CHARACTERIZING SLEEP:

FROM NEUROCHEMISTRY TO SPECIAL POPULATIONS

By

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ABSTRACT

Sleep is a necessary function that has specifically been shown to be important for learning and memory. However, we are only beginning to understand the underlying processes regulating sleep. Sleep and wake states appear to be controlled by fast acting neurotransmitters (e.g. GABA and glutamate), and transitions between NREM and REM sleep involve a mutually inhibitory switch between GABAergic neurons. Better understandings of sleep mechanisms allow us to try to explain sleep disturbances in special populations. Individuals with Down syndrome (DS) have many sleep impairments and cognitive deficits. This thesis sought to further characterize sleep in children with DS by examining separate NREM-REM sleep cycles across the night. Children with DS appear to have an increase in REM sleep latency due to an extremely long duration for the first NREM sleep episode. This may indicate impairment in the initial transition from NREM to REM sleep and help explain the lack of sleep benefit for learning after naps observed in children with DS. Overall, further research into this phenomenon and the mechanisms driving sleep transitions is needed to obtain a more complete understanding of sleep across populations.

WHAT IS SLEEP

Sleep is a universal process that is required for sustained life and optimal functioning. Yet, we are only beginning to scrape the surface of this necessary and complex state. Wake involves voluntary motor activation and response to internal and external stimuli (Scammell, Arrigoni, & Lipton, 2017), while sleep is traditionally defined as a behavioral state that is characterized by immobility or reduced behavioral responsiveness to external stimuli (Vyazovskiy, 2015). However, sleep is much more intricate and involves complex neurological states and processes, most of which are unknown or not completely understood.

Sleep is discussed in the context of three states that differ in electroencephalographic (EEG) activity: wake, non-rapid eye movement (NREM) sleep, and rapid eye movement (REM) sleep. Wake EEG is characterized by low amplitude, high frequency activity, eye movement, and swallowing (Britton et al., 2016). NREM is divided into three stages. Stage 1 (N1) occurs during the sleep-wake transition, has the lowest threshold for arousal and lasts between 30 seconds to 5 minutes (Mindell & Owens, 2015). Stage 2 (N2) is characterized by fluctuating fast activity (sleep spindles) and high amplitude slow-wave spikes (K complexes) (Mindell & Owens, 2015), and lasts between 5 and 25 minutes (Mindell & Owens, 2015). Stage 3 (N3), also referred to as deep or slow wave sleep (SWS), is dominated by delta EEG waves, has the highest arousal threshold, and lasts between 30 and 45 minutes (Mindell & Owens, 2015). Following SWS is a transition into a lighter stage of sleep known as REM sleep. REM sleep is characterized by desynchronized but high amplitude cortical activity, high brain metabolic rate, dreaming, absence of skeletal muscle tone, lack of normal thermoregulation, and bursts of phasic eye movement, and the first REM sleep episode occurs 70 to 100 minutes after sleep onset (Mindell

& Owens, 2015). While we are able to recognize and measure each sleep state, the exact and overarching function of sleep is still unknown.

INITIATION AND MAINTENANCE OF SLEEP AND WAKE STATES

There is still much we do not understand about the mechanisms of sleep, but here I present current understandings of the neurochemicals that promote and drive sleep and wake states. As mentioned above, differing EEG activities allow us to define states and sleep stages, serving as a biomarker in which frequencies can be observed and measured to monitor the effects of neuromodulator manipulation on brain states.

The primary wake promoting pathway ascends through the paramedian region of the midbrain and splits into the dorsal pathway, which innervates the thalamus and is responsible for the content of consciousness (e.g. sensation, motor response, cognition, etc.), and the ventral pathway, which innervates the hypothalamus, basal forebrain (BF), and cortex, and is responsible for the behavioral state of wake (consciousness itself) (Scammell, Arrigoni, & Lipton, 2017). The reticular formation was thought to integrate sensory information and drive general arousal and motor responses (Scammell, Arrigoni, & Lipton, 2017). The ascending reticular activating system model of wake suggests that neurons in the upper brainstem reticular formation project to forebrain targets that promote wake (Fuller et al., 2011). Most of the neurons in these pathways are monoaminergic or cholinergic. However, it is more likely that projections form the parabrachial nucleus (PB) to the BF to the cortex are critical for the arousal process, and that the reticulo-thalamo-cortical pathway has a limited role in arousal (Fuller et al., 2011).

