Title

A Search for Histopathology in the SCN of Three Neurodevelopmental/Neurodegenerative Diseases with Circadian Dysfunction

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A Search for Histopathology in the SCN of Three Neurodevelopmental/Neurodegenerative Diseases with Circadian Dysfunction

A thesis submitted in partial satisfaction of the

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Physiological Science

by

Zoë Alexandra MacDowell Kaswan

2016

ABSTRACT OF THE THESIS

A Search for Histopathology in the SCN of
Three Neurodevelopmental/Neurodegenerative Diseases with
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by

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Master of Physiological Science
University of California, Los Angeles, 2016
Professor Christopher S. Colwell, Co-Chair
Professor Gene D. Block, Co-Chair

Circadian rhythms are endogenously-generated rhythmic bodily processes that occur with a period of approximately 24 hours. They occur at every level of biological organization, and fundamentally underlie almost every physiological, neurological, and behavioral function undergone by an organism. It should not be surprising, therefore, that circadian disturbances are a common symptom of diseases and disorders of the nervous system, and that this disturbance feeds back into the disease mechanisms to worsen symptoms. Circadian phenotypes of mouse models of Rett syndrome, Autism Spectrum Disorder, and Huntington's disease have been extensively studied in our lab. This thesis further examines these models to determine if a common underlying histopathology is present in the suprachiasmatic nucleus (SCN), the central clock of the mammalian brain. Tracing of Nissl stains was used to determine if there were any

changes in the gross morphology of the SCN, and immunohistochemistry and immunofluorescence were used to look for possible loss of SCN neuropeptide-expressing subpopulations of neurons. Ultimately, no common histoanatomical phenotype was observed between the three mouse models, leading to the conclusion that separate underlying phenotypes must be converging on common patient symptoms.

The thesis of Zoë Alexandra MacDowell Kaswan is approved.

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Introduction

The rotation of the earth on its axis creates rhythmic environmental changes, most notably the light/dark (LD) cycle, with a period of 24 hours. As life evolved, it came to reflect these environmental changes in its own organization, thus optimizing utilization of environmental resources, internal efficiency, and survival. Circadian rhythms, endogenously generated rhythms with a period of ~24 hours, are found at every level of biological organization and allow organisms to be predictive of, rather than simply react to, their needs and environment. In mammals, the most fundamental circadian rhythm is that of the molecular clock: a transcriptional-translational negative feedback loop that gives each cell its own rhythm. Cellular rhythms are coordinated into tissue-wide rhythms, rhythms of organ function, and ultimately behavioral rhythms.

The molecular clock has two primary components: a positive arm and a negative arm. The positive arm is composed of the genes *Clock* and *Bmal1* and their corresponding proteins. CLOCK and BMAL1 bind to the E-box promoter region of the genes *Period1,2,&3* (abbreviated *Per1, Per2,* and *Per3*), and *Cryptochrome1&2* (abbreviated *Cry1* and *Cry2*). PER and CRY proteins dimerize in the cytoplasm and translocate back into the nucleus, where they bind to CLOCK and BMAL1 dimers to inhibit their own transcription, thus constituting the negative arm of the clock. This feedback loop takes roughly 24 hours to complete. Together, these genes and their proteins are the "core molecular clock"; the many other genes and proteins which they regulate are secondary clocks. The function of the molecular clock can be observed by measuring the rhythmic expression of core clock genes, most commonly *Per2* (Brown, Kowalska, & Dallmann, 2012; Buhr & Takahashi, 2013).

Although the core molecular clock is found in every cell, slight cell-to-cell variation would cause them to drift apart without a common reference point, and no tissue-wide rhythm would be able to form. In mammals, this common reference point is provided by the surprachiasmatic nucleus (SCN), the so-called Master Clock of the brain. The SCN is a small bilateral nucleus located in the ventral hypothalamus, just above the optic chiasm and lateral to the third ventricle, wherefrom it communicates directly and indirectly with all other areas of the brain and body via both neural and hormonal signals. By ensuring that they have a consistent phase relationship (even in the absence of external stimuli), the SCN is able to coordinate other rhythms in the body, and synchronize them to the LD cycle (Okamura, 2007; Dibner, Schibler, & Albrecht, 2010; Colwell, 2011).

The SCN can be divided into two anatomical subregions, the ventrolateral "core" and the dorsomedial "shell." Neurons in the core receive environmental input, most notably from intrinsically-photosensitive retinal ganglion cells (ipRGCs) in the retina, but also from brain regions related to eating, sleep, and other rhythmic behaviors. Core neurons typically have weaker molecular rhythms than those of the shell, which allows them to be easily entrained to this environmental information. They then synapse onto the shell, ensuring a cohesive SCN rhythm. Shell neurons have strong endogenous molecular rhythms, allowing them to maintain a clear rhythm for the body even in constant conditions (Abrahamson & Moore, 2001; Lee, Billings, & Lehman, 2003; Yan et al., 2007).

Core and shell neurons also express different neuropeptides. Vasoactive Intestinal Peptide (VIP) is found in many core neurons, particularly those closest to the optic chiasm, and is necessary for intraSCN synchrony. Mice lacking VIP mice are unable to establish coherent behavioral rhythms in constant conditions, and have altered light entrainment. VIP+ neurons

send dense projections throughout the entire SCN and into other brain targets (Vosko et al., 2007; Vosko et al., 2015). Arginine Vasopressin (AVP) is found in many shell neurons, and is a key peptide for communicating the rhythmic SCN activity to other brain regions (particularly the paraventricular nucleus) (Caldwell et al., 2008).

One way that the SCN is able to coordinate and synchronize the many other bodily rhythms is through inducing rhythmic hormonal release. Melatonin, a hormone released by the pineal gland during the dark phase, is tightly regulated by the SCN (Moore, 1996). It is in turn an important hormone for signaling time information to organs and brain regions, and induces nighttime sleepiness. Melatonin levels can be measured in a laboratory setting to assess the internal clock in humans; however, since its release is light-sensitive, the subject must remain in constant dim conditions for proper circadian assessment.

Considering the fundamental nature of circadian rhythms, it should not be surprising that their disturbance is a common symptom of a diverse array of neurological and physiological conditions. Circadian disruption can cause ill health even in otherwise healthy animals and people (Evans & Davidson, 2013), and the idea that disease-induced circadian dysfunction feeds back into disease mechanisms and further exacerbates the initial condition is gaining traction in the medical and scientific community (see Musiek, 2015 for a neurodegenerative disease perspective). This view is supported by research in animal models demonstrating that circadian insults worsen disease symptoms, while circadian alignment ameliorates symptoms and improves health outcomes (Foster & Kreitzman, 2014).

Three disorders in which a majority of patients experience similar sleep disturbances, but which otherwise have distinct mechanisms and symptoms, are Rett syndrome (RTT), autism spectrum disorder (ASD), and Huntington's disease (HD). Our lab has characterized the

circadian phenotype for mouse models of each of these conditions, more thoroughly than can be done in human patients, and found both similarities and differences in the extent of their circadian dysfunction. This thesis examines the gross morphology of the SCN and its VIP and AVP-expressing neural subpopulations in each of these three mouse models and their WT littermates to determine if there are any common histoanatomical differences that might underlie the observed circadian symptoms.

Materials and Methods

Animals

All experiments were approved by the UCLA Animal Research Committee (ARC). Animal use and welfare followed all guidelines set by the UCLA Division of Laboratory Animal Management (DLAM) and National Institutes of Health (NIH). Animals were either bred from colonies at UCLA or purchased from Jackson Laboratories. Genotyping was performed at 15 days of age by tail snip. All animals were housed in light-proof, sound-proof, temperature- and humidity-controlled chambers. The lighting conditions were set to a 12-hour light, 12-hour dark (LD) cycle at 300 lux intensity. All times are given in zeitgeber time (ZT), the time in reference to lights-on (lights come on at ZT0 and go off at ZT12).

Nissl stain and SCN measurements

Perfusion was done at ZT14 (early night) for Nissl staining. After anesthetization by isoflorene, animals were perfused intracardially with 20 mL phosophate buffered saline (PBS; 0.1 M, pH 7.4) and 25-30 mL 4% paraformaldehyde (PFA) in PBS. Brains were removed and post-fixed overnight in 4% PFA at 4 °C, then cryoprotected for a minimum of 24 hours in 20% sucrose in PBS at 4 °C. Brains used for Nissl staining were sliced coronally at 20-50 μm on a cryostat (Leica, Buffalo Grove, IL, USA) and 12 sequential slices containing SCN were collected. Standard cresyl violet staining protocol was followed (Li et al., 2015; Kuljis et al., 2016). Images were taken on a Zeiss Axioskop mounted with a CCD camera (Axiocam) using the AxioVision software (Zeiss, Pleasanton, CA, USA). AxioVision software was used to make SCN measurements. Because the exact borders of the SCN are somewhat arbitrary, two observers who did not participate in the staining or image-taking, and were masked to genotype,

traced the SCN in consecutive slices of each brain. Area was calculated by tracing the perimeter of the SCN as determined by cell density; height was defined as the distance between the most ventral and dorsal points of the SCN; width was defined as the distance between the most medial and lateral points of the SCN. Except where stated otherwise, area measurements used for analysis were produced by identifying the two most central sections (those having the largest area) and then adding the two sections immediately anterior and two-three posterior (6-7 slices per final measurement).

