had here.

Most of all, I thank my family and my friends in Taiwan, who are like brothers and sisters for me. I cannot image having gone through this study abroad without the support, encouragement. All of them gave me the strength to reach the end of this thesis and my program.

Thank you all!
1. Introduction

1.1 Circadian System

Circadian rhythms are generated by an intrinsic timing system with a period about 24 hours. This timing system produces the daily cycles of sleep-wake, body temperature, and hormonal secretion. In order to function adaptively, this timing system must be synchronized to the local environment. Light is the most potent Zeitgeber (time giver) of circadian system. The melanopsin-containing intrinsically photosensitive retinal ganglion cells (ipRGCs), which are sensitive to the blue-light wavelength, receive light signals and transmit them to the central clock located in suprachiasmatic nucleus (SCN). SCN then sends information to other brain regions and the peripheral effectors to synchronize animal’s body with the environmental cues [1].

1.1.1 The ipRGCs with the photopigment melanopsin directly send photic information to the central oscillator

Among the environmental cues, light functions as the primary environmental synchronizer of the circadian system. In fact, the daily changes of photic environment resulting from the rotation of earth subject all creatures to cycles of light and dark. The master clock of the mammalian brain (SCN) uses light information to synchronize its own internal oscillation to ensure that organisms exhibit a particular behavior at an appropriate time of the day.
Rods and cones used to be considered the exclusive photoreceptors in the mammalian eyes. However, studies showing that genetically engineered mice without rods and cones still had normal circadian rhythms, suggesting that the photoreceptor for circadian entrainment is distinct [2]. This novel photoreceptor, which turned out to be melanopsin, was first discovered in *Xenopus* skin melanophores. It was then shown to present in the layer of retinal ganglion cell (RGC) of mouse and primates. In the same study, Provencio and colleagues [3] first determined that the nucleotide sequence of melanopsin was different from rhodopsin and cone opsins. In addition, their results of RT-PCR of different body tissues suggested that the expression of melanopsin was restricted to the eyes. Further *in situ* hybridization histochemistry revealed that melanopsin was expressed in the inner retina of both laboratory monkey and mouse [3]. The link between this novel photoreceptor and circadian system was determined when another team found impaired capability of photoentrainment in a mouse model (*Opn4*−/−) that had normal rhodopsin and cones but lacked melanopsin [4]. The locomotor activity rhythm of *Opn4*−/− mice was similar to the control mice under regular light/dark (LD) cycle or constant darkness. In addition, the acute light suppression of activity which was generated by a 300-lux white light pulse during the dark phase was also preserved. However, the period of locomotor activity rhythm of *Opn4*−/− mice was shorter than the control mice when they were housed under constant lighting condition, suggesting that lengthening effects of light were attenuated in the *Opn4*−/− mice. The results that the capacity of light synchronization was impaired rather than completely destroyed indicated that melanopsin cannot be the only light detector for the circadian system.

Melanopsin is an opsin class of G-protein-coupled receptor (GPCR) [5]. It uses 11-*cis* retinaldehyde as the light detector (chromophore). Activated by lights, the conformational change of 11-*cis* retinaldehyde into all-trans retinal leads to downstream signaling transduction. Through the signal transduction, these ipRGCs depolarize their membrane and directly send the photic information to the SCN via the retinohypothalamic tract (RHT).
addition, this photopigment shows peak spectral sensitivity at 480nm, which lies in the blue/green range of the visible light. In other words, lights with this wavelength have the strongest impact on the circadian system of humans and other mammals. In fact, blue light exposure has been shown to have acute impact on the physiological and psychological state including core body temperature, heart rate, alertness, and cognition [6, 7].

1.1.2 The molecular clock is composed of three core feedback loops

SCN rhythms in cellular and molecular expression are generated by transcription/translation feedback loops involving genes and their products [8-10]. Sharing core components, the molecular clockwork is broadly conserved from flies to mammals. In mammals, there are three core interlocking feedback loops responsible for the core oscillator. (Fig. 1) The central loop is comprised of the CLOCK:BMAL1 heterodimer and their driving repressors (PERs and CRYs), which negatively feedback on their own expression. The CLOCK (circadian locomotor output cycles kaput) protein and BMAL1 (brain and muscle ARNT-like-1) protein heterodimerize and activate the expression of PERs (period 1-3) and CRYs (cryptochrome 1-2) by interacting with the E-box domain (CACGTG) of those genes’ promoters. The resulting proteins form complexes that accumulate in the cytoplasm and are later translocated back to the nucleus to interrupt the transcriptional activities of CLOCK:BMAL1. With the increasing levels of PERs and CRYs overtime, the transcriptional activities of CLOCK:BMAL1 are gradually repressed by PERs and CRYs. To recover their transcriptional activities, CRYs are degraded by an ubiquitin process. This allows a new 24 hour cycle to begin.

Additionally, as a secondary feedback loop, CLOCK:BMAL1 also promote the expression of REV-ERB (α, β) and ROR (retinoic acid receptor-related orphan receptor) (α, β, γ). They translocate back into the nucleus and interact with the ROREs (ROR response elements) on the promoter region of BMAL1 and regulate its transcription. RORs are activators of BMAL1
whereas REV-ERBs function as repressors. The third loop consists of the DBP (D-box binding protein) and the NFIL3 (nuclear factor interleukin 3). The expression of DBP is regulated by the E-box; on the other hand, the expression of NFIL3 is mediated by ROREs. The DBP activates whereas NFIL3 represses the expression of D-box and regulate the expression of PERs.

Fig. 1: The molecular clock is composed of three core feedback loops. In mammals, there are three core interlocking feedback loops responsible for the core oscillator. The figure is modified from the review by Curtis et al 2014. [10].

1.1.3 SCN outputs maintain coherence between peripheral clocks

The identification of SCN as the central clock was first shown in rodents. In 1972, studies by Moore and Stephan demonstrated that removal of the SCN would cause a permanent loss of the rhythmicity of behavior and endocrine activity [11, 12]. Their findings suggest that this structure might be the internal pacemaker for physiological and behavioral activities. In
addition, an SCN graft rescues rhythmicity of the SCN-lesioned host, and the restored behavior exhibits the period of the donor instead of the host [13]. Most importantly, SCN neurons function as intrinsic oscillators [8]. In both diurnal and nocturnal animals, the spontaneous firing rate of SCN displays rhythmic pattern: it shows peak action potentials in the middle of the day, and becomes silent at night. Moreover, SCN neurons preserve their circadian rhythm in electrical activity even when they are isolated from their circuit. Put them all together, the evidence suggests that the SCN is the central clock which plays necessary role in circadian system.

Anatomically, SCN is a bilaterally paired nucleus made up of ventral (core) and dorsal (shell) regions [14]. The majority of neurons express GABA (gamma-aminobutyric acid) and use GABA as a transmitter. The ventral SCN neurons have lower amplitude rhythms and express VIP (vasoactive intestinal peptide) or GRP (gastrin-releasing peptide); on the other hand, the dorsal SCN neurons have robust rhythm, and express vasopressin or PK2 (prokineticin 2). Functionally, the ventral SCN integrates the environmental signals and communicates with the dorsal SCN. Both ventral and dorsal SCN have projections to the subparaventricular zone and other medial hypothalamic structures surrounding them. In addition to SCN, there are other oscillators in peripheral organ systems such as our heart, immune cells, and liver. These peripheral oscillators have their own rhythm that regulates tissue-specific gene expression and mediate their functions. Through autonomic nervous system (ANS) and hypothalamus-pituitary-adrenal (HPA) axis and other hormones, the SCN outputs send environmental information to the rest of the brain and the peripheral organ systems. The hormone epinephrine and norepinephrine in the ANS as well as glucocorticoid in the HPA axis act as synchronizers to the peripheral clocks. As a result, the central clock, SCN, plays a critical role in maintaining coherence between peripheral clocks and thus robust circadian rhythmicity in the organism as a whole [15].
1.1.4 Inappropriate light cues result in physiological disruption and may trigger mood disorders.

Light is the strongest Zeitgeber and has broad impact on both physiology and psychology. Besides the visual function via rods and cones, the melanopsin-containing RGCs project to SCN and communicate the effects of light on the circadian system. Light at inappropriate times can cause desynchronization between the SCN and the peripheral oscillators resulting in disorders in sleep [16], the immune system [17], the cardiovascular function [18] and mood [19]. For example, the artificial light that people are exposed in the evening can delay their sleep onset. Melatonin is a hormone released by pineal gland at night. It is important for sleep regulation, and can be acutely suppressed by nighttime light exposure. Accordingly, a study investigating the effects of light in the evening on 22 male and female subjects reported suppressed melatonin levels and delayed sleep onset [20].