Monoaminergic neurons are generally thought to be promote higher brain activity states (primarily wake), and have been shown to have high firing rates during wake, slow firing rates during NREM, and stop firing altogether during REM (Scammell, Arrigoni, & Lipton, 2017).

These neurons innervate the cortex, BF, lateral hypothalamus (LH), and thalamus to drive wake. Noradrenergic neurons of the locus coeruleus (LC) project to the forebrain to promote cortical activity and the wake state (Carter et al., 2010). Optogenetic activation of these neurons in mice rapidly wakes the animals from sleep, immediately facilitating the sleep to wake transition. Activation of these neurons was also found to be necessary to maintain wake episodes while inhibition did not increase wake duration (Carter et al., 2010).

Serotonin, produced by the median dorsal raphe nucleus (mDRN), similarly promotes wake in the forebrain (Scammell, Arrigoni, & Lipton, 2017), in which there is a causal relationship between mDRN neuron firing, cortical activity, and sleep to wake transitions. Optogenetic activation of mDRN serotonergic neurons caused excitation of other wake promoting neurons, significantly increasing wake and fragmenting NREM sleep (Ito et al., 2013). However, serotonin may also play a role in regulating REM sleep state initiation. Serotonin (5-HT1A) receptor responsive neurons in the pedunculopontine tegmental nucleus (PPT) become maximally active immediately before and during REM sleep (Grace, Liu, & Horner, 2012). Grace, Liu, & Horner (2012) selectively silenced REM sleep active neurons in the PPT using 5-HT1A agonists, and found that the percentage of REM sleep out of total sleep time was significantly increased due to an increase in the frequency of REM sleep episodes but not episode duration. This suggests REM sleep initiation but not maintenance is affected by this population of neurons. Furthermore, the increased amount of REM sleep episodes was due to periods of low REM sleep drive. These results indicate that 5-HT1A receptors in PPT neurons function to inhibit REM sleep by increasing the threshold for REM sleep initiation (Grace, Liu, & Horner, 2012).

Lastly, histamine most likely promotes generalized arousal as levels are observed to be high during wake (Scammell, Arrigoni, & Lipton, 2017). Histamine is produced in the tuberomammillary nucleus (TMN), and these neurons excite neurons in the cortex and thalamus during wake to promote arousal (Scammell, Arrigoni, & Lipton, 2017). A recent study by Yu et al. (2015), indicates that co-release of GABA from histamine neurons further promotes cortical activation and wake.

Several neuropeptides also influence wake and sleep states. Orexins are neuropeptides found in the LH that excite wake promoting regions and are important for maintaining wake and regulating REM sleep (Sasaki et al., 2011). Activation of these neurons increases wake and strongly suppresses REM sleep. Activating orexin neurons has been shown to awaken mice from REM sleep, while animals with disrupted orexin signaling have shorter wake bouts and sudden transitions from wake into REM like sleep states (Scammell, Arrigoni, & Lipton, 2017). Sasaki et al. (2011) demonstrated that excitation of orexin A and orexin B producing neurons increased the amount of time spent in wake and decreased NREM and REM sleep times, while inhibition of these neurons decreased wake time and increased NREM sleep time.

Neurons that produce the neuropeptide melanin concentrating hormone (MCH) are intermixed with orexins in the LH but have the opposite effect. MCH producing neurons may promote REM sleep through innervation of the sublaterodorsal nucleus (SLD), the primary REM sleep promoting region (Scammell, Arrigoni, & Lipton, 2017). Vetrivelan et al. (2016) selectively activated and destroyed MCH neurons in mice and observed activation to cause increases in REM sleep, with no alteration in NREM sleep, while deletions of these neurons altered the diurnal rhythm of wake and REM sleep but didn’t change total amounts. Therefore,

MCH neurons appear to drive REM sleep and may be necessary for normal expression of diurnal variation of REM sleep and wake (Vetrivelan et al., 2016).