Immunohistochemistry (IHC)

Animals were perfused at ZT6 as described above. Brains were sliced coronally at 50 μm on a cryostat (Leica, Buffalo Grove, IL, USA) and 12 sequential slices containing SCN were collected. Slices were first incubated in an endogenous peroxidase blocking solution (10% methanol, 10% hydrogen peroxide (30% w/w concentration) in PBS). They were then incubated in a serum blocking solution (3% NGS, 0.1% TritonX in PBS) for a minimum of 1 hour at room temperature, after which they were incubated in primary antibody solution (rabbit polyclonal anti-VIP, 1:2000 in serum blocking solution; Alpha Diagnostics, San Antonio, TX, USA or Guinea pig polyclonal anti-AVP, 1:1000 in serum blocking solution; Peninsula Laboratories, San Carlos, CA, USA) for 48 hours at 4 °C. Primary antibody was omitted for the first slice of each brain to serve as a negative control. Three washes in PBS were done to remove excess primary before slices were incubated in the corresponding biotinylated secondary antibody for two hours at room temperature (anti-rabbit or anti-Guinea pig 1:500 in serum blocking solution; Vector Laboratories, Burlingame, CA, USA). Slices were then incubated in avidin-biotin solution following manufacturer's instructions (ABC kit, Vector Laboratories). Positive staining was

visualized using 3,3'-diaminobenzidine (DAB) intensified with nickel(II) chloride (Fisher Scientific). Slices were then mounted and coverslipped with Depex mounting medium (Fisher Scientific).

Immunofluorescence (IF)

Animals were perfused at ZT6 as described above. Brains were sliced coronally at 50 μm on a cryostat (Leica, Buffalo Grove, IL, USA) and 12 sequential slices containing SCN were collected. Slices were incubated in a serum blocking solution (0.3% TritonX, 1% BSA, 10% NDS in PBS) for a minimum of 1 hr and then incubated in primary antibody solution (rabbit polyclonal anti-VIP, 1:1000; Alpha Diagnostics, San Antonio, TX, USA and Guinea pig polyclonal anti-AVP, 1:500; Peninsula Laboratories, San Carlos, CA, USA) for 2 hrs at 37 °C (primary antibody solution contained antibody at the stated dilution in a solution of 0.3% TritonX, 1% BSA, and 5% NDS in PBS). After washing, slices were incubated in secondary antibody solution (donkey anti-rabbit Alexa488 1:200 and donkey anti-Guinea pig Cy3 1:300; Jackson ImmunoResearch, West Grove, PA, USA) for 30 min at room temperature (secondary antibody solution contained antibody at the stated dilution in a solution of 0.3% TritonX, 1% BSA, and 5% NDS in PBS). Slices were washed again to remove excess secondary antibody and then coverslipped with Vectashield containing DAPI.

Counting VIP and AVP+ neurons

Stereological analysis was performed by a single investigator using StereoInvestigator software (MicroBrightfield Biosciences, Williston, VT, USA) controlling an AxioImager M2 ApoTome microscope (Zeiss) with a motorized stage. The SCN was identified by location and

cell density, and StereoInvestigator's built-in stereology workflow was followed. Due to the low number of immunopositive neurons, their uneven distribution, and the small area of interest, stereological parameters (counting frame size and grid size) were designed to cover the entire area of interest so that each cell was counted directly. The area of interest was traced at 10X magnification and counting was performed at 40X magnification. For IHC, counting was done under Köhler illumination.

Statistics

Due to the small sample sizes, normalcy of distributions and variance could not be determined. Mann-Whitney rank sum analysis and/or Welch's t-test were performed using GraphPad Prism 7 to test the hypothesis that mutant/KO values were not distinguishable from those of their WT littermates. Statistical significance was set at $p < 0.05$, and values are reported as mean ± SEM.

Rett Syndrome / MeCP2 KO

Background

Rett syndrome (RTT) is a genetic developmental disorder leading to severe mental retardation, autonomic dysfunction (particularly during the night), microcephaly, stereotyped non-specific hand movements, and abnormal gait (Hagberg et al., 1983). The vast majority of cases are due to a de novo mutation of the X-linked gene methyl CpG binding protein 2 (*MECP2*). Since this mutation is typically embryonically lethal in males, RTT is almost exclusively found in females for whom X-inactivation makes the phenotype relatively variable (Amir et al., 1999). RTT is usually diagnosed between ages 1 and 4, although symptoms may then be traced back to 6-18 months of age. Beginning at age of diagnosis, RTT patients experience a progressive loss of language and meaningful hand movements followed by impaired autonomic function (Neul et al., 2010). The majority (~80%) of RTT patients also experience sleep disruptions including sleep fragmentation and excessive daytime sleepiness (Young et al., 2007).

Additionally, it is worth noting that under normal circumstances *MECP2* has a role in regulating the core molecular clock: it binds to and activates transcription of *Per1* and *Per2* (Alvarez-Saavedra et al., 2011). *MECP2* is highly expressed in the SCN (Dragich et al., 2007), and is phosphorylated during the light phase and upon photic stimulation (Zhou et al., 2006).

The MeCP2 Knockout (KO) Mouse Model

The MeCP2 mouse model carries a null mutation of the *MeCP2* gene caused by mutation of exon 3. Females are heterozygous for the mutation, while males are hemizygous (-/Y genotype; KO). Male MeCP2 KO mice recapitulate patient symptoms well: they have reduced

activity, abnormal gait, stereotyped movements, anxiety, cognitive deficits, and microcephaly (Stearns et al., 2007). The male MeCP2 KO has a more consistent and extreme phenotype than the female heterozygote, and has been used for more of the current RTT research. We chose to begin our characterization of the circadian disruption in RTT using the male KO because of this greater wealth of background information.

Circadian Phenotype

We have shown that MeCP2 KO mice recapitulate the sleep phenotype of RTT patients; although they slept for the same total amount per day as their WT littermates, they had increased sleep fragmentation and delayed sleep (they fell asleep later and stayed asleep longer into the active phase). Mutant mice also had a significant reduction in behavioral rhythms, with four out of the eight animals studied becoming completely arrhythmic and the other four only weakly rhythmic (Fig. 1). These mice also showed reduced response to light, as evidenced by a reduction in masking behavior; WT mice showed a pronounced reduction in wheel running behavior if exposed to a light pulse, while the KO mice did not (Li et al., 2015).

Reduction in rhythmic behavioral activity was paralleled at the cellular and molecular levels. Spontaneous firing rate of SCN neurons was decreased during both the day and night, although a day/night difference did remain. Bioluminescence rhythms of Per2 expression in SCN explants were also still present, but again at a much lower amplitude than in the WT SCN (Fig. 2) (Li et al., 2015).

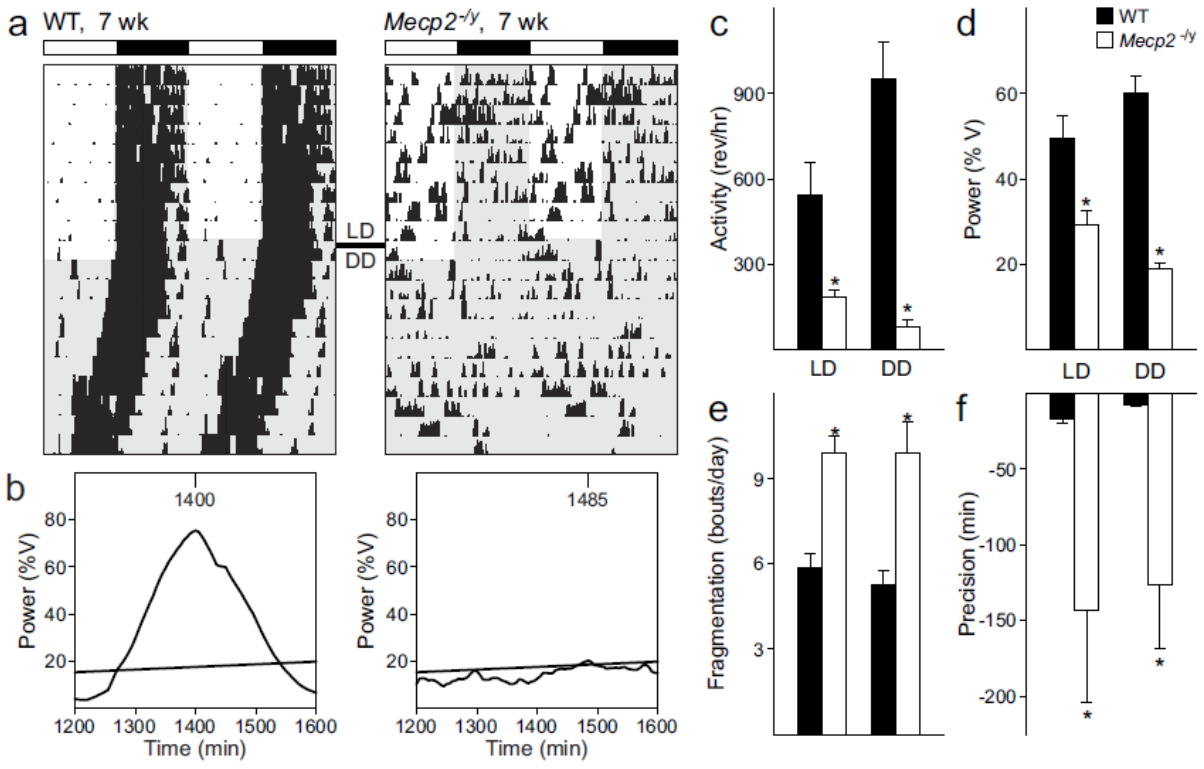

Figure 1: MeCP2 KO mice show reduced behavioral rhythmicity compared to WT (A&B), with decreased overall activity (C) and power (D), and increased fragmentation (E). *Reproduced from Li et al., 2015.*

