Research paper

Blue light therapy improves circadian dysfunction as well as motor symptoms in two mouse models of Huntington's disease

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ARTICLE INFO

ABSTRACT

Patients with Huntington's disease (HD) exhibit movement disorders, psychiatric disturbance and cognitive impairments as the disease progresses. Abnormal sleep/wake cycles are common among HD patients with reports of delayed sleep onset, fatigue during the day, and a delayed pattern of melatonin secretion all of which suggest circadian dysfunction. Mouse models of HD confirm disrupted circadian rhythms with pathophysiology found in the central circadian clock (suprachiasmatic nucleus). Importantly, circadian dysfunction manifests early in disease, even before the classic motor symptoms, in both patients and mouse models. Therefore, we hypothesize that the circadian dysfunction may interact with the disease pathology and exacerbate the HD symptoms. If correct, early intervention may benefit patients and delay disease progression. One test of this hypothesis is to determine whether light therapy designed to strengthen this intrinsic timing system can delay the disease progression in mouse models. Therefore, we determined the impact of blue wavelength-enriched light on two HD models: the BACHD and Q175 mice. Both models received 6 h of blue-light at the beginning of their daily light cycle for 3 months. After treatment, both genotypes showed improvements in their locomotor activity rhythm without significant change to their sleep behavior. Critically, treated mice of both lines exhibited improved motor performance compared to untreated controls. Focusing on the Q175 genotype, we sought to determine whether the treatment altered signaling pathways in brain regions known to be impacted by HD using NanoString gene expression assays. We found that the expression of several HD relevant markers was altered in the striatum and cortex of the treated mice. Our study demonstrates that strengthening the circadian system can delay the progression of HD in pre-clinical models. This work suggests that lighting conditions should be considered when managing treatment of HD and other neurodegenerative disorders.

1. Introduction

Huntington's disease (HD) patients suffer from progressive neurodegeneration that inflicts cognitive, psychiatric, cardiovascular and motor dysfunction (Margolis and Ross 2003; Bourne et al., 2006; Grimbergen et al., 2008; Fisher et al., 2014). HD is caused by a CAG repeat expansion within the first exon of the *Huntingtin* (*Htt*) gene and when translated, produces a polyglutamine repeat that leads to protein misfolding, soluble aggregates, and inclusion bodies detected throughout the body (Saft et al., 2005; Ciammola et al., 2006). The mutated HTT protein leads to dysfunction of a large range of cellular processes,

including cytoskeletal organization, protein folding, metabolism and transcriptional activities. HD symptoms start at a range of ages, with an average onset at 40 years of age. Generally, the longer the CAG repeat, the earlier the age of onset and the greater the severity of the symptoms (Duyao et al., 1993; Langbehn et al., 2010). Still, even among patients with the same CAG repeat length, there is considerable range in the onset of symptoms (around a decade) and their severity (Wexler et al., 2004; Gusella et al., 2014). This variability raises the possibility of environmental modifiers to the disease and suggests that optimal disease management can increase the health span of the patients. This possibility is important to pursue as there are no known cures for

HD.

Sleep disorders are extremely common in HD and have detrimental effects on daily functioning and the quality of life of patients and their caregivers (Cuturic et al., 2009; Aziz et al., 2010; Goodman et al., 2011). Disruptions in the timing of sleep are common and often become apparent years before the onset of motor symptoms. Mouse models of HD also exhibit a progressive and rapid breakdown of the circadian rest/activity cycle that mimics the condition observed in human patients typified by loss of consolidated sleep, increased wakeful activity during the sleep phase, and more sleep during the active/awake phase (Morton et al., 2005; Kudo et al., 2011; Loh et al., 2013). Disorganized circadian timing causes a number of undesirable effects throughout the body (Colwell 2015) altering the function of key organ systems including the heart, pancreas, liver and lungs as well as the brain. This body of work supports the hypothesis that circadian dysfunctions might interact with HD disease pathology and exacerbate the symptoms. If this hypothesis is correct, we would expect to modify the HD disease progression by employing treatments that improve the sleep/wake cycle.