Cholinergic neurons also have been thought to play an important role in regulating EEG activity for both sleep and wake states. Cholinergic neurons fire in association with fast cortical rhythms during wake and REM sleep but much less during NREM sleep (Scammell, Arrigoni, & Lipton, 2017). This has led to the belief that activation of these neurons, specifically those in the PPT and laterodorsal tegmentum (LDT), suppress slow frequency EEG activity observed in NREM sleep, thus destabilizing the NREM state and promoting more active states (i.e. wake) (Scammell, Arrigoni, & Lipton, 2017). Activating cholinergic neurons in the PPT reduced EEG slow waves during NREM sleep (Saper & Fuller, 2017). Cholinergic neurons fire just before REM sleep or wake and promote the transition into these states. Optogenetic selective activation of cholinergic neurons in the PPT/LDT during NREM sleep increased the number of REM sleep episodes (increased the probability of REM) but did not change the length of the REM sleep episode, indicating that these neurons are important for REM sleep generation but not REM sleep maintenance (Van Dort et al., 2015).

The basal forebrain (BF), which also contains cholinergic neurons, plays a crucial role in cortical activation. Selective lesions in rats of cholinergic neuronal projections from the BF to the cortex caused a decrease in gamma EEG power (high frequency waves) across all sleep and wake states (Bernston, Shafi, & Sarter, 2002). Activation of these neurons is sufficient to suppress SWS and promotes wake and REM sleep (Yu et al., 2015). Inactivation of these neurons results in prolonged SWS and decreased probability of awakening of from this state but does not alter the duration of wake or REM sleep, or the probability of transitioning into these states (Yu et al., 2015). Similar findings were demonstrated by Chen et al. (2016) who also found

that activation of these neurons caused a decrease in EEG delta activity during NREM sleep and slightly increased wakefulness in mice, while inhibition of these neurons increased EEG delta power and slightly decreased wakefulness. Optogenetic selective stimulation of BF cholinergic neurons increases the transition between NREM sleep and wake (Zant et al., 2016). Overall, the primary role of cholinergic BF neurons appears to be the termination of SWS.

However, the BF also contains GABAergic and glutamatergic neurons that contribute to sleep and wake regulation, including interactions between neurons within the BF itself. Yang et al. (2014), found that the cholinergic neurons in the BF also excite neighboring GABAergic neurons containing parvalbumin (PV), a calcium binding protein. Zant et al. (2016) coupled optical stimulation of cholinergic BF neurons, which increases local acetylcholine levels, and reverse micro-dialysis of cholinergic receptor antagonists into the BF, and found that the wake promoting effect of stimulation of theses neurons requires both local release of acetylcholine and activation of non-cholinergic neurons (GABA/PV) projecting to the cortex.

The role of GABA in sleep and wake regulation is becoming increasingly favored. Although the neuromodulators mentioned above, monoamines and acetylcholine, may still play a modulatory role in regulation of neural sleep and wake states, more recent understandings of sleep-wake circuitry implicate fast neurotransmitters (e.g. GABA and glutamate) as the primary instigators of regulatory systems controlling sleep and wake states (Saper & Fuller, 2017). It is GABAergic neurons that appear to exert control of initiation, maintenance, and regulation of these states.

36% of GABAergic neurons in the BF have activity patterns that correlate with cortical gamma oscillations (active during wake), while other GABA neurons in this region fire fastest during sleep (Saper & Fuller, 2017). Stimulation of GABAergic and glutamatergic neurons in the

BF that influence other BF neurons and innervate the cortex were shown to promote arousal (Scammell, Arrigoni, & Lipton, 2017). These neurons indirectly promote activation of the cortex (wake state) by reducing the activity of inhibitory cortical-thalamic interneurons (Scammell, Arrigoni, & Lipton, 2017). Other GABAergic neurons in the BF are mostly active during NREM sleep, begin firing right before the NREM sleep state and fire maximally throughout NREM sleep (Scammell, Arrigoni, & Lipton, 2017).