Results

Brains of male MeCP2 KO mice and their male WT littermates were Nissl stained and the SCN was traced to determine area. The MeCP2 KO SCNs (575,321 ± 25,669.5 μm; n=8) were significantly smaller than those of WTs (662,961 ± 25,724.5 μm; n=7) by Welch's t-test ($p = 0.0315$) (Fig. 3A). This difference in area was particularly seen as a change in height, which was also significant ($p < 0.05$), whereas the change in width was not (Table 1) (Li et al., 2015).

Because MeCP2 KO mice, like RTT patients, exhibit microcephaly (caused by smaller neurons rather than neurodegeneration), it is possible that this change in size was due to microcephaly rather than cell loss. Core neurons expressing VIP are the most likely neuronal population to cause the circadian phenotype we observed if lost. Thus, brains of male MeCP2

KO mice and their male WT littermates were stained for VIP and the number of immunopositive neurons was counted. The number of VIP+ neurons in MeCP2 KO brain (287.8 ± 22.03; n=5) was significantly fewer than that in WT brains (356.8 ± 5.407; n=5) by Welch's t-test (p = 0.0330), and bordering on significance by Mann-Whitney rank sum analysis (p = 0.0556) (Li et al., 2015) (Fig. 3B).

It was also observed that most MeCP2 KO brains had reduced staining intensity compared to WT, both when looking at the SCN as a whole (as would be expected with fewer VIP+ cells) and within the immunopositive cells themselves (Fig. 3B).

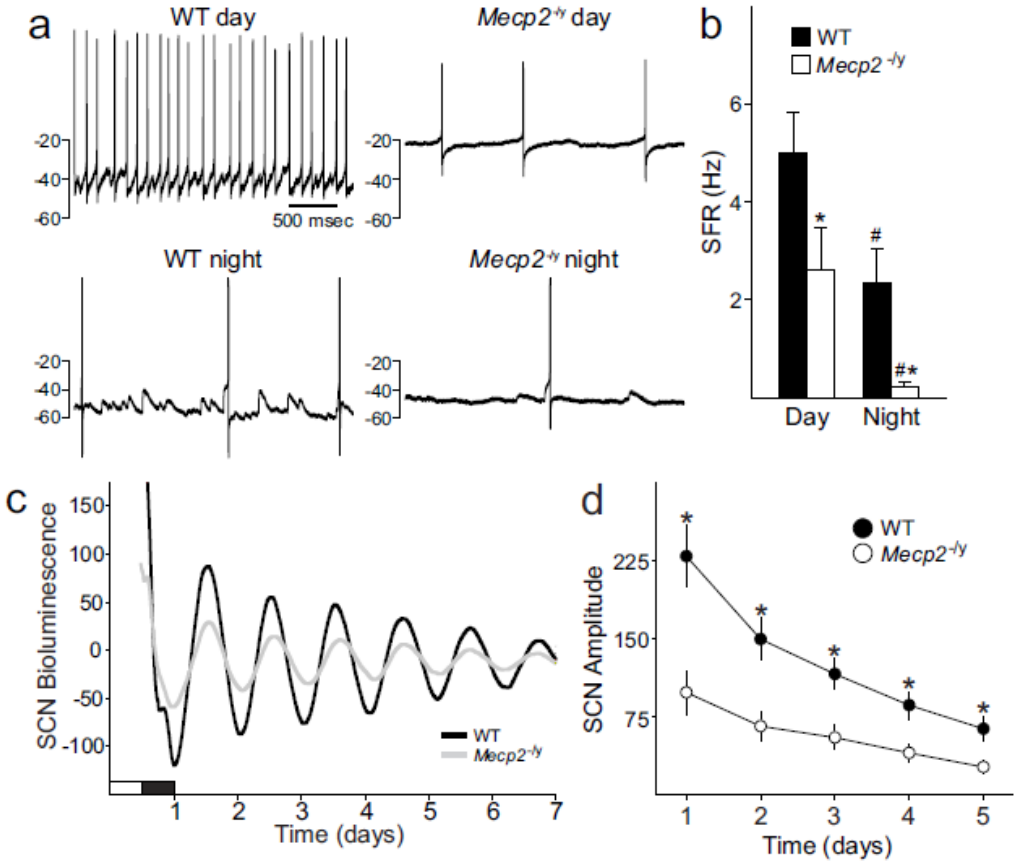

Figure 2: MeCP2 KO mice have a significant reduction in SCN spontaneous firing rate, although a day/night difference does remain (A&B). SCN Per2 rhythms, as measured by Per2:Luc rhythms, are dampened (C&D). *Reproduced from Li et al., 2015*.

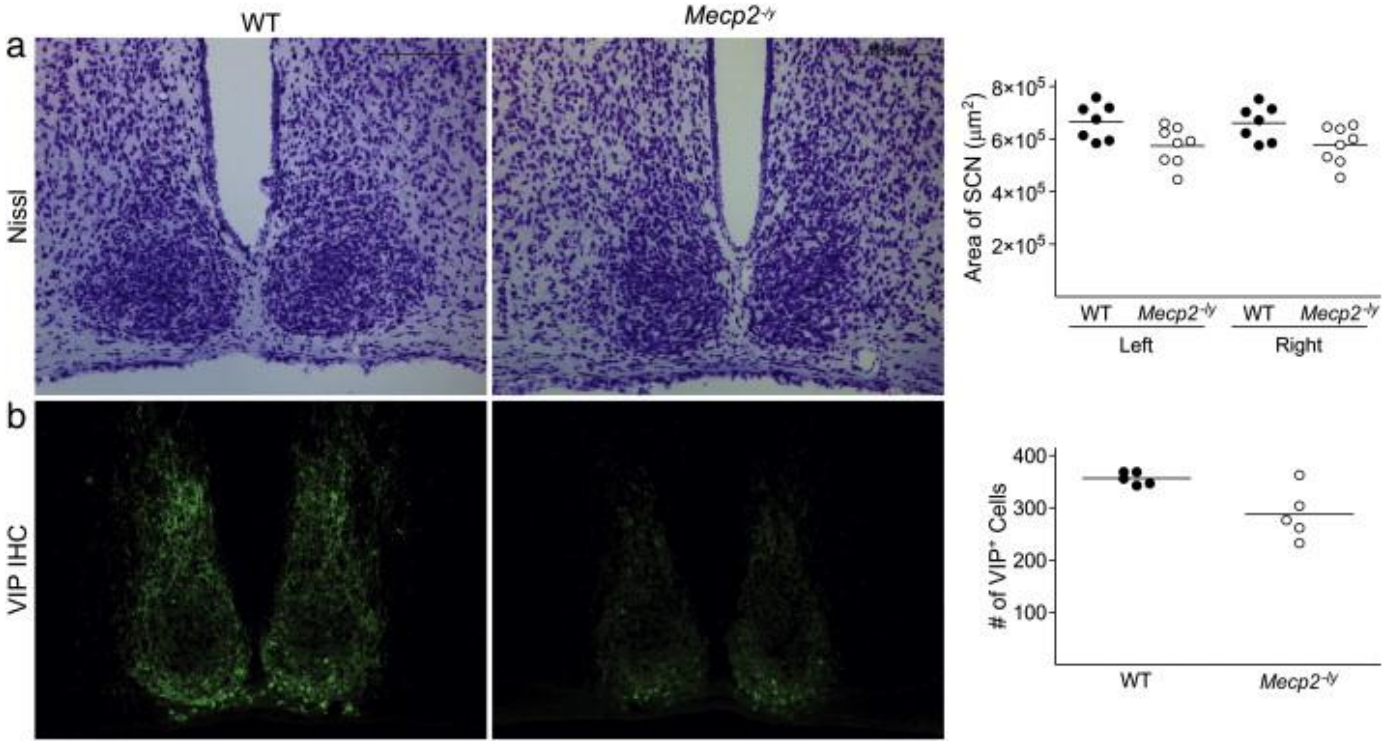

Figure 3: A: The SCNs of MeCP2 KO mice are significantly smaller than those of their WT littermates. WT (closed circles): area of 662,961 ± 25,724.5 (mean ± SEM; n=7); KO (open circles): area of 575,321 ± 25,669.5 (mean ± SEM; n=8), p = 0.0315. There is no hemispheric difference in the size of the SCN for either genotype. B: MeCP2 KO SCNs have fewer VIP+ neurons than those of their WT littermates. WT (closed circles): 356.8 ± 5.407 (n=5); KO (open circles): 287.8 ± 22.03 (n=5), p = 0.0330. Values given as mean ± SEM. Although not quantified, it was also observed that many of the KO brains also had lighter staining. *Reproduced from Li et al., 2015*.