Inappropriate light exposure also impacts the immune system. In fact, the circadian system and the immune system are tightly and reciprocally coupled: an immune challenge can induce a phase delay of the circadian oscillation; on the other hand, immune cells show daily variations in cell numbers in the blood. Many pro-inflammatory cytokines also display diurnal variation. Their peak levels are found during the rest phase of rodents (day time) and human (night time). Light disruptions at night can result in an abnormal immune response. Nighttime exposure of light suppressed the numbers and the cytotoxic activities of splenic NK cells in rats.[17] Jet lag, a syndrome that happens when rapidly crossing different time zones, involves the desynchronization between the internal circadian rhythm and the external light-dark (LD) cycle. A chronic jet lag paradigm is one where the LD cycle is regularly advanced or delayed with an insufficient interval to allow the animal's circadian system to become entrained. Animals housed in constant lighting (LL) conditions or chronic jet lag conditions were reported to have more severe inflammatory response and increased mortality than those housed in regular LD cycle [21].
Many studies have reported that inappropriate light exposure affects cardiovascular rhythm [22]. Because daily rhythms in cardiovascular function are mediated by the SCN, the phase-shifting effect of light can thus alter cardiovascular rhythms. Moreover, light also has an acute influence on cardiovascular system. In human studies, light exposure at night increase heart rate (HR) and heart rate variability (HRV). Another study done on human volunteers showed that light affected cardiac tone. The light in early morning increased the sympathetic cardiac tone, which partially responsible for the increased heart rate [18]. Consistently, light also influences the cardiovascular system in animal models. For example, rats housed under regular LD cycle had higher systolic blood pressure, diastolic blood pressure, and heart rate in the dark phase than in the light phase. The rats under LL condition for 17 weeks exhibited disrupted rhythms. Specifically, their systolic and diastolic blood pressures were increased during resting phase whereas their heart rate was decreased during active phase. Nevertheless, transferring these mice from LL conditions to a regular LD cycle could fully restore the normal cardiovascular parameters within 1 week [23].

Seasonal mood fluctuation provides some of the evidence that light exposure affects psychiatric states [16]. Many people who live in high latitude areas, where there are more dramatic seasonal daytime changes, have more frequent depression during the fall comes (shorter daytime). Shift workers, who are exposed to artificial lights during their resting phase, are reported to have sleep as well as mood disorders [24-26]. In the lab, extreme lighting conditions have been well-studied and have been shown to be detrimental to mood and cognitive function. Depression-like behaviors, anxiety-like behaviors and impaired learning and memory have been observed in rodents that were exposed to inappropriate photo-environment such as LL, nighttime dim light exposure, short daylight exposure, or Jet lag [27-29].
1.2 Huntington’s Disease

1.2.1 HD is a neurodegenerative disease resulting from abnormal CAG repeats

Huntington’s disease (HD) is a neurodegenerative disorder characterized with clinical triad of symptoms: movement disorders, psychiatric disturbance and cognitive impairments. In HD patients, the non-motor symptoms such as sleep deficits, depressed mood and metabolic disorders start earlier than the onset of motor symptoms [14]. HD was first discovered in 1692, but the full and accurate description was published by George Huntington in 1872. HD shortens life span with death usually occurring within 15–20 years of showing signs of the disease. HD gene, as known as IT-15 (interesting transcript 15), was found on chromosome 4 (p16.3) by the Huntington’s Disease Collaborative Research Group in 1993 [30]. The genetic deficits of HD are resulted from the abnormal and unstable expansion of the cytosine-adenine-guanine (CAG) repeated sequence in the region encoding huntingtin protein (htt). The unstable repeat is translated into a polyglutamine (polyQ) stretch of the htt, resulting in mutant form (mhtt). There is a direct relationship between the length of CAG repeats and the age of disease onset [31]. Subjects with less than 26 copies of the CAG repeats are considered normal. Subjects with 27-35 copies are regarded as intermediate risk and 36-39 repeats are regarded as high risk. More than 39 CAG repeats results in HD, and the longer the repeats, the earlier onset of the disease [32-34]. Importantly, the study by Wexler et al. suggested that environmental stresses also played a role. It reported that the environmental factors contribute to nearly 40% of the age of HD onset [35].

The precise function of htt within cells remains elusive. Importantly, mice with targeted knockout of htt gene are embryonic lethal [36]. In addition to the necessary role in development, HTT functions as a scaffolding protein essential for intracellular and synaptic
vesicular trafficking. The mechanism responsible for neurodegeneration, however, is not well-understood. Although htt mRNA is expressed in almost all tissues of the body and homogenously throughout the brain of people with or without HD, the neuropathology is selective. Pathologically, there is an evident neuronal loss in the striatum, which is usually accompanied by cell loss in the cerebral cortex and widespread brain atrophy. The aggregation of mhtt causes progressive loss of GABA containing medium spiny neurons (MSNs) in caudate and putamen, and finally resulting in great atrophy of the striatum. The neurodegeneration can also be found in the globus pallidus, cerebral cortex, hippocampus and hypothalamus. As a result, the degenerative process leads to the three clinical symptoms [37-41].

1.2.2 Both HD patients and animal models exhibit circadian deficits

There are three different types of functions that are affected with HD: movement disorder, psychiatric disturbance and cognitive impairments.

1.2.2.1 Movement Disorder

Uncontrollable movement is a prominent feature of HD [42]. The early motor deficits include difficulties in fine motor control and gait disturbance. Chorea is the most well-known motor impairment, and it has been seen in more than 90% of patients. Other motor disturbance such as bradykinesia (a slowness of voluntary movement) and rigidity (a resistance to passive joint movements) often become dominant in the late stages of HD [43]. Motor deficits suggest that the region of the brain controlling muscle and coordination is impaired. Focused movements require executing desired movements (direct pathway) while suppressing unwanted movements (indirect pathway) (Fig. 2). In the direct pathway, MSNs inhibit globus pallidus interna (GPI) and substantia nigra pars reticulata (SNr) through GABA,
and thus disinhibit the ventral anterior (VA) and ventral lateral (VL) thalamic nuclei. As a result, direct pathway excites motor cortex (Fig. 2A). In the indirect pathway, MSNs inhibit globus pallidus externa (GPe), which in turn disinhibits the subthalamic nucleus (STN). The exciting STN then activates GPi and SNr via glutamate, and thus inhibits VA/VL thalamic nuclei and motor cortex [44] (Fig. 2B). The coordinating direct pathway and indirect pathway function as a balanced system in order to executing desired movements while suppressing unwanted movements. Therefore, the loss of striatal MSNs in HD is likely to underlie the motor abnormalities. They lost their ability to do fine motor movements and have irrepressible undesired chorea (Fig. 3).

Fig. 2: The functional direct and indirect pathway of basal ganglia circuitry. Fine movements require executing desired movements (direct pathway) while suppressing unwanted movements (indirect pathway). (A) Direct pathway excites motor cortex. (B) Indirect pathway inhibits motor cortex. Figure is modified from the review by Raymond et al 2011. [45]
Fig. 3: Impaired basal ganglia circuitry in HD patients. In HD, loss of striatal MSNs disrupts the coordination of direct pathway and indirect pathway, and results in motor abnormalities. Figure is modified from the review by Raymond et al 2011. [45]

In my thesis project, I performed the accelerating rotarod test [46] and the challenging beam test [47], to assess the motor performance of BACHD mice. Briefly, both tests assessed the motor coordination and balancing ability of BACHD mice. In the accelerating rotarod test, mice were put on a rotating rod. The better the motor coordination and balance, the longer the mice could stay on the rod. The challenging beam test determined the ability of mice to cross a bridge of decreasing width, with re-entry into the home cage as the motivating factor. It scored the stepping errors when a mouse passed the beam that was suspended above the ground. The wire hang test was also performed to evaluate motor deficits in BACHD mice. In this test, a BACHD mouse was placed on the top center of a mesh, and then the frame was
inverted. A mouse with better motor function should stay longer on the frame than mouse with poor motor function.

1.2.2.2 Psychiatric Disturbance

HD patients are reported to exhibit psychological and behavioral disturbances including anxiety, depression, aggression, and apathy. Aggression and irritability might severely destroy the relationship between HD patients and their family and caregivers. Suicide attempts are also more common in patients. These kinds of personality changes can manifest even more than 10 years before the motor symptoms [48-52]. In my study, the anxiety-like behavior was assessed by using the open-field test. The aggressive behavior of BACHD mice was assessed by a territorial challenge, namely intruder test.