Light is a powerful regulator of our physiology and behavior. Besides visually driven behaviors, there are also a number of non-visual light responses including those involved in the regulation of circadian behaviors (Duffy and Wright 2005; Roenneberg et al., 2013). The importance of the intrinsically photoreceptive retinal ganglion cells (ipRGCs) that make use of melanopsin as a photopigment has become clear (Rollag et al., 2003; Schmidt et al., 2011). These ipRGCs underlie circadian light detection as well as the impact of light on mood and perhaps cognition (LeGates et al., 2014). Melanopsin phototransduction is maximally sensitive to blue wavelength light (Panda et al., 2005). However, ipRGCs can also respond to rod- and cone-driven signals (Dacey et al., 2005). The ipRGCs integrate this light information (Lucas et al., 2012) and send a direct projection to the central circadian clock (suprachiasmatic nucleus, SCN) where this signal has a profound impact on electrical activity and gene expression (Colwell 2011). Clinically, timed exposure to bright white light has previously been applied to treat the sleep/wake disturbances in aging and neurodegenerative disease with somewhat mixed results (Willis and Turner 2007; Riemersma-van der Lek et al., 2008; McCurry et al., 2011; Zhou et al., 2012). More recent work has turned to utilizing blue light of lower intensity to improve the sleep/wake cycle in human subjects (Royer et al., 2012; Gabel et al., 2013; Figueiro et al., 2014; Sloane et al., 2015). Unfortunately, there has been little work applying blue light-therapy to pre-clinical disease models to optimize treatments or explore underlying mechanisms.

We previously established an age-dependent progression of circadian and motor symptoms for two mouse modes of HD: BACHD and Q175 (Kudo et al., 2011; Loh et al., 2013; Kuljis et al., 2016). In the present study, we sought to determine whether blue-enhanced lighting conditions can alter this disease trajectory by applying this light treatment for 3 months (mo) starting just prior to the genotypic-specific decline in activity rhythms (BACHD, 3 mo; Heterozygote Q175, 6 mo). For nocturnal animals, light initiates sleep through a melanopsin-dependent mechanism (Lupi et al., 2008). In order to suppress undesired day-time activity and reinforce photic cues to the circadian timing system of the HD mutants, we applied blue-enriched light treatment during the first 6 h (h) of the light cycle. After the treatment, we assessed the impact of blue-enriched lighting on HD-related decline by comparing locomotor activity, sleep behavior, and motor performance with their age-matched controls. At the end of the study, we collected cortex and striatum tissue samples from the Q175 line for analysis of the expression of 100 previously identified HD transcriptional markers (Langfelder et al., 2016).

2. Material and methods

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

2.1. Animals

All mice used in this study were males on the C57BL6/J background. BACHD mice that we employed for this study express a transgenic copy of the full length human mutant huntingtin gene encoding 97 glutamine repeats under the control of endogenous regulatory machinery (Gray et al., 2008). The Q175 mice arose from a spontaneous expansion of the CAG repeat in the CAG140 transgenic knock-in line (Menalled et al., 2012). The Q175 mice have previously been shown to have around 175 CAG repeats and we used mice heterozygous (Het) for the Q175 allele. Mutant mice were obtained from the Jackson Laboratory (Bar Harbor, Maine) from a colony managed by the CHDI Foundation. To confirm previous findings of daily rhythm and motor performance decline in both HD models, we also examined WT mice at 3, 6, and 9 mo of age (Supplemental Table 1).

2.2. Housing conditions

The animals were singly housed within light-tight chambers with independently controlled lighting conditions: 12 h of light followed by 12 h of dark (12:12 LD). The chambers were in the same animal housing facility with controlled temperature and humidity, and each chamber held 8 cages of mice, grouped together by lighting treatment. All animals received cotton nestlets, and rodent chow and water were made available ad libitum. Animals were assessed for cage activity, sleep behavior, and motor performance under these baseline conditions prior to beginning treatments. After baseline measurements were collected, the animals were housed under one of two different lighting conditions for 3 months: the control group was maintained under 12:12 LD using white light, while the blue-enriched light-treated group was also maintained under the 12:12 LD and received additional exposure to blue light during the first half of the light phase (Zeitgeber time, ZT 0–6, where ZT 0 refers to the start of the light cycle). We have previously shown that the BACHD line exhibits abnormally high activity during the beginning of the light period (Kudo et al., 2011). Both control and blue-enriched light chambers were lit from the top of the cabinet using white fluorescent lamps providing approximately 350 lx of full spectrum light. The blue LED lamps (peak at 470 nm wavelength) were vertically positioned on the top of each single cage at the same height as the white lamps providing an additional 150 lx. These readings were obtained when wheel running cages were present. The spectral irradiance was measured using a spectrophotometer (International Light Technologies, Peabody, Massachusetts; Supplemental Fig. 1) while photopic illuminance (lux) intensity was measured by using a light meter (BK precision, Yorba Linda, CA). These measurements were made without the cages in the chamber and thus represent the maximum irradiance to which the mice could have been exposed.

All motor tests were performed in the middle of the night, during ZT 18–22, under dim red light (3 lx). The behavioral tests were performed before the start of treatment to ensure that there were no differences between the untreated mutants and then again after 3 mo of treatment. These final tests were performed while the animals were still under blue-enriched lighting conditions.

2.3. Cage activity

Locomotor activity was recorded using infrared sensors and analyzed using the El Temps (A. Diez-Nogura, Barcelona, Spain;