Study	Genotype	Area (μm)	Height (μm)	Width (μm)
MeCP2	WT (n=7)	662,961 ± 25,724.5	1,113 ± 13.5	878.1 ± 22.1
	KO (n=8)	575,321 ± 25,669.5	1,007.5 ± 23	825.2 ± 19.6
Cntnap2	WT (n=3)	627,855 ± 10,914	3,035.899 ± 7.25	2,661.795 ± 37.25
	KO (n=4)	653,219 ± 18,106	3,101.423 ± 46.36	2,680.177 ± 72.84

Table 1: Summary of Nissl measurements for MeCP2 and Cntnap2 studies. Values given as mean ± SEM.

Autism Spectrum Disorder / Cntnap2 KO

Background

Autism spectrum disorder (ASD) is an umbrella term for a diverse grouping of developmental disorders sharing three core symptoms: impaired communication, impaired social interactions, and restrictive or repetitive behaviors (American Psychiatric Association, 2013). Diagnosis is not considered reliable until age 2, and many children are diagnosed much later (Lord et al., 2006). Although severity and progression of symptoms is typically highly variable, 50-80% of patients experience sleep disturbances in addition to the core ASD phenotype. Sleep complaints most frequently involve resistance to going to sleep, difficulty falling asleep, and difficulty maintaining sleep (Krakowiak et al. 2008; Cohen et al. 2014). On days following a night of poor sleep, ASD symptoms such as behavioral problems, hyperactivity, and restricted/repetitive behavior are exacerbated, while adaptive skills decline (Schreck, Mulick, & Smith, 2004; Mazurek & Sohl, 2016).

A 2009 study by Hu et al. that separated patients by severity of symptoms found 15 circadian genes that are expressed differently in the most severe ASD subgroup (including *CRY1*, *PER3*, the brain-expressed *CLOCK* analog *NPAS2*, and the key melatonin-synthesis enzyme *AANAT*). A 2016 study by Yang et al. also found differences in circadian genes in ASD patients. A total of 36 mutations were found in 11 circadian-related and core clock genes, including 6 found only in ASD patients with sleep disorders.

Melatonin levels and regulation may also be altered in ASD. While not many studies on the relationship between ASD and melatonin have been done, and those that have are typically too small to draw strong conclusions from and also often fail to measure sleep quality, some studies have shown lowered levels of melatonin and its primary metabolite 6-sulfoxymelatonin

in the blood of ASD patients compared to typically-developing peers. Other studies suggest that polymorphisms and mutations of melatonin-regulation genes are related to ASD, although these results are not consistently replicated. Melatonin supplements do help to treat many patients' sleep symptoms, but not all children who benefit from melatonin show decreased endogenous melatonin levels; therefore it may be working in ways other than simply correctly a deficiency (see Veatch et al., 2015 for a review of studies on the relationship between ASD and melatonin).

The Cntnap2 KO Mouse Model

The Cntnap2 KO mouse model carries a null mutation of Contactin-associated protein-like 2 (*Cntnap2*), one of the first identified Autism susceptibility genes (Alarcón et al., 2008). Cntnap2 KO mice recapitulate core ASD symptoms: they have reduced vocalizations as pups (impaired communication), repetitive grooming behavior and impaired spontaneous alternation (repetitive and restrictive behavior, respectively), and impaired social preference (impaired social interactions). They also have neuronal migration abnormalities and reduced expression of inhibitory interneuron markers at P14 (Peñagarikano et al., 2011).

Circadian Phenotype

Cntnap2 KO mice had reduced overall activity compared to WT littermates, and a reduction in behavioral rhythmicity due to an increase in daytime activity (Fig. 4). This was accompanied by an apparent shift in the electrical activity rhythms of the SCN. Furthermore, exposure to dim light at night (a circadian disruptor) caused an increase in repetitive behavior and a decrease in social behavior in Cntnap2 KO mice but not in WT littermates subjected to the same disruption. This is consistent with the human finding that ASD symptoms worsen on days

following poor sleep (see above), and, importantly, suggests a direct circadian influence (unpublished data).

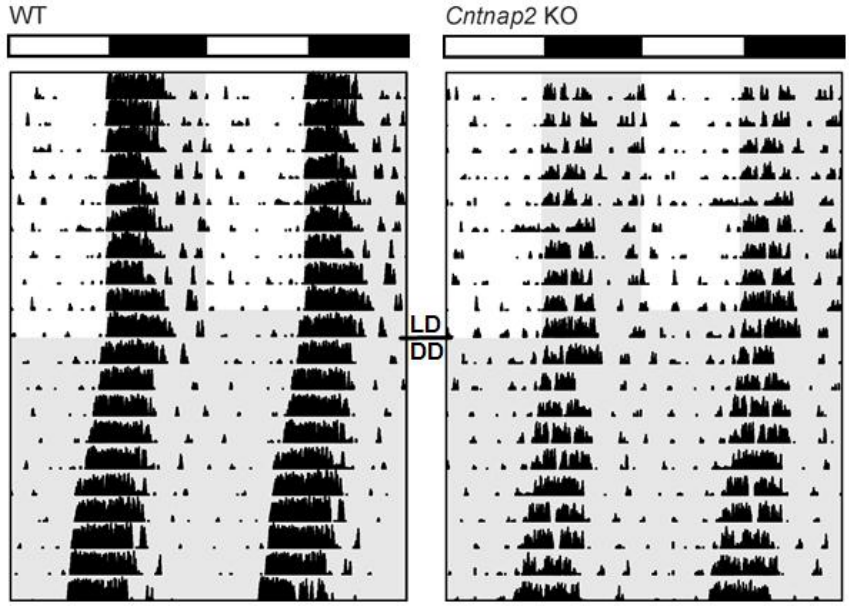

Figure 4: Cntnap2 KO mice display reduced behavioral rhythms and increased daytime activity compared to WT littermates. *Unpublished data courtesy of Dawn H. Loh and Christopher S. Colwell.*

Results

Brains of male Cntnap2 KO mice and their male WT littermates were Nissl stained, and the SCN was traced to determine area. The area of all slices was added to produce the final area measurement. The difference between the areas of Cntnap2 KO (653219 ± 18106 μm; n=4) and WT (627,855 ± 10914 μm; n=3) SCNs was not significant by Mann-Whitney rank sum analysis ($p = 0.2286$) (Fig. 5A&B). Since it is possible for total area to remain the same but a change in shape to still occur, we also compared height (WT: 3,035.899 ± 7.25, KO: 3,101.423 ± 46.36) and width (WT: 2,661.795 ± 37.25, KO: 2,680.177 ± 72.84), but did not see a difference (Table 1). The loss of Cntnap2 did not cause any gross morphological changes to the SCN.

To determine if there was any change in VIP or AVP+ cell populations, brains of male Cntnap2 KO mice and their male WT littermates were stained for VIP and AVP by double-immunofluorescence, and the number of immunopositive neurons was counted. There was no significant difference in the number of VIP+ neurons between Cntnap2 KO (397 ± 26.15; n= 3) and WT (326.3 ± 51.97; n=3) SCNs ($p = 0.4$ by Mann-Whitney ran sum analysis). Neither was there a difference in the number of AVP+ neurons between Cntnap2 KO (317 ± 8.622; n=3) and WT (288.7 ± 53.72; n=3) SCNs ($p > 0.9999$ by Mann-Whitney ran sum analysis) (Fig. 5C-E). The loss of Cntnap2 did not change the number of VIP or AVP-expressing neurons in the SCN.