1.2.2.3 Cognitive Impairments

The decline of cognitive capabilities is progressive and occurs in all HD patients. Impaired learning and memory are most noticed, and HD patients are reported to have difficulty in retrieving stored information several years before the manifestation of their motor symptoms. Cognitive impairment worsens as the disease develops and eventually leads to dementia [53, 54]. In addition, disturbed sleep and circadian rhythm are also prominent features of the disease that deleteriously affect the quality of life of both patients and caregivers [55]. In the present study, the novel object recognition (NOR) test was performed to measure the deficits in the episodic memory of BACHD mice. Their sleep and activities were recorded as well in order to characterize their sleep pattern, temporal activities, and the underlying circadian disturbances.
1.2.3 BACHD mouse model of HD was chosen for the study

There are 3 general types of mouse models of HD [56], and the bacterial artificial chromosome (BAC) HD mouse model is one of them. This model is developed in Professor William Yang’s lab in UCLA in 2008 [57]. BACHD mice are the transgenic mice that express the entire human htt gene, including all the introns and exons as well as the regulatory upstream and downstream sequences. This transgenic design is expected to ensure appropriate regulation of the mhtt expression. In this model, the transgenic mice express mhtt with 97 CAG repeats. With the stable CAG repeats, this sophisticated HD model has been proved to show progressive motor disturbance as well as cognitive and psychiatric dysfunction [43]. These progressive phenotypes are parallel to what has been described in HD patients and make BACHD mice a good model to study. The motor symptoms nearly start at 2 months of age, and the circadian deficits begin at 3 months of age. Although R6/2 mouse model is the very first HD mouse model and has been well studied, its lifespan is too short for long-term treatments. For this reason, the BACHD mouse model was favored for circadian-based treatment.
1.3 Links between Circadian system and HD

1.3.1 Circadian dysfunctions might interact with HD and alter disease progression.

The first connection between circadian disorders and HD patients came from the study by Morton et al. 2005, showing that HD patients exhibited abnormal sleep/wake cycle. Particularly, those patients were found delayed sleep onset, fragmented sleep, more night time activities, and greater sleepiness during the waking phase. These abnormalities are early HD features and correlate with cognitive dysfunction and disease severity [58, 59].

Consistent with the abnormal sleep/wake cycle, the changed circadian pattern of melatonin has also been reported in early-stage HD. Melatonin is a hormone that responses to the light cues, and plays major role in the regulation of sleep and circadian phase-shifting. In addition, studies have demonstrated the ability of melatonin to protect neurons under conditions of enhanced oxidative stress. It suggests that abnormal melatonin secretion may also influence the neurodegenerative process underlying HD [55, 60, 61]. The decreased number of VIP and AVP neurons has also been observed in the SCN of HD patients whereas they are unchanged in other brain areas [62]. Therefore, it seems that the disrupted circadian rhythm might arise from the SCN.

The prior studies suggest that the circadian system is compromised in HD. Poor circadian rhythm can cause negative impacts on both central nervous system and peripheral organ systems. For example, people who suffer from disrupted circadian rhythms, such as shift workers, have lower capacity of learning and memory and tend to be more anxious [24]. The consequences of circadian disruption also include cardiovascular system disorders and abnormal immune response. Notably, all these symptoms are similar to the phenotypes observed in HD patients. Moreover, previous work by Wexler et al. reported that the environmental factors contribute to nearly 40% of the age of HD onset [35]. It suggests that
the environmental stress might participate to the disease onset and progression. Therefore, circadian dysfunctions itself might interact with the HD disease and make it worse.

1.3.2 Previous studies in BACHD mice support that the circadian rhythm in HD is broken

HD patients are reported to have delayed sleep onset, fragmented sleep, more night time activities, and greater sleepiness during the waking phase [59]. Consistently, our previous study has revealed that both R6/2 mice and BACHD mice showed low amplitude as well as fragmented wheel running activities under both LD and DD conditions. The diseased mice also had more daytime activities than WT. In addition, our lab found that sleep onset was delayed [56]. The deficit in the onset of sleep is similar to the sleep phenotype of HD patients. In the same study, we also reported disrupted circadian regulation of the cardiovascular system in BACHD mice. Heart rate and core body temperature exhibit robust circadian rhythm: lower during sleep phase and higher during active phase. The difference between day and night, however, was compromised in BACHD mice. They had higher heart rate and body temperature during their sleep phase, suggesting a loss of circadian control. Moreover, daytime spontaneous firing rate was reduced in the SCN of BACHD mice. The SCN exhibits higher firing rate during daytime compared with the nighttime. While WT mice showed clear day-night difference, BACHD mice didn’t show diurnal changes. This is the first finding showing that SCN outputs are disrupted in the HD mouse model.

Briefly, our previous studies support the hypothesis that BACHD mice have a compromised circadian system. If the hypothesis that circadian dysfunctions contribute to HD symptoms is correct, we would expect that it might be possible to rescue HD by improving their sleep/wake cycle. If our hypothesis that we can rescue BACHD mice by reinforcing and correcting their circadian rhythm is confirmed, it would be potential and promising benefit for human patients.
1.3.3 Light therapy is useful for many disorders including neurodegenerative diseases

Light has potent and profound impacts on central nervous systems [16, 63, 64] and peripheral organ systems [23, 65]. Previous studies have shown that inappropriately timed lighting can cause undesired disorders whereas optimized lighting condition can be a powerful non-pharmacological therapy to many disorders. In general, light therapy uses a light box which emits 2500-10000 lux of light at a specified distance. The entraining effects of light depend on the timing, intensity, duration, wavelength, and distance from the device. The principle behind the light therapy is that the light exposure activates the SCN and promotes the synchronization between internal circadian rhythm and environmental LD cycle, which can result in enhanced physical and psychiatric outcomes. Indeed, light therapy has been proved to stabilize the circadian system and improve sleep deficits, including improvement in reducing the difficulties in sleep onset and difficulties in sleeping through the night. It is has been applied and shown to be effective and beneficial to those who are suffering from circadian rhythm sleep disorders (CRSD), including delayed or advanced sleep-phase disorder, irregular sleep-wake disorder, and free-running rhythm disorder [66, 67]. In addition, light therapy is also useful to resolve the circadian deficits of shift workers and those suffering from Jet lag. It also ameliorates depressive symptoms in patients with seasonal affective disorder (SAD) and Jet lag [68]. Because circadian rhythm is tightly related to emotions, it has been shown to be beneficial for other symptoms besides depression, such as irritability and aggressive behavior. They suffer from sleep disorders in the evening and likely to become aggressive during the day. In addition, although more data are required, light therapy has been proposed and may provide better control of seizures [69].

While bright light therapy attempts to simulate the sunlight without harmful components, the blue-enriched light therapy has evolved more recently based on the discovery of
melanopsin-containing RGCs in human. With the sensitive peak at wavelength 450 to 500nm, which is the blue to green range of the light spectrum, the melanopsin-contain RGCs are primarily responsible for encoding environmental light to circadian information. Studies have compared the phase-shifting effects among red light (660 nm), green light (525 nm), blue/green light (497 nm) and blue light (470nm). The results showed that light with shorter wavelengths are more effective than with longer wavelengths in suppressing melatonin and in phase shifting. Therefore, the human circadian system is more sensitive to the blue light than red light. Based on these findings, a low intensity blue enriched light should be as effective as a standard bright light therapy. Furthermore, the blue-enriched light therapy potentially benefits current light therapy by shorter therapy sessions, more comfortable light intensity, and energy saving [70]. Therefore, some studies have started to investigate the effectiveness of blue-enriched light therapy on many disorders that have been accomplished in the past with standard light therapy. When treating SAD, 750 lux of blue-enriched light has been shown to be equally effective as 10000 lux standard light therapy [71]. In addition, blue-enriched light therapy is capable of improving mood disorders and cognitive functions [16].