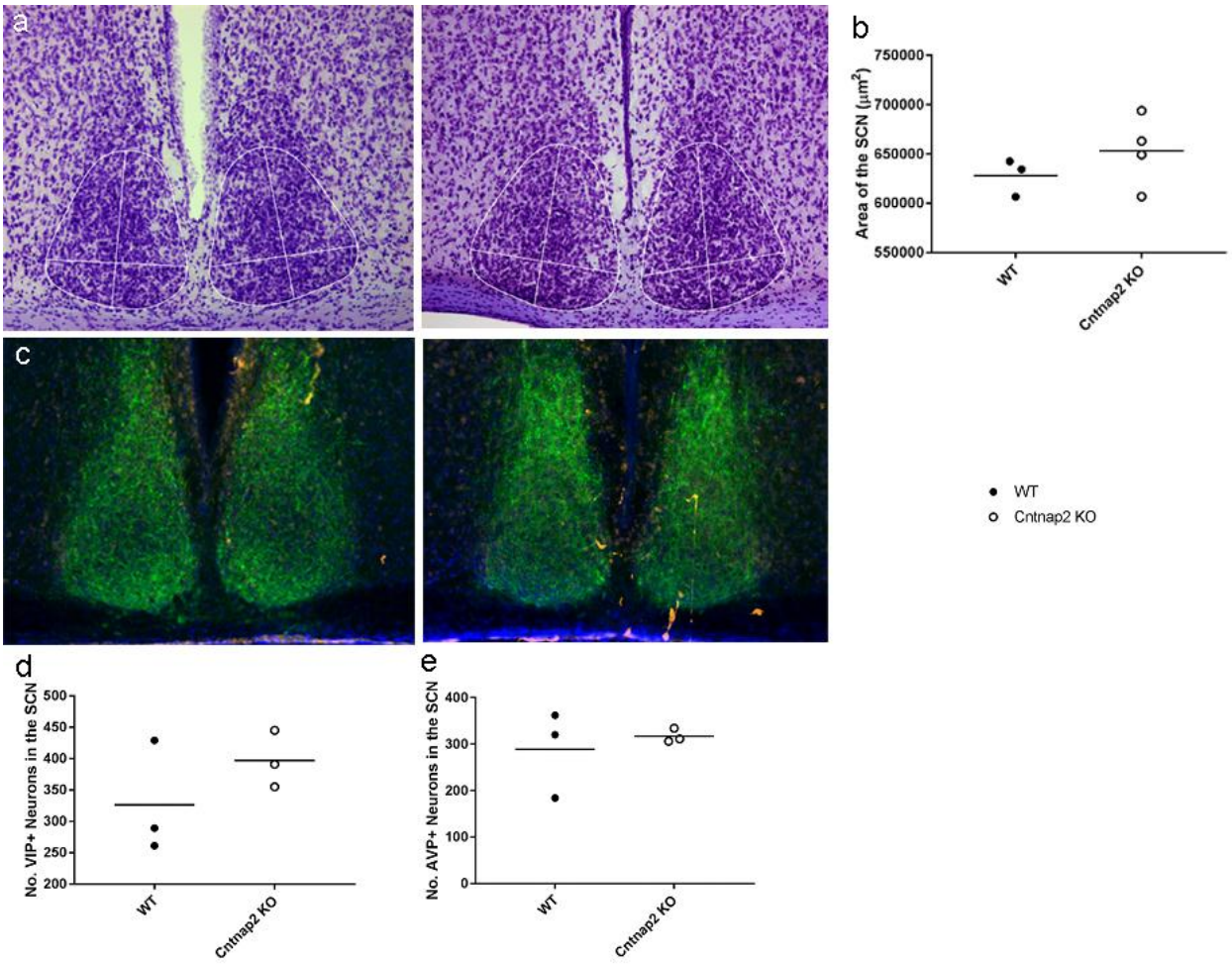

Figure 5: There is no difference between the area of WT (left) and Cntnap2 KO (right) SCNs (A&B). Neither is there a difference in the number of VIP (C&D) or AVP (C&E) neurons. B: Area of SCN. WT: 627,855 ± 10,914 (n=3), KO: 653,219 ± 18,106 (n=4), $p = 0.2286$. D: Number of VIP+ neurons in the SCN. WT: 326.3 ± 51.97 (n=3), KO: 397 ± 26.15 (n=3), $p = 0.4$. E: Number of AVP+ neurons in the SCN. WT: 288.7 ± 53.72 (n=3), KO: 288.7 ± 53.72 (n=3), $p > 0.9999$. Closed circles: WT, open circles: Cntnap2 KO. Values given as mean ± SEM.

Huntington's Disease / BACHD

Background

Huntington's disease (HD) is an autosomal dominant progressive disease caused by a CAG repeat expansion (>36 repeats) in exon 1 of the *Huntingtin* (*Htt*) gene. Although the normal function of HTT is still not fully understood, mutant *Htt* causes protein misfolding, soluble aggregates, and inclusion bodies found throughout the brain and body. Nevertheless, HD is primarily considered a neurodegenerative disease since its key feature on autopsy is neurodegeneration in the striatum and cortex (Huntington's Disease Collaborative Research Group, 1993). The hallmark motor symptom of the disease, Huntington's chorea, typically begins in middle age; however, many non-motor symptoms, including depression and cognitive and psychiatric deficits may be present years earlier (Aziz et al., 2010). Patients may report sleep disturbances up to a decade before the onset of motor dysfunction and up to 90% of patients experience sleep disturbances during the course of their disease (Morton, 2013).

Sleep disturbances typically include delayed sleep onset, sleep fragmentation, and increased daytime sleepiness, lowering quality of life for both patients and caregivers. These aspects of sleep are regulated by the circadian system, and when studied further patients are shown to have altered melatonin rhythms – further suggesting that the sleep symptoms are due to an underlying circadian dysfunction (Morton, 2013). Post-mortem analysis of HD brains have shown loss of VIP and AVP+ neurons in the SCN (van Wamelen et al., 2013). The R6/2 mouse model of HD, an early-onset and rapidly advancing model, showed loss of VIP+ neurons and $VPAC_2$ receptor at 11 weeks of age (late in disease progression, as motor and circadian symptoms develop at 4 weeks) (Fahrenkrug et al., 2007). However, it is not clear from these data

if this cell loss is the primary cause of circadian dysfunction or if it occurs towards the end of life.

The BACHD Mouse Model

The BACHD mouse model of HD is a transgenic model that uses a bacterial artificial chromosome (BAC) to insert the full-length human *Huntingtin* gene with a stable 97 CAG/CGG repeats. These mice recapitulate the core HD symptoms including progressive motor dysfunction and neurodegeneration in the striatum and cortex late in disease progression. Onset of motor symptoms is at approximately 3 months of age (Gray et al., 2008). Unlike the R6/2 mouse model mentioned above, the BACHD model has a normal lifespan and allows for examination of disease progression.

The BACHD mouse model has also been used to study the relationship between HD and the cardiovascular system, as HD patients experience cardiovascular events at a rate higher than the normal population. We found that as young as 3 mo, BACHD mice have larger hearts with thicker walls, and reduced left ventricular ejection fraction (a measure of functionality) than their WT littermates. This increase in size and decrease in functionality continued through 15 mo (Schroeder et al., 2016).

Circadian Phenotype

Three month old male BACHD mice slept less during the sleep phase, and more during the wake phase, than their WT littermates. This change was specifically due to delayed sleep onset and offset, similar to patient reports. Male BACHD mice also exhibited a progressive loss of behavioral rhythmicity over 3, 6, 9, and 12 months of age, and took longer to re-synchronize

to 6 h phase advances and delays (Kudo et al., 2011). Female BACHD mice also experienced a progressive loss of behavioral rhythmicity beginning at 3 months, but this deterioration was less severe than in age-matched males (Fig. 6A) (Kuljis et al., 2016).

Both male and female BACHD mice had a loss of SCN electrical rhythms, specifically due to reduction of their daytime spontaneous firing rate (Fig. 6B) (Kuljis et al., 2016). However, in males, there was no change in SCN PER2 expression as determined by IHC at peak and trough indicating that the molecular clock was still functioning normally (Kudo et al., 2011). Nissl staining and tracing of the SCN revealed that BACHD males had a significantly smaller SCN than their WT counterparts (by about 13%) at 3 mo, while females did not (Fig. 6C) (Kuljis et al., 2016).

The cardiovascular system also saw a decline in its circadian rhythms. At 5-6 mo, BACHD mice showed a loss of heart rate variability (HRV) in both LD and DD conditions. Healthy (WT) mice have high HRV during active periods and lower HRV during rest, caused by circadian variation in the autonomic drive to the heart. Thus this is indicative of impaired autonomic cardiovascular regulation, including in its temporal patterning (Kudo et al., 2011), possibly leading (or contributing) to the cardiac phenotype described above and patients' increased risk of cardiac events.

Results

Brains of male and female BACHD mice and their WT littermates were stained for VIP and AVP, and the number of immunopositive neurons was counted (Table 2). 2-way ANOVA found no interaction between sex and genotype, and two-tailed t-tests showed no differences within sex or genotype for either neuropeptide (Fig. 7) (Kuljis et al., 2016). This indicates that

mutant *Htt* did not causes changes to the number of VIP or AVP-expressing neurons and that, although the SCN is a sexually dimorphic area by size, the sexes did not differ in expression of these neuropeptides.

Genotype	Sex	VIP+ Neurons (#)	AVP+ Neurons (#)
WT	Male	390.7 ± 59.28 (n=6)	734 ± 104.9 (n=5)
	Female	410.8 ± 14.44 (n=5)	839.2 ± 61.76 (n=5)
BACHD	Male	386.8 ± 27.1 (n=6)	657.6 ± 101.8 (n=5)
	Female	427 ± 62.09 (n=5)	889.4 ± 71.28 (n=5)

Table 2: Number of VIP and AVP-expressing neurons in the SCN of WT and BACHD males and females. There was no difference between sex or genotype for either neuropeptide. Values given as mean ± SEM.

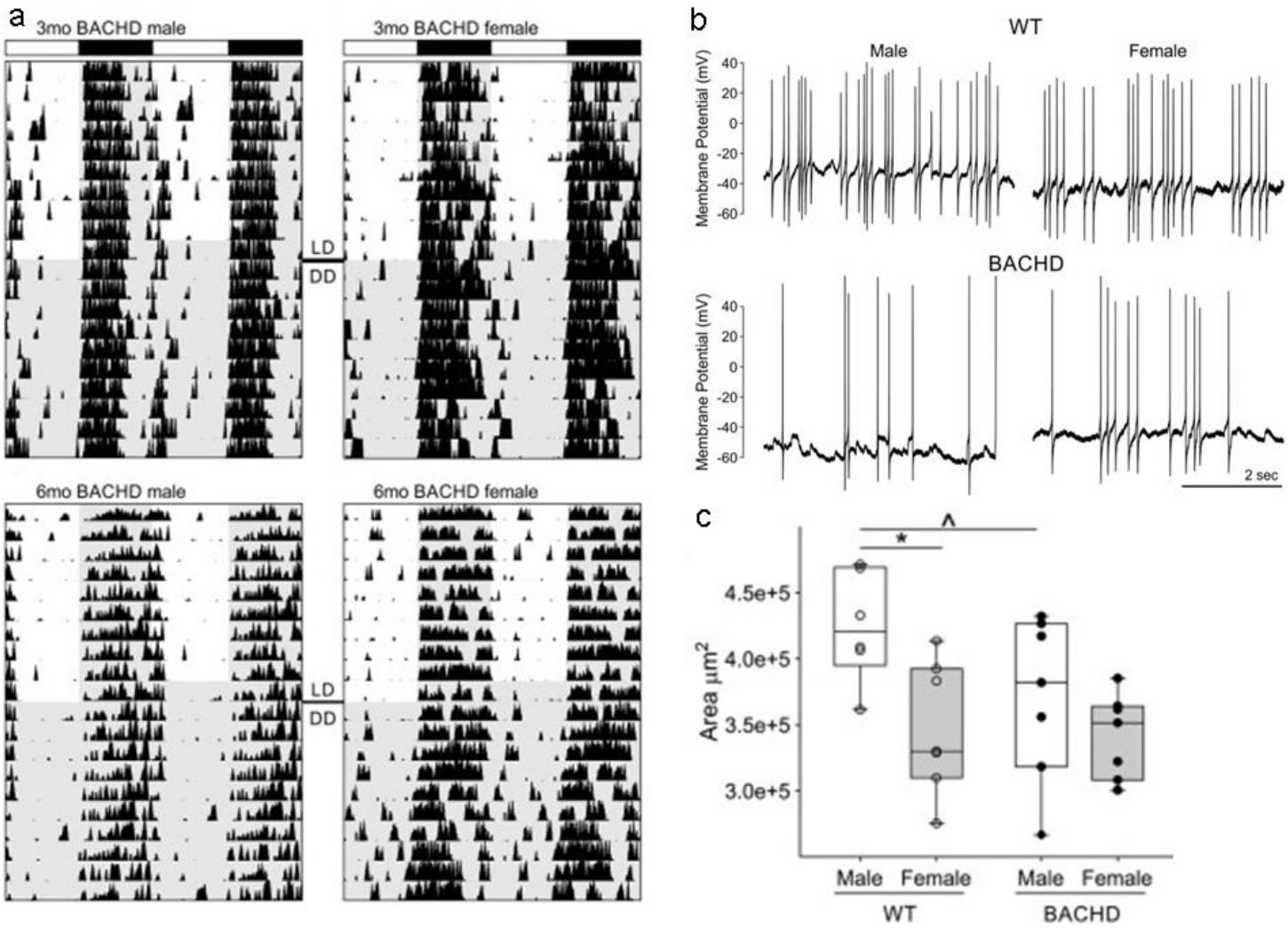

Figure 6: BACHD mice show reduced behavioral rhythmicity starting at 3 months, though females show a delayed decline compared to males (A). Both male and female BACHD mice show reduced daytime spontaneous firing rate in the SCN (B). Only male BACHD mice have a genotype-associated reduction in SCN size at 3 mo (C). Reproduced with modification from Kuljis et al., 2016.

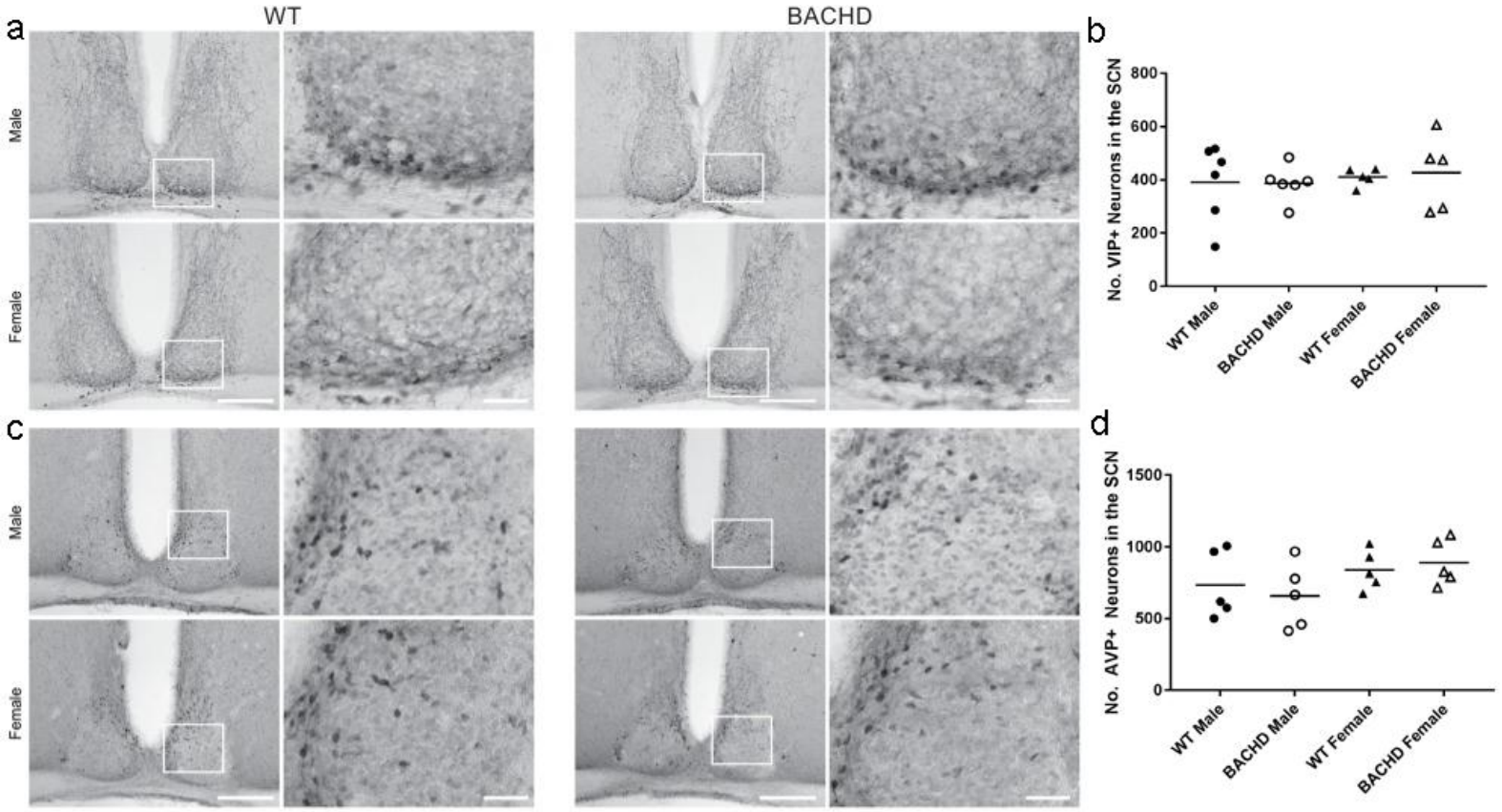

Figure 7: There was no difference in the number of VIP (A&B) or AVP (C & D) immunopositive neurons in either male or female BACHD or WT mice. B: Number of VIP+ neurons. WT Male (closed circle): 390.7 ± 59.28 (n=6), BACHD male (open circle): 386.8 ± 27.1 (n=6), WT female (closed triangle): 410.8 ± 14.44 (n=5), BACHD female (open triangle): 427 ± 62.09 (n=5). D: Number of AVP+ neurons. WT Male (closed circle): 734 ± 104.9, BACHD male (open circle): 657.6 ± 101.8, WT female (closed triangle): 839.2 ± 61.76, BACHD female (open triangle): 889.4 ± 71.28, n=5 for each group. Values given as mean ± SEM. *Reproduced with modification from Kuljis et al., 2016.*

Discussion

Rett syndrome (RTT), autism spectrum disorder (ASD), and Huntington's disease (HD) are three very different conditions. Although RTT and ASD are both developmental disorders that typically manifest in early childhood, HD symptoms generally begin in middle age. RTT and HD are both caused by genetic mutations, but the mutation the leads to RTT is usually de novo while HD is inherited; ASD is usually believed to be multifactorial in origin. RTT and HD also both cause distinct gross neuroanatomical changes, but this change is microcephaly due to small neurons in RTT and neurodegeneration of striatum and cortex in HD, and neither of these gross abnormalities are found in ASD. Behavioral symptoms are also vastly different among the three conditions. Yet despite these many differences, patients of all three disorders experience similar sleep problems which lower their quality of life, the quality of life of their caregivers, and likely exacerbate their other symptoms.