Light therapy has been applied to neurodegenerative disease such as Parkinson’s disease (PD) and Alzheimer’s disease (AD) as well. In fact, circadian deficits are also observed in both PD and AD patients. The symptoms include excessive daytime sleepiness, sleep fragmentation, reduced total sleep time, and delayed sleep onset. In PD patients, light therapy seems to be effective in treating sleep disorders and depression. Some studies even pointed that light therapy have positive influence on motor function [72]. In Alzheimer’s disease related disorder (ADRD) patients, clinical research has demonstrated that light therapy can consolidate the sleep-activity pattern. Light exposure in the morning also helps them to sleep better at night. Moreover, there are studies reporting that morning light significantly improves aggressive behavior, depression and even aberrant motor behavior [73].
HD is one of neurodegenerative diseases. These positive results from PD and AD studies suggested that light therapy might also work in HD. In fact, the combination of bright light and scheduled exercise through limited wheel assess has been shown to lead to improvements in the R6/2 HD mouse model [74]. The standard light therapy, however, requires very bright white light which is likely abhorrent to HD patients. In addition, the lifespan of R6/2 mice are too short to assess detailed impact of circadian treatment. For this reason, the BACHD mouse model was favored for circadian-based treatment because of their normal lifespan. Since the blue wavelength light has the strongest impact on the circadian system; for my thesis study, I applied low intensity of blue-enriched light rather than a high intensity standard light on BACHD mice to assess the detailed impact of the treatment.
2. Specific Aim and Hypothesis

2.1 Specific Aim

Based on prior work showing that the environmental factors contribute to nearly 40% of the age of HD onset [35], the environmental stress might accelerate to the disease onset and progression. If the compromised circadian rhythm interacts with HD and exacerbates the manifestations of the disease, we would expect that restoration of a normal circadian rhythm might improve HD while disrupting the circadian system would negatively impact HD progression. Compared to other Zeitgebers (e.g. food and temperature), light is the strongest one among them. In fact, inappropriate photo-environment has been reported to cause a negative influence on mood, memory, cardiovascular system, immune system, and sleep [6, 16, 19, 21]. Therefore, my thesis tested that whether inappropriate lighting conditions can disrupt the circadian system and might exacerbate the symptoms of HD. In addition, my thesis study also tested whether light therapy could benefit HD symptoms in BACHD mice. I applied low intensity of blue-enriched light during the resting phase of BACHD mice in the present study. By examining the bidirectional impact of lighting conditions on BACHD mouse model, my thesis project explores a potential therapy for HD symptoms.

2.2 Thesis Hypothesis

**Hypothesis 1:** Constant dim light can disrupt the circadian system of BACHD mice and make their symptoms worse.

**Hypothesis 2:** Blue light can reinforce the entrainment and improve the sleep/wake cycle of BACHD mice and improve their symptoms.
3. Material and Methods

All experimental protocols used in this study were approved by the University of California Los Angeles Animal Research Committee (ARC 2009-022). Every effort was made to minimize pain and discomfort. Experiments followed University of California Los Angeles Division of Laboratory animal recommendations for animal use and welfare, as well as National Institutes of Health guidelines.

3.1 Animals

The BACHD mice were on the C57BL6/J background. They were obtained from our colony at UCLA. All animals were singly housed within light-tight chambers with controlled lighting conditions. Animals were assessed for cage activity, motor performance, cognition, affect, and sleep patterning between 2-3 months of age prior to beginning treatments. After all the pre-treated data were collected, the BACHD mice were housed under different lighting conditions for 3 months. They are reassessed at 6 months of age after 3 months of continuous treatment. All the tests except sleep recording and cage activity were run during ZT 18 – ZT 22 under dim red light (10–30 lux).

3.2 Housing Conditions

All animals received cotton nestlets and rodent chow ad libitum. Before any treatment of lighting conditions, animals were housed under regular 12:12 LD cycle. After all the pre-treated data were collected, the BACHD mice were housed under different lighting conditions for 3 months: The control group was housed under regular lighting condition without any treatment. The constant dim light-treated group was housed under dim light of 20 lux during both active phase and sleep phase. The blue light-treated group was housed
under regular light-dark cycle with 6 hours of blue light exposure during the light phase (ZT 0 - ZT 6, 500 lux). The light intensity of blue light plus bright light is 1000-1200 lux in this group.

3.3 Cage Activity

Adult male BACHD mice (n=40) at 2 month of age were singly housed in cages and locomotor activity was recorded as previously described [47] using infrared sensors. The cage activities were recorded in order to determine the period, rhythmic strength and nocturnality of their activities. Mice were exposed to 12:12 hour LD cycle and entrained for 2 weeks before the collection of data under LD conditions. Zeitgeber time 0 (ZT 0) was the onset of lights turning on, and ZT 12 was the time when lights turn off under the LD conditions.

Briefly, cage activity was recorded in 3 min bins, and 10 days of data was averaged for analysis. Free-running period (tau, \( \tau \)) was determined using the \( \chi^2 \) periodogram and the power of the rhythm was determined by multiplying the amplitude, Qp, by 100/n, where \( n \)=number of data points examined using the El Temps program (A. Diez-Noguera, Barcelona, Spain). Activity amount was determined by averaging 10 days of cage activity. Activity duration (alpha, \( \alpha \)) was determined by the duration of activity over the threshold of the mean using an average waveform of 10 days of activity. Nocturnality was determined from the average percentage of activity conducted during the dark. Precision was determined by calculating the daily variation in onset from a best-fit regression line drawn through 10 days of activity using the ClockLab program (Actimetrics, Wilmette, IL). Fragmentation was defined by bouts/day, where each bout was counted when activity was separated by a gap of 21 minutes or more (max gap setting of 21 min).

3.4 Video Measurement of Immobility-defined Sleep
Mice were singly housed in see-through plastic cages containing bedding. A side-view of each cage was obtained. Video capture was accomplished using cameras with visible light filters (Gadspot Inc., City of Industry, CA) connected to the video-capture card (Adlink Technology Inc., Irvine, CA) on a Dell Optiplex computer system. The ANY-maze software (Stoelting Co., Wood Dale, IL) was used to track the animals as previously described. Briefly, immobility-defined sleep in our study was defined as 95% of the area of the animal maintaining immobile for a minimum of 40 seconds. Continuous recording and tracking of the mice under a 12:12 LD cycle was performed for 4 days. We used data collected from days 2 and 3 for further analysis. Immobility-defined sleep data were exported in 1 min bins, and total sleep was determined by summing the duration of sleep in the day (ZT 0–ZT 12) or night (ZT 12–ZT 24). Number of sleep bouts was determined using ClockLab at the resolution of 1 min bins of ANY-maze data (minimum of 40 seconds sleep per bin). Average bout duration (number of consecutive bins with at least 40 seconds of sleep per bin) was determined within day or night.

### 3.5 Motor tests

Accelerating rotarod test and challenging beam test were applied to determine the progression of motor dysfunction in BACHD mice [47]. On the first day of accelerating rotarod test, the mice were trained on the rotarod (Ugo Basile, Varese, Italy) with 5 trials. The accelerating rotarod went from 5 rpm to a maximum of 38 rpm. The maximum length of each trial was 600 seconds. On the second day, mice were tested on the rotarod and the latency to fall from the rotarod was recorded from 5 trials. Data of each mouse was analyzed after averaged the time of all 5 trials.

The challenging beam test was run to determine the ability of mice to cross a bridge of decreasing width. The beam narrows in 4 intervals from 33 mm > 24 mm > 18 mm > 6 mm, with each segment spanning 253 mm in length. The home cage of each mouse was put on
the end of the beam as the motivating factor. Animals were trained on the beam for 5 consecutive trials on two consecutive days. During each trial, each mouse was placed on the widest end of the beam and allowed to cross with minimal handling by the experiment runner. On the testing day, a metal grid (10 X 10 mm spacing) was overlaid on the beam, and the mice were tested and challenged to cross the beam. 5 consecutive trials were recorded by a camcorder. The videos were scored by two independent observers for the time to cross the beam and touch the home cage, the number of steps taken by the left hind limb, and the number of missteps (errors) made by each mouse. An error was scored when more than half of the foot dipped below the grid. The number of errors was averaged across the 5 trials per mouse to give the final reported values.

The wire hang test seeks to evaluate motor function and deficit in BACHD mice. A 0.25 inch framed steel mesh and a 15-inch deep bucket were used as the apparatus. The test consisted of three trials with 5-minute inter-trial intervals. Animals were trained for 3 consecutive trials on 2 consecutive days. Day 3 is the testing day. Each mouse was placed on the top center of the mesh with all four paws, the frame is then inverted and placed over the container. The timer is started and when the mouse has dropped the time is recorded. Data of each mouse was analyzed after averaged the time of all 3 trials.

3.6 Novel Object Recognition Test

The novel object recognition (NOR) test was performed to assess their recognition memory. The animals were individually placed in a large arena (3 feet x 4 feet). On Day 1 and day 2, mice were allowed to habituate the arena for 10 minutes. On day 3 and day 4, a pair of identical objects was induced in the arena, and the mice were habituated to them for 10 minutes. On day 5, the mice were tested by changing one object with a novel object. They were given 5 minutes to explore the arena and objects. By using the ANY-maze software, the animals are tracked and scored for their duration in which they spend with each object. A
discrimination index (DI) of 0.5 means they spend equal amount of time with both objects. A higher DI indicates they favor the novel object over the other familiar object.