Patients with these disorders all have difficulty falling asleep and maintaining sleep at night, followed by excessive sleepiness during the day (Young et al., 2007; Cohen et al., 2014; Morton, 2013). This shift in when sleep occurs is suggestive of an underlying circadian dysfunction rather than simply problems in the sleep/wake-generating regions of the brain. ASD and HD patients also often have other health problems commonly found with chronic circadian disruption: gastrointestinal problems in ASD patients, and depression in HD patients. ASD patients have been found to have more genetic variability of the melatonin gene than the general population (Veatch et al., 2015), although the consequences of this are unknown, and HD patients have been shown to have altered melatonin rhythms. Unfortunately, due to the manner in which melatonin has to be measured, such studies are difficult to do in children – and especially

children with developmental disorders – so we don't know much about what melatonin rhythms are like in ASD patients, and nothing about them in RTT patients.

As the central clock in mammals, the SCN plays a role in setting other rhythms: sleep timing, melatonin secretion, and autonomic regulation of the heart (and other organs) among them. It is therefore possible that a single dysfunction in the SCN could result in the wide variety of circadian symptoms seen in the patients and mouse models. *MeCP2*, *Cntnap2*, and *Htt* are all expressed in the SCN, which should therefore experience some manner of deficit with their mutation. This makes the SCN the first logical place to look for pathology leading to circadian deficits. Indeed, circadian deficits were seen in mouse models of each disorder, but they were not consistent between models.

Mouse models of the three disorders recapitulated the sleep complaints of patients, showing delayed sleep onset (where a rhythm could be determined) and increased fragmentation (Li et al., 2016; Kudo et al., 2011; Kuljis et al., 2016). All models also showed a reduction in circadian rhythmicity, which was more pronounced at an earlier age in the MeCP2 and Cntnap2 KO mice than the BACHD, as expected since RTT and ASD patients also exhibit symptoms at a significantly younger age than do HD patients. Electrophysiological rhythms of spontaneous firing rate in the SCN were altered in each model, but in different ways (Li et al., 2016; Kudo et al., 2011; Kuljis et al., 2016). In the MeCP2 KO, firing rate was decreased during both day and night, although a rhythm remained. In the BACHD however, the rhythm was lost in both males and females due to reduction of the daytime firing rate only. Cntnap2 KO SCN's didn't show a reduction in rhythms, but rather a phase shift. MeCP2 mice showed a reduction in amplitude of Per2 bioluminescence rhythms, while IHC in BACHD males showed no difference. These data

demonstrate that although patients experience very similar sleep symptoms, their underlying pathology may be quite different.

No common underlying SCN histoanatomical pathology was seen between the three mouse models. Although Nissl staining and tracing showed that both MeCP2 KO mice and male BACHD mice had smaller SCNs than their WT counterparts, female BACHD mice and Cntnap2 mice did not. MeCP2 mice had fewer VIP+ neurons and reduced staining compared to their WT littermates, but no other mouse models showed any difference in number of VIP or AVP-expressing neurons. Although the electrophysiological pathology seen in each mouse model clearly demonstrates that there are some disorder-related changes at the level of the SCN, this does not seem to be due to loss of the neuropeptides VIP or AVP.

It is important to note that these experiments were likely all underpowered. Monte Carlo bootstrapping simulation of the MeCP2 VIP+ cell counting data (10,000 repetitions) shows a power of only 43.6% for Mann-Whitney rank sum analysis, and 36.7% for Welch's t-test. To achieve close to 80% power, at least n=10 would be necessary. Although significance was observed in this case, only ~40% of replication experiments using the same sample size could be expected to get the same result. If this is representative of the BACHD and Cntnap2 cell counting data as well, we should not be surprised to have gotten negative results; even if a difference does exist between the two genotypes, such a low power made it unlikely for us to have detected that difference. This is a widespread problem in the neuroscience field, where a desire to reduce the number of animals used ultimately leads to conflicting results, the need for a great number of replications, and weakening of the scientific field as a whole. By being aware of the problem, we can take steps to address it in future studies.

Overall, the sleep problems observed in RTT, ASD, and HD patients (Young et al., 2007; Cohen et al., 2014; Morton, 2013), while outwardly similar, must be being caused by different disease mechanisms affecting different extra-SCN brain regions or affecting the SCN in different ways. It is likely that each disorder will have to be approached differently when trying to alleviate patient symptoms. This process should be guided by the additional circadian alterations that we were able to see in the mouse models. In MeCP2 mice, where we did see a reduction in size of the SCN and number of VIP+ neurons, future studies should look for loss of other neuropeptide-expressing subpopulations, as well as an examination of how other brain regions are affected by this change. To help patients, means of entrainment other than light may be particularly beneficial (such as timed eating and exercise). In Cntnap2, the phase of the SCN needs to be determined and the pineal gland should be studied directly. Since C57B/6 mice (the strain used for this line) do not produce endogenous melatonin, it may be useful to cross the line onto a background that does so this can be studied more effectively. In BACHD, since we did not see a loss of cells but did see a loss of spontaneous firing rhythms, it would be most useful to study ionic currents and attempt to "boost" the circadian signal. Cardiovascular-regulating regions of the brain stem, such as the nucleus of the solitary tract, should also be examined.

Understanding the cause of circadian dysfunction is key to effectively treating patients. For example, if the SCN signal is low or mistimed due to partial cell loss or a phase shift, boosting input to the SCN through light therapy or non-light zeitgebers may be most effective at restoring healthy rhythms. Alternatively, if the SCN is working fine, but the ability of the pineal gland to produce melatonin is altered, then light therapies would likely be ineffective and timed melatonin supplements would be more helpful. It is also useful to know if disorders with similar symptoms also have similar pathologies, so that we can expect to treat them similarly. This thesis

begins the long process of addressing these questions by examining the gross morphology of the SCN as well as its VIP and AVP+ neuronal populations in mouse models of three disorders.

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References

Abrahamson, E. E., & Moore, R. Y. (2001). Suprachiasmatic nucleus in the mouse: retinal innervation, intrinsic organization and efferent projections. Brain Research, 916(1–2), 172–191.

Alarcón, M., Abrahams, B. S., Stone, J. L., Duvall, J. A., Perederiy, J. V., Bomar, J. M., … Geschwind, D. H. (2008). Linkage, association, and gene-expression analyses identify CNTNAP2 as an autism-susceptibility gene. American Journal of Human Genetics, 82(1), 150–159. http://doi.org/10.1016/j.ajhg.2007.09.005

Alvarez-Saavedra, M., Antoun, G., Yanagiya, A., Oliva-Hernandez, R., Cornejo-Palma, D., Perez-Iratxeta, C., … Cheng, H.-Y. M. (2011). miRNA-132 orchestrates chromatin remodeling and translational control of the circadian clock. Human Molecular Genetics, 20(4), 731–751. http://doi.org/10.1093/hmg/ddq519

Amir, R. E., Van den Veyver, I. B., Wan, M., Tran, C. Q., Francke, U., & Zoghbi, H. Y. (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nature Genetics, 23(2), 185–188. http://doi.org/10.1038/13810

Aziz, N. A., Anguelova, G. V., Marinus, J., Lammers, G. J., & Roos, R. A. C. (2010). Sleep and circadian rhythm alterations correlate with depression and cognitive impairment in Huntington's disease. Parkinsonism & Related Disorders, 16(5), 345–350. http://doi.org/10.1016/j.parkreldis.2010.02.009

Brown, S. A., Kowalska, E., & Dallmann, R. (2012). (Re)inventing the circadian feedback loop. Developmental Cell, 22(3), 477–487. http://doi.org/10.1016/j.devcel.2012.02.007

Buhr, E. D., & Takahashi, J. S. (2013). Molecular components of the Mammalian circadian clock. Handbook of Experimental Pharmacology, (217), 3–27. http://doi.org/10.1007/978-3-642-25950-0_1

Caldwell, H. K., Lee, H.-J., Macbeth, A. H., & Young, W. S. (2008). Vasopressin: Behavioral Roles of an "Original" Neuropeptide. Progress in Neurobiology, 84(1), 1–24. http://doi.org/10.1016/j.pneurobio.2007.10.007

Cohen, S., Conduit, R., Lockley, S. W., Rajaratnam, S. M., & Cornish, K. M. (2014). The relationship between sleep and behavior in autism spectrum disorder (ASD): a review. Journal of Neurodevelopmental Disorders, 6(1), 44. http://doi.org/10.1186/1866-1955-6-44

Colwell, C. S. (2011). Linking neural activity and molecular oscillations in the SCN. Nature Reviews. Neuroscience, 12(10), 553–569. http://doi.org/10.1038/nrn3086

Dibner, C., Schibler, U., & Albrecht, U. (2010). The mammalian circadian timing system: organization and coordination of central and peripheral clocks. Annual Review of Physiology, 72, 517–549. http://doi.org/10.1146/annurev-physiol-021909-135821

Dragich, J. M., Kim, Y.-H., Arnold, A. P., & Schanen, N. C. (2007). Differential distribution of the MeCP2 splice variants in the postnatal mouse brain. The Journal of Comparative Neurology, 501(4), 526–542. http://doi.org/10.1002/cne.21264

Evans, J. A., & Davidson, A. J. (2013). Health consequences of circadian disruption in humans and animal models. Progress in Molecular Biology and Translational Science, 119, 283–323. http://doi.org/10.1016/B978-0-12-396971-2.00010-5

Fahrenkrug, J., Popovic, N., Georg, B., Brundin, P., & Hannibal, J. (2007). Decreased VIP and VPAC2 receptor expression in the biological clock of the R6/2 Huntington's disease mouse. Journal of Molecular Neuroscience: MN, 31(2), 139–148.