3.7 Open Field Test

Open field was used to assess the anxiety-like behavior and locomotor activity of groups post treatments. Animals were individually placed in a large arena (3 feet x 4 feet) for duration of 10 minutes. By using the ANY-maze software, the animals are tracked and scored for their traveled distance and moving velocity. Mice were observed for 10 min during exploration of an open field. Their locomotor activity was assessed by their travel distance. Their Anxiety was assessed by the time spent in the center zone of testing arena [75].

3.8 Intruder Test

The intruder test was performed to assess the aggression of groups post treatments. The resident (BACHD) mice were allowed to acclimate and adapt to the open cage 5 minutes before the test began. Unfamiliar C57 WT mice with lighter body weight were selected as the intruder mice. The test started when the intruder mouse was introduced to the resident’s cage and proceed for 5 minutes. The tests were recorded by a camcorder and manually scored for their aggression behavior by two independent observers. Among those mice showed aggression behavior, we report the exploration time before first attack, the total time of attack, and the duration of the longest attack. Data was analyzed after averaged the scoring of the two observers.

3.9 LPS Challenge and Multiplex Assay
Lipopolysaccharide (LPS) (List Biological Laboratories, CA, USA) was reconstitute by saline and stored in a -20°C fridge. At ZT1, 5mg/kg of LPS was administered into post-treated animals (6 month of age) by intraperitoneal (IP) injection. 24 hrs later, blood sample was collected during deep anesthesia by using the facial vein technique with an 18G syringe needle. Blood was allowed to clot for 30 minutes at room temperature, and serum was collected following centrifugation at 10000 rpm for 15 minutes and frozen at -80°C until further analysis. A volume of 25µL serum was used to assess cytokine levels of IL4, IL-6, IL-10, RANTES, and TNF-α by using the Millipore cytokine multiplex kit (EMD Millipore Corporation, Billerica, MA, USA) and the Biorad Bio-plex 200 systems machine (Hercules, CA, USA).

### 3.10 Statistical methods

A Student’s t-Test was used to analyze the comparison between age-matched Ctl group and lighting treatments (LL group and BL group). A two-way ANOVA was used to analyze the effect of treatment and week of treatment on body weight changes, and the effect of treatment and ZT time on sleep time and duration of sleep bouts. If the data did not pass normality test or variance test, a Mann-Whitney Rank Sum Test was applied to determine the significance. Following ANOVA analysis, post hoc Holm-Sidak was used to identify significant different groups. Values are reported as mean ± standard error of the mean (SEM). SigmaStat (version 3.5, SYSTAT Software, San Jose, CA) was used to run statistical analyses.
4. Results

In my thesis study, the body weights of BACHD mice were recorded weekly until the end of the study. Before applying any lighting treatment, activities and sleep of BACHD mice were recorded to assess their baseline circadian function. We also assessed their motor performance, cognitive function, and anxiety at 3 month of age. After their baseline data were collected, BACHD mice were housed under different lighting treatments for 3 months. To assess the negative impact of inappropriate photo-environment, one batch of BACHD mice was housed under constant dim light (LL). On the other hand, to assess the benefits of blue light treatment, this group was housed under regular LD cycle with blue light during the first 6 hours of light phase. After 3 months of treatments, we performed all the tests again to assess the consequence of different lighting treatments. In addition, at 6 month of age, their aggressive behavior was assessed by the intruder test. And in the end of this study, their blood was sampled after immune challenge with LPS. And then, their serum levels of cytokines were measured by using multiplex ELISA kit.

4.1 Constant dim light significantly decreased the body weight of BACHD mice.

First, we found LL altered the body weight of BACHD mice (Fig. 4). Animal’s body weights were recorded weekly until the end of the study. The change of body weight from the baseline was analyzed to assess the effect of lighting treatments. Week 0 is the beginning of the study, and week 14 is the end of the study. We found that, although untreated BACHD mice had increased body weight over time, the BACHD mice under LL displayed reduced body weight (Fig. 4A). Starting from the week 7, the difference between LL group and Ctl group became significant (P < 0.05). As a result, in the end of the study, LL group mice decreased 13.5 ± 4.5% of body weight whereas Ctl group increase 5.6 ± 4.9 % body weight compared with their baseline. This difference in body weight changes between LL group and Ctl group was significant different (P < 0.05). On the other hand, the effect of blue light (BL)...
on their body weight changes was not apparent. After 3 months of treatment, the body weight change was not significantly different from the control group (Fig. 4B). By using 2 way-ANOVA, we found that the effect of treatments was significant ($F = 31.683, P < 0.001$) (Table 1).

4.2 Constant dim light dampened whereas blue light treatment reinforced activity and sleep rhythms of BACHD mice.

We evaluated their activity rhythm by their power of rhythm, percentage of activity in ZT0-ZT12, fragmentation, and precision of activity cycle onset. Before any lighting treatment, there was no difference in those parameters (Fig. 5A). After 3 month of treatment, LL group displayed arrhythmic activity pattern (Fig. 5B, middle), and the periodogram analysis showed that most animals in this group did not have significant rhythm. In addition, the power of rhythm was reduced ($P < 0.01$) (Fig. 5C). However, fragmentation of activity was not different. On the other hand, we found consolidating effect of blue light treatment on their activity pattern (Fig. 5B, right). In fact, blue light group showed improved power of rhythm ($P < 0.01$), and the onset of activity tended to be more precise than the Ctl group. But their fragmentation was not different from Ctl group (Fig. 5C).

In addition to activity rhythm, we examined immobility-defined sleep in BACHD mice to determine if lighting conditions altered the amount or temporal pattern of sleep. In our results, the Ctl group showed a diurnal rhythm of sleep time at 6 month of age. The Ctl group spent more time on sleep during ZT0- ZT12 than ZT12- ZT24 ($P < 0.05$) (Fig. 6A). But, their bout duration was not different between ZT0-12 and ZT12-24. In LL group, after 3 month of treatment, their sleep rhythm was compromised (Fig. 6B). In fact, LL group showed no diurnal difference in sleep time, and it was due to their increasing time of sleep in ZT 12- ZT 24. ($P < 0.05$) (Fig. 6A). Moreover, there was higher number of sleep bouts during in LL group than Ctl group ($P < 0.001$), suggesting that LL group had more fragmented sleep than
Ctl group (Fig. 6D). On the other hand, blue light group had robust sleep rhythm (Fig. 6C). In fact, the blue light treatment preserved the diurnal changes on sleep time. Moreover, the blue light group displayed longer average duration of sleep bouts in ZT0-12 than ZT12-24 (P < 0.05) (Fig. 6A). In addition, blue light group had less number of sleep bouts than the Ctl group. Therefore, our result suggested that the blue light treatment improved the sleep rhythm. By using 2 way ANOVA, at 6 month of age, the effect of ZT time was significant in the amount of time spent on sleep (F = 32.27, P < 0.001) and the average duration of sleep bouts (F = 5.51, P < 0.05). The effect of treatment was not significant on either sleep time or bout duration (Table 2).

4.3 Constant dim light but not blue light may have an effect on their motor performance.

We performed the accelerating rotarod test, challenging beam test, and wire hang test to assess the animals’ motor function. In the accelerating rotarod test, a mouse with better motor function would stay longer on the accelerating rotarod than mouse with poor motor function. The results of latency to fall were normalized to the body weight of each mouse. The mean latency to fall was shorter at 6 month of age than at 3 month of age in all groups, but LL group did not reached significance (Fig. 7A). To assess the consequence of different lighting treatments, we compared the results of LL group and blue light group with Ctl group (Fig. 7B). As a result, at 6 month of age, Ctl group (3.87 ± 0.93 sec/g) and LL group (5.00 ± 0.63 sec/g) spent similar time staying on the rotarod. The BL group also spent similar time as the control group on the rotarod (3.72 ± 0.81 sec/g). Therefore, in this accelerating rotarod test, the motor performance of BACHD mice was not altered by the lighting treatments.

The challenging beam test forced the BACHD mice to cross a bridge of decreasing width. An error was scored when more than half of the foot dipped below the grid and making more
errors indicates that the mouse had poor motor coordinating capacity. After 3 month of treatments, the LL group made significantly more errors than their baseline (P < 0.001). Although Ctl group and blue light group also tend to make more errors than their baseline, they did not reach significance (Fig. 8A). In fact, LL group made 85.7 ± 18.6 % more errors whereas group mice only made 33.1 ± 26.9% more errors than their baseline (P = 0.065) (Fig. 8B). In addition, at 6 month of age, the mean number of errors was significantly higher in the LL group than in the Ctl group (P < 0.01) (Fig. 8C). The blue light group, however, had similar error scoring as the Ctl group. Therefore, in this challenge beam test, our data suggested that LL treatment seemed to have an aggravating effect whereas blue light did not alter their motor performance.

The wire hang test was also performed to evaluate motor deficit in BACHD mice. BACHD mouse was placed on the top center of the mesh, and then the frame is inverted; thus, a mouse with better motor function would stay longer on the frame than the mouse with poor motor function. The results of latency to fall were normalized to the body weight of each mouse. After 3 months of treatments, LL group (1.78 ± 0.21) and blue light group (1.86 ± 0.30 sec/g) had similar latency to fall as Ctl group (1.77 ± 026 sec/g) (Fig. 9). There is no significant effect of lighting treatments on their motor performance in the wire hang test.

4.4 No significant impairment or effect of lighting treatments on recognition memory on BACHD mice at 3 or 6 month of age.

We assessed their recognition memory as the cognition test. In this recognition test, the preference of investigating novel object was described by using the discrimination index (DI). A DI of 0.5 indicated that BACHD mouse spend equal amount of time with both objects. A higher DI suggested that they favor the novel object over the familiar one. Before any lighting treatment, since the DI of untreated BACHD mice was greater than 0.5, it suggested that they had normal recognition memory at 3 month of age (Fig. 10A). At 6 month of age, the DI
of control group was still higher than 0.5 (0.70 ± 0.07). In addition, after 3 months of treatments, the DI of LL group (0.78 ± 0.04) and blue light (0.63 ± 0.05) was also higher than 0.5 (Fig. 10B). Therefore, our data suggested no impairment or effect of lighting treatment on their recognition memory at 3 or 6 month of age.

4.5 Constant dim light resulted in declined locomotor activity but no effect on anxiety in BACHD mice

The open field test was performed during the active phase of the circadian rhythm in dark environment for 10 minutes. This test assessed the locomotor activity (traveled distance) and anxiety (time spent on center zone). A mouse with less anxiety would stay longer in the center zone of testing arena. At 3 month of age, there was no difference in traveled distance or center staying time among groups (Fig. 11A). After 3 month of treatments, LL group had declined traveled distance (31.74 ± 2.09 m) compared with Ctl group (P < 0.05); however, their time spent in the center zone (109.49 ± 18.94 sec) was not different from Ctl group (137.33 ± 10.96 sec) (Fig. 11B). A closer look revealed that the declined traveled distance existed in both central and peripheral zones (P < 0.05) (Fig. 11C). In short, our data suggested that LL declined the locomotor activity but had no effect on anxiety in BACHD mice. On the other hand, blue light group had similar traveled distance (42.63 ± 3.38 m) and center staying time as Ctl group (134.23 ± 8.38 sec). It suggested that blue light treatment did not alter their locomotor activity or anxiety.

4.6 Constant dim light increased the percentage of BACHD mice showing aggressive behavior

We tested the hypothesis that LL-treated BACHD mice may become more aggressive than untreated BACHD mice by giving a territorial challenge. Aggressive behavior was assessed
by scoring how much time spent on exploration before the first attack and how much time spent on attacking. An aggressive mouse would spend less time on exploration and more time on attacking than a non-aggressive mouse. As a result, LL group had similar latency before the first attack (48.5 ± 6.7) as Ctl group (81.5 ± 41.5). In addition, time spent on attacking was similar in the LL groups (89.2 ± 19.6) as the Ctl group (101.2 ± 35.6) (Fig. 12A). However, the number of mice showing aggression was higher in the LL group than the Ctl group. While there were 62.5% BACHD mice showing aggressive behavior in the Ctl group, there were 87.5% BACHD mice showing aggression in the LL group (Fig. 12B).

4.7 Lower level of LPS-challenged IL4 in blue light-treated BACHD mice.

In the end of the study, blood was sampled following the immune challenge by using LPS as a probe. The serum levels of proinflammatory cytokine of post treatment groups were measured by multiplex ELISA assay. Both anti-inflammatory cytokines (IL4 and IL10) and proinflammatory cytokines (IL6, TNFα, and Rantes) were selected based on literature reviews. As a result, compared with Ctl, the level of IL4 was lower in blue light group; however, there was no impact of lighting treatments on the levels of other cytokines (Fig. 13, Table 3).
Fig. 4: BACHD mice treated with constant dim light display declined body weights. (A) Weekly body weight changes from their baseline. Week 0 is the beginning of the study, and week 14 is the end of the study. More details are shown in table 1. Control group (Ctl) is shown in black. Constant dim light group (LL) is shown in dark red. Blue light group (BL) is shown in blue. (B) Fold change of body weight between week 0 and week 14. *P< 0.05, comparison with Ctl group.
Fig. 5: Constant dim light dampened whereas blue light treatment reinforced activity rhythms. (A) Representative baseline actogram of BACHD mice at 3 month of age (left), and measurements of activity parameters (right). The actogram of cage cavity was double plotted from BACHD mice under 12:12 LD cycle. Gray shading indicates lights off. (B) Representative actogram of Ctl group (left), LL group (middle), and blue light group (right) at 6 month of age. (C) Measurements of circadian parameters at 6 month of age. Power of rhythm was measured by the $X^2$ periodogram. Fragmentation was measured by the number of activity bouts per day. **$P < 0.01$, comparison with Ctl group.
Fig. 6: Sleep rhythm was improved by blue light treatment at 6 month of age. (A) Measurements of sleep time (left) and duration of bouts (right) at 6 month of age. (B) Hourly running averages of immobility-defined sleep of Ctl group and LL group at 6 month of age. (C) Hourly running averages of immobility-defined sleep of Ctl group and BL group at 6 month of age. (D) Number of sleep bouts per day at 6 month of age. *P<0.05, comparison with Ctl group; ^P <0.05, comparison between ZT time within treatment.
Fig. 7: No effect of lighting treatments on motor performance in accelerating rotarod test. (A) Results of accelerating rotarod test at 3 and 6 month of age. Latency to fall was normalized to body weight of each mouse. Each dot indicates the result of each animal. (B) Comparison of latency to fall/BW between groups at 6 month of age. *P<0.05, comparison between baseline (3mo) and post treatment (6 mo).
Fig. 8: Constant dim light showed a deteriorating effect on motor performance in challenging beam tests. (A) Number of errors made by each group when they crossed the bridge at 3 or 6 month of age. (B) Fold changes of number of errors between 3 and 6 month of age. (C) Comparison of the number of errors of each group at 6 month of age. ###P<0.001, comparison between baseline (3mo) and post treatment (6 mo). *P<0.05, comparison with Ctl group.
Fig. 9: No effect of lighting treatments on motor performance in wire hang test.

Comparison of latency to fall/BW between groups at 6 month of age. Latency to fall was normalized to body weight of each mouse.
Fig. 10: No significant impairment or effect of lighting treatments on recognition memory of BACHD mice at 3 or 6 month of age. Results of novel object recognition test at 3 (A) and 6 (B) month of age. Discrimination index is used to describe the preference of novel object. A discrimination index of 0.5 indicates that mice spend the same amount of time on each object.
Fig. 11: Constant dim light declined locomotor activity but had no effect on anxiety in BACHD mice. (A) Traveled distance and time sent on center zone of testing arena at 3 month of age. (B) Traveled distance and time sent on center zone of testing arena at 6 month of age. (C) Traveled distance in the center zone (left) and peripheral zone (right) of testing arena at 6 month of age. Yellow area indicates the testing zone. *P<0.05, comparison with Ctl group.
Fig. 12: Constant dim light likely made BACHD mice more irritable in a territorial challenge. (A) Latency before first attack indicates the exploration time before their first attack (left). Aggression was scored by time spent on attacking the resident mouse (right). The intruder test was performed at 6 month of age. (B) Percentage of BACHD mice showing aggressive behavior in the intruder test. White area indicates the percentage of BACHD mice without displaying aggressive behavior in both groups. Black area indicates the percentage of BACHD mice displaying aggression in the Ctl group. Red area indicates the percentage of BACHD mice displaying aggression in the LL group.
Fig. 13: Lowered level of LPS-challenged IL4 in blue light-treated BACHD mice. The immune challenge was given by IP injection of LPS (5mg/kg) at ZT1, and blood was sampled after 24 hours. The serum levels of cytokines were measured by using the multiplex ELISA kit. *P<0.05, comparison with Ctl group.
**Table 1:** Fold change of body weight beginning at 3 month of age. *P < 0.05, comparison with Ctl group within the same week.