Foster, R. G., & Kreitzman, L. (2014). The rhythms of life: what your body clock means to you! Experimental Physiology, 99(4), 599–606. http://doi.org/10.1113/expphysiol.2012.071118

Gray, M., Shirasaki, D. I., Cepeda, C., Andre, V. M., Wilburn, B., Lu, X.-H., … William Yang, X. (2008). Full Length Human Mutant Huntingtin with a Stable Polyglutamine Repeat Can Elicit Progressive and Selective Neuropathogenesis in BACHD Mice. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 28(24), 6182–6195. http://doi.org/10.1523/JNEUROSCI.0857-08.2008

Hagberg, B., Aicardi, J., Dias, K., & Ramos, O. (1983). A progressive syndrome of autism, dementia, ataxia, and loss of purposeful hand use in girls: Rett's syndrome: report of 35 cases. Annals of Neurology, 14(4), 471–479. http://doi.org/10.1002/ana.410140412

Hu, V. W., Sarachana, T., Kim, K. S., Nguyen, A., Kulkarni, S., Steinberg, M. E., … Lee, N. H. (2009). Gene Expression Profiling Differentiates Autism Case–Controls and Phenotypic Variants of Autism Spectrum Disorders: Evidence for Circadian Rhythm Dysfunction in Severe Autism. Autism Research : Official Journal of the International Society for Autism Research, 2(2), 78–97. http://doi.org/10.1002/aur.73

Huntington's Disease Collaborative Research Group. (1993). A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell*, *72*(6), 971–983.

Krakowiak, P., Goodlin-Jones, B., Hertz-Picciotto, I., Croen, L. A., & Hansen, R. L. (2008). Sleep problems in children with autism spectrum disorders, developmental delays, and typical development: a population-based study. Journal of Sleep Research, 17(2), 197–206. http://doi.org/10.1111/j.1365-2869.2008.00650.x

Kudo, T., Schroeder, A., Loh, D. H., Kuljis, D., Jordan, M. C., Roos, K. P., & Colwell, C. S. (2011). Dysfunctions in Circadian Behavior and Physiology in Mouse Models of Huntington's Disease. Experimental Neurology, 228(1), 80–90. http://doi.org/10.1016/j.expneurol.2010.12.011

Kuljis, D. A., Gad, L., Loh, D. H., MacDowell Kaswan, Z., Hitchcock, O. N., Ghiani, C. A., & Colwell, C. S. (2016). Sex Differences in Circadian Dysfunction in the BACHD Mouse Model of Huntington's Disease. PLoS ONE, 11(2). http://doi.org/10.1371/journal.pone.0147583

Lee, H. S., Billings, H. J., & Lehman, M. N. (2003). The suprachiasmatic nucleus: a clock of multiple components. Journal of Biological Rhythms, 18(6), 435–449. http://doi.org/10.1177/0748730403259106

Li, Q., Loh, D. H., Kudo, T., Truong, D., Derakhshesh, M., Kaswan, Z. M., … Colwell, C. S. (2015). Circadian rhythm disruption in a mouse model of Rett syndrome circadian disruption in RTT. Neurobiology of Disease, 77, 155–164. http://doi.org/10.1016/j.nbd.2015.03.009

Lord, C., Risi, S., DiLavore, P. S., Shulman, C., Thurm, A., & Pickles, A. (2006). Autism from 2 to 9 years of age. Archives of General Psychiatry, 63(6), 694–701. http://doi.org/10.1001/archpsyc.63.6.694

Mazurek, M. O., & Sohl, K. (2016). Sleep and Behavioral Problems in Children with Autism Spectrum Disorder. Journal of Autism and Developmental Disorders, 46(6), 1906–1915. http://doi.org/10.1007/s10803-016-2723-7

Moore, R. Y. (1996). Neural control of the pineal gland. Behavioural Brain Research, 73(1–2), 125–130.

Morton, A. J. (2013). Circadian and sleep disorder in Huntington's disease. Experimental Neurology, 243, 34–44. http://doi.org/10.1016/j.expneurol.2012.10.014

Musiek, E. S. (2015). Circadian clock disruption in neurodegenerative diseases: cause and effect? Frontiers in Pharmacology, 6. http://doi.org/10.3389/fphar.2015.00029

Neul, J. L., Kaufmann, W. E., Glaze, D. G., Christodoulou, J., Clarke, A. J., Bahi-Buisson, N., … RettSearch Consortium. (2010). Rett syndrome: revised diagnostic criteria and nomenclature. Annals of Neurology, 68(6), 944–950. http://doi.org/10.1002/ana.22124

Neurodevelopmental Disorders. (2013). In American Psychiatric Association, Diagnostic and Statistical Manual of Mental Disorders (Fifth Edition). American Psychiatric Association. Retrieved from http://psychiatryonline.org/doi/abs/10.1176/appi.books.9780890425596.dsm01

Okamura, H. (2007). Suprachiasmatic nucleus clock time in the mammalian circadian system. Cold Spring Harbor Symposia on Quantitative Biology, 72, 551–556. http://doi.org/10.1101/sqb.2007.72.033

Schreck, K. A., Mulick, J. A., & Smith, A. F. (2004). Sleep problems as possible predictors of intensified symptoms of autism. Research in Developmental Disabilities, 25(1), 57–66.

Schroeder, A. M., Wang, H. B., Park, S., Jordan, M. C., Gao, F., Coppola, G., … Colwell, C. S. (2016). Cardiac Dysfunction in the BACHD Mouse Model of Huntington's Disease. PLoS ONE, 11(1). http://doi.org/10.1371/journal.pone.0147269

Stearns, N. A., Schaevitz, L. R., Bowling, H., Nag, N., Berger, U. V., & Berger-Sweeney, J. (2007). Behavioral and anatomical abnormalities in Mecp2 mutant mice: a model for Rett syndrome. Neuroscience, 146(3), 907–921. http://doi.org/10.1016/j.neuroscience.2007.02.009

van Wamelen, D. J., Aziz, N. A., Anink, J. J., van Steenhoven, R., Angeloni, D., Fraschini, F., … Swaab, D. F. (2013). Suprachiasmatic nucleus neuropeptide expression in patients with Huntington's Disease. Sleep, 36(1), 117–125. http://doi.org/10.5665/sleep.2314

Veatch, O. J., Goldman, S. E., Adkins, K. W., & Malow, B. A. (2015). Melatonin in Children with Autism Spectrum Disorders: How Does the Evidence Fit Together? Journal of Nature and Science, 1(7), e125.

Vosko, A. M., Schroeder, A., Loh, D. H., & Colwell, C. S. (2007). Vasoactive intestinal peptide and the mammalian circadian system. General and Comparative Endocrinology, 152(2–3), 165–175. http://doi.org/10.1016/j.ygcen.2007.04.018

Vosko, A., van Diepen, H. C., Kuljis, D., Chiu, A. M., Heyer, D., Terra, H., … Colwell, C. S. (2015). Role of vasoactive intestinal peptide in the light input to the circadian system. The European Journal of Neuroscience, 42(2), 1839–1848. http://doi.org/10.1111/ejn.12919

Yan, L., Karatsoreos, I., Lesauter, J., Welsh, D. K., Kay, S., Foley, D., & Silver, R. (2007). Exploring spatiotemporal organization of SCN circuits. Cold Spring Harbor Symposia on Quantitative Biology, 72, 527–541. http://doi.org/10.1101/sqb.2007.72.037

Yang, Z., Matsumoto, A., Nakayama, K., Jimbo, E. F., Kojima, K., Nagata, K., … Yamagata, T. (2016). Circadian-relevant genes are highly polymorphic in autism spectrum disorder patients. Brain & Development, 38(1), 91–99. http://doi.org/10.1016/j.braindev.2015.04.006

Young, D., Nagarajan, L., de Klerk, N., Jacoby, P., Ellaway, C., & Leonard, H. (2007). Sleep problems in Rett syndrome. Brain & Development, 29(10), 609–616. http://doi.org/10.1016/j.braindev.2007.04.001

Zhou, Z., Hong, E. J., Cohen, S., Zhao, W.-N., Ho, H.-Y. H., Schmidt, L., … Greenberg, M. E. (2006). Brain-specific phosphorylation of MeCP2 regulates activity-dependent Bdnf transcription, dendritic growth, and spine maturation. Neuron, 52(2), 255–269. http://doi.org/10.1016/j.neuron.2006.09.037