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<th>BL</th>
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Table 2: Sleep parameters in BACHD mice at 6 month of age. **P<0.01, comparison with Ctl group; ^P <0.05, compared with ZT 12-24 within treatment.

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<td>Treatment x ZT</td>
<td>F</td>
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</table>

Average Duration of Sleep Bouts

| ZT 0-12     | 14.34±6.40 | 10.21±1.06 | 19.14±1.53^ |
| ZT 12-24    | 15.46±2.76 | 12.62±0.83 | 5.57±0.59  |

<table>
<thead>
<tr>
<th>Treatment x ZT</th>
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<td>Treatment</td>
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<td>Treatment x ZT</td>
<td>F</td>
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</table>
Table 3: Levels of LPS-challenged cytokines of BACHD mice at 6 month of age. *P < 0.05, comparison with Ctl group

<table>
<thead>
<tr>
<th></th>
<th>Ctl</th>
<th>LL</th>
<th>BL</th>
</tr>
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<tbody>
<tr>
<td>IL4</td>
<td>2.57 ± 0.79</td>
<td>2.50 ± 1.82</td>
<td>0.79 ± 0.17*</td>
</tr>
<tr>
<td>IL10</td>
<td>131.03 ± 22.77</td>
<td>172.68 ± 30.42</td>
<td>180.37 ± 76.69</td>
</tr>
<tr>
<td>IL6</td>
<td>3554.75 ± 2004.42</td>
<td>5962.76 ± 3785.38</td>
<td>4917.02 ± 4342.97</td>
</tr>
<tr>
<td>TNFα</td>
<td>20.59 ± 7.28</td>
<td>16.71 ± 3.28</td>
<td>24.71 ± 20.88</td>
</tr>
<tr>
<td>Rantes</td>
<td>653.27 ± 125.79</td>
<td>711.17 ± 87.09</td>
<td>735.02 ± 213.99</td>
</tr>
</tbody>
</table>
5. Discussion

My thesis study evaluated the effect of manipulating lighting conditions on several symptoms exhibited by the BACHD model of HD. I found that dim light treatment disrupted rhythms in activity and sleep in the BACHD mice. I also found that dim light treatment caused the BACHD mice to lose body weight and increased errors in one test of motor performance. Moreover, constant dim light made the BACHD mice become more irritable in the intruder test. In contrast, blue light successfully improved the sleep/wake rhythms in the mutant mice. This treatment did not alter motor performance in the BACHD mice.

5.1 Long-term exposure of constant dim light might diminish food intake of BACHD mice and contribute to their declined body weights at 6 month of age

Although untreated BACHD mice had increasing body weight overtime as described in prior studies [75], our data indicated that BACHD mice exposed to constant dim light did not gain weight from 3 month of age to 6 month of age. Instead, BACHD mice lost 13.5 ± 4.5% of their body weight after 3 month of exposure of constant dim light. How the metabolic profile is affected by mHtt especially in the prodromal period and early stages of the disease is not fully understand and needs further investigation; however, HD patients are known to display weight loss while BACHD mice display weight gain. Although we did not record their food intake through the experiment, the declined body weight might be as a result of diminished food intake.

Nevertheless, obesity can be a result of reduced activity, increased food intake, a reduced metabolic rate, or a combination of these factors. However, Ctl group did not display reduced activity in the open field at 6 months of age. Instead, the developed obesity of BACHD mice
might be as a result of early increase in food intake.

Hult et al 2000. reported that both male and female BACHD mice had significantly higher food intake than WT mice as early as at 2 months of age, and this difference continued at 4 months of age [76]. This finding suggested that the obesity of BACHD mice might be as a result of early increase in food intake. On the other hand, a long term exposure of LL cycle is reported to have a diminishing effect on total energy intake in WT mice [77]. This similar decreased food intake had also found in rats exposure to 6-7 weeks of LL cycle. In this study, they also found an association between LL cycle and reduced energy expenditure. As a result, the LL group had gained less weight and resulted in lower body weight than the control group in the end of the experiment [78]. In consistent, our data indicated that LL-treated BACHD mice showed significant difference in body weight compared with untreated BACHD mice after 7 weeks of treatment. Even though body weight can also be influenced by amount of activity and metabolic rate, LL-treated BACHD mice showed declined locomotor activity in open field test. In addition, our sleep recording indicated that they spent more time on sleep, which had lower metabolic rate, during active phase. Therefore, decreased body weight found in LL-BACHD mice could be due to diminished energy intake or changed metabolism. Our future study is addressing this issue by measuring their food consumption.

5.2 Blue light treatment might prevent circadian rhythm from breakdown and benefit both general health and life quality of HD patients

Importantly, although not altering motor symptoms, my thesis study revealed a beneficial effect of blue light treatment on sleep/wake cycle of BACHD mice. In HD patients, documented circadian abnormalities include daytime sleepiness and nighttime sleep
disturbances. This disturbance of activity patterns severely jeopardizes life qualities of both patients and their caregivers. Moreover, the detrimental effects of circadian dysfunction on general health also suggest that circadian abnormalities associated with HD can exacerbate the disease. Indeed, many of the symptoms of circadian dysfunction are the same as the symptoms of HD, including decreased motor control, memory problems, and mood changes. Therefore, it is even possible that circadian dysfunction itself is primarily responsible for some of HD symptoms. This hypothesis raises the interesting possibility that treating circadian dysfunction might improve the lives of HD patients.

Light therapy has been shown to be an efficient non-pharmacological therapy in many disorders such as seasonal affective disorder and chronic depression. To avoid undesired effect of strong intensity of bright light, we applied low intensity of blue light in my thesis study. In human studies, light therapy is usually applied in the early morning [74]; to be consistent in our study, the blue light treatment was applied in the begin of light phase on BACHD mice. As a result, we showed that circadian behavioral abnormalities were prevented by blue light therapy: compared with untreated BACHD mice, the blue light-treated BACHD mice had consolidated sleep/wake cycle. The beneficial effect of blue light might due to the fact that the blue light wavelength and higher light intensity more effectively act on the SCN, and promotes the synchronization between internal circadian rhythm and environmental LD cycle, which can result in enforced sleep/wake cycle. However, whether blue light treatment slows HD progression remains unknown. Our data indicated that early-staged 3 months of blue light treatment did not alter other functional outputs such as motor performance. Investigation on the aggregation of mHtt or microarray analysis on the changes of pathways might help to address this question.

Also, the light intensity may have to be strengthened to have more effective results. In clinical studies, light therapy uses a light box which emits 2500-10000 lux of light at a specified distance [70, 79]; however, in my thesis study, the light intensity of blue light plus
white light was 1000-1200 lux in the blue light group. Although one of advantages of using blue light treatment is to avoid too strong light intensity, 1200 lux of light intensity may not be strong enough to achieve treating effect. In addition, impaired light-input pathway is one possibility to explain the disrupted activity pattern of BACHD mice, which can also dampen the effect of light therapy. In fact, our previous study has shown reduced sensitivity to the phase-shifting effect of light in BACHD mice [56]. Our lab has investigated the retinohypothalamic tract terminals in the SCN as one of future studies. For these two reasons, higher light intensity might be required to be effective.

Nevertheless, it is important to point out that the potential effect of preventing circadian rhythm breakdown by blue light treatment will certainly benefit both general health and life quality of patients as well as their caregivers. Unlike HD mouse model, the effect of light therapy on human subjects with HD is less-studied. Our promising results suggest that blue light therapy may have value to HD patients.

5.3 The beneficial effect of blue light on motor performance may require longer treating duration and become more dominant at later age

Consistent with prior studies, we found motor performance deficits in untreated BACHD mice. The mice exhibited less latency to fall in rotarod tests and trended to make more errors in challenge beam test at 6 month of age than at 3 month of age. The correlation between neurodegeneration and circadian clock dysfunction prompted us to answer whether circadian system itself contributes to pathophysiology of HD. Since assessment of motor dysfunction is an important component in the diagnosis of HD, measurement of motor function in BACHD mice was therefore a logical way to test our hypothesis.
In challenge beam test, animals require high motor coordination to cross a bridge with decreasing width without many errors. In consistent with our hypothesis, constant dim light had a deleterious effect in making BACHD mice worse at the challenge beam test than untreated BACHD mice. The circadian system has been shown to regulate mitochondrial function and immune response. Although we did not have biomarkers from our BACHD mice, disrupting the circadian system by constant dim light may contribute to chronic inflammation, mitochondrial dysfunction, increased oxidative stress and elevated DNA damage. These negative results are all likely accelerating the pathology of HD. On the other hand, although blue light therapy seems promising in AD and PD studies, we found no rescue effect on the motor performance of BACHD mice. In fact, blue light-treated BACHD mice had as poor motor performance as untreated BACHD mice at 6 month of age. Of course, a longer duration of blue light treatment may have a beneficial effect. In my thesis study, I applied 3 month of blue light exposure during the first 6 hours of the light phase. There was no evidence indicating that blue light treatment can decrease the aggregation of mHtt; however, it might be beneficial by decreasing oxidative stress and thus delaying disease progression. We assessed post treatment results at 6 month of age, which was still an early stage of HD in BACHD mice. Even though we found improvement in their activity and sleep rhythm, the beneficial effect of delaying disease progression requires longer time. Therefore, the delaying effect of blue light treatment on HD progression would become more dominant with longer duration of treatment and at later stage.

Another possibility for ineffective impact of blue light treatment in this study might due to the hypothesis that BACHD mice have deficits in the light-response. In our previous study, BACHD mice required longer time to resynchronize to phase shifting (6h advance or delay) in the LD cycle than WT controls. Furthermore, BACHD mice in DD also showed reduced sensitivity to the light pulse treatment compared with the WT controls [56]. In summary, there is evidence suggesting that BACHD mice could have weakened light response. Since we
applied low intensity of blue light in my thesis study, it is possible that the impaired light-input pathway dampened the effect of blue light treatment. Therefore, a strengthened blue light is required to achieve optimized effects. In addition, there are several tests can be done in the future study to address the hypothesis that BACHD mice have impaired light-response. For example, we can compare the number of AVP and VIP neurons between BACHD mice and WT controls. It is also interesting to look at the retinohypothalamic tract by using the fluorescent tracing staining. Finally, we can look at the gene expression within SCN after giving a light pulse test.

5.4 The influence of lighting treatments on the recognition memory of BACHD mice might be more dominant after 9 month of age.

Early HD patients are reported to have impaired recognition memory [80]. Our study revealed no recognition impairment in BACHD mice at either 3 or 6 month of age. The result of untreated BACHD mice is consistent with prior study that BACHD mice don’t display their impairment of novel-object preference until at 9 month of age [81].

However, neither constant dim light nor blue light treatment had impact on their recognition capacity at early age. The circadian system regulates learning and memory though several mechanisms. In addition to sleep-dependent consolidation of memory, circadian system itself plays a role in memory formation and consolidation. For example, animals housed under disruptive lighting environment have been shown to exhibit impaired contextual, spatial and time-related memory. Targeted disruption of circadian genes further supports that circadian system is involved in synaptic plasticity and cognitive function [82, 83]. Our blue light treatment did not show rescue effect might due to the fact that BACHD mice had not shown symptoms of impaired recognition memory yet. However, LL-treated BACHD did not show impairments either. The results suggest that the influence of constant dim light might not be strong enough at this early stage. Nevertheless, the disruptive effect of
constant dim light should be considered in later age of BACHD mice. In fact, BACHD mice have been reported to have deficiency in spatial learning [81] and reversal learning [84] at 9 month of age. Since cognitive dysfunction severely impact HD patients’ daily life and capacity of working, it is important to address that careless lifestyle such as dim light at night can result in exacerbating effect.

5.5 Constant dim light likely made BACHD mice more irritable to the surrounding events

Aggression is one of common symptoms reported in early-stage HD patients [85]. It not only destroys the relationship between patients and their family but also put their caregivers at risk of assault. Circadian disruption has been well linked to increased aggressive behavior including physical and verbal violence. We thus suspected that the aggressive behavior of HD patients might be exacerbated by the lighting conditions of the hospital environment, which can be disruptive. For example, patients spend more time indoor during both daytime nighttime, and the artificial lights can be considered as light pollution. The hypothesis whether an inappropriate photo-environment can worsen the aggressive symptoms of HD was tested on our BACHD mice by giving a territorial challenge. As a result, we found that LL-treated BACHD mice had a similar degree of aggression as untreated BACHD mice. In other words, they have similar latency before the first attack and similar time spent on attacking the intruder mouse. However, the number of mice showing aggression increased with the dim light perturbation. While there were 62.5% BACHD mice showed aggressive behavior in the untreated group, there were 87.5% BACHD mice showed aggressive behavior in LL group. Thus, inappropriate lighting treatments likely made BACHD mice more irritable to the surrounding events rather than increasing their time spent on attack.
In fact, this observation is similar to what is reported from the HD patients. Due to impairment in caudate nucleus, which is important in emotion control, HD patients are more susceptible to emotional fluctuation. Therefore, they can escalate into rage easily; however, the emotional fluctuation of rage can be short, and they can also recover from their anger quickly. A bigger sample size is needed to demonstrate our finding; but yet, this is the first study assessing the aggressive behavior phenotype on this HD mouse model. In fact, compared to extensive reports on HD patients, there are much fewer reports assessing aggressive behavior on HD mouse model [86]. My thesis study thus provides additional information for drug development targeting on psychiatric disorders.

5.6 The effect of lighting treatments on immune response could be masked by existed higher levels of cytokines and abnormal immune system in BACHD mice.

Overactive and impaired immune functions have been proposed to contribute to the neuronal degeneration in HD [87]. In fact, Levels of IL-6, IL-8 and TNF-α in the plasma and striatum of HD patients have been reported to be higher than in non-HD controls [88], and evidence of elevated cytokine levels in pre-symptomatic patients suggests that the inflammation is a precursor rather than a consequence of brain disease. Although the level of proinflammatory cytokines in BACHD mouse model is still not known, their impaired migration of macrophages to an inflammatory stimulus was reported [89]. This migration deficit of macrophage underlies immunological changes in BACHD mice. In addition, an increased level of IL-6 release has been reported in other HD mouse model such as R6/2 [90] and YAC128 mice [91], suggesting that nuclear factor-κ B (NF-κ B) dependent pathways might be upregulated in HD. And it was recently demonstrated that disruption of circadian clock function leads to abnormal immune response and activated (NF-κ B) dependent pathways [92]. For example, Cry1 −/− Cry2 −/− mice exhibit increased levels of
IL6 and TNFα. In addition, weekly phase-shifts result in elevated immune response to LPS challenge and mortality. In addition, dim light at night has also been shown to shift basal inflammatory tone in the brain and periphery. [93]. Therefore, we tested the hypothesis that disruptive lighting treatments might exaggerate the immune response to the LPS challenge. Although we found lower level of IL4 in blue light group, there was no effect of lighting treatments on the levels of other cytokines. Our results suggested that the effect of lighting inputs on their immune response may not be dominant in this neurodegenerative model. It is worth emphasizing that the comparison was made between the HD mutants rather than WT controls. In other words, the exaggerating effect of lighting treatments on immune response could be masked by existing higher levels of cytokines and abnormal immune system of BACHD mice. Therefore, the influence of lighting treatments on their immune response can be elucidated by comparing the results with age-matched WTs that are treated with the same lighting treatments in future study.

Furthermore, it is worth considering whether the immune system itself can contribute to HD pathology and symptoms. Based on the genetic construction of BACHD mice, we can either specifically express or remove mHtt in microglia. For example, we can use genes such as Cd11b (CD11b-cre transgenic mouse) to target myeloid cells in the brain, including microglia [89, 94-96]. If BACHD mice with mHtt-expressed microglia can recapitulate HD pathology and symptoms or BACHD mice with mHtt-negative microglia can slow down disease progression, it would help reveal the intrinsic and bidirectional contribution of the immune system to HD. What’s more, in a future study, it is also worth considering extracting immune cells from a healthy control, and examining whether these cells become dysfunctional in the HD environment.
6. Conclusion

In conclusion, my thesis study replicates and extends published results in adult BACHD mice. We showed that inappropriate photo-environment such as constant dim light can make HD symptoms worse. HD patients are constantly exposed to such light pollution because they spend more time indoor both daytime and nighttime with artificial lights. The results of my thesis study would suggest HD patients to have a more careful controlled photic environment. More importantly, my results showed that blue light treatment improves the sleep/wake cycle in a mouse model of HD. These finding suggest that blue light therapy should be evaluated in HD patients as an inexpensive sleep aid.
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