GABA and glutamate in the PPT/LDT also fire in association with fast EEG activity. GABAergic neurons in the PPT/LDT mostly fire during wake, but some fire during REM rather than NREM (Scammell, Arrigoni, & Lipton, 2017). However, the role of GABA and glutamate transmission in REM sleep in this region is still unclear. Similarly, glutamatergic neurons projecting from the PB to the BF are active during wake and REM sleep. Inhibition of the transmission of these neurons through the deletion of the glutamate transporter (Vglut2), caused wake to be decreased and delta EEG power during NREM sleep to be increased (Scammell, Arrigoni, & Lipton, 2017). Activating these neurons resulted in profound wakefulness while inhibition produced an increase in REM sleep (Scammell, Arrigoni, & Lipton, 2017).

GABAergic neurons in the LH innervate the thalamic reticular nucleus (TRN). Activation of these neurons rapidly wakes mice from NREM, while inhibition increases slow waves and the length of NREM sleep episodes (Herrera et al., 2016). Herrera et al. (2016) showed that GABAergic neurons in the LH exert inhibitory control over TRN GABAergic neurons, and optogenetic activation of these neurons induced rapid arousal from NREM sleep, but not REM, while silencing LH signaling to the TRN increased the duration of NREM sleep and amplitude of delta oscillations. Other GABAergic neurons in the LH innervate and inhibit sleep promoting

regions, including the ventrolateral preoptic area (VLPO), and activation of these neurons promote wake while inhibition increased sleep (Venner et al., 2016).

In contrast, sleep is initiated by sleep active neurons in the anterior hypothalamus and rostral medulla which inhibit wake promoting systems (Weber, 2017). GABAergic and galanin producing neurons in the ventrolateral preoptic nucleus (VLPO) and median preoptic nucleus (MnPO) are essential for promoting NREM sleep states, and do so by innervating and inhibiting arousal promoting brain regions (e.g. cholinergic neurons in the BF, orexin neurons, the TMN, and the median dorsal raphe) (Scammell, Arrigoni, & Lipton, 2017). GABAergic and galanin neurons in the extended VLPO are active during REM sleep and innervate the mDRN, LC, and ventrolateral periaqueductal gray and lateral pontine tegmentum (vlPAG/LPT) to suppress wake during this sleep state (Scammell, Arrigoni, & Lipton, 2017).

GABAergic and glycinergic neurons in the parafacial zone (PZ) in the caudal brainstem express are thought to promote the NREM sleep state by inhibiting wake promoting neurons in the PB and neuronal projections to the BF (Scammell, Arrigoni, & Lipton, 2017). Activation of these neurons induces periods of NREM sleep with high slow wave EEG activity, while inhibition of these neurons decreases NREM sleep (Scammell, Arrigoni, & Lipton, 2017).

Although most cortical neurons are active during wake, a subset of GABAergic cortical interneurons that produce nitric oxide synthase (nNOS) are active during NREM sleep. These neurons are thought to respond to the homeostatic sleep drive and synchronize slow cortical rhythms through intercortical projections and release of GABA and nitric oxide (NO) (Scammell, Arrigoni, & Lipton, 2017).

Neural circuits in the pons are also critical for REM sleep generation and control, particularly glutamatergic neurons in the SLD, which are essential for generating muscle atonia

(descending projections) and EEG activity (ascending projections) during REM sleep (Scammell, Arrigoni, & Lipton, 2017). During REM sleep, these neurons inhibit REM suppressing neurons in the vlPAG/LPT, and are conversely inhibited by these same structures during wake (Scammell, Arrigoni, & Lipton, 2017). Cholinergic neurons promoting REM sleep and monoaminergic neurons suppressing this state (promoting wake) are now thought to play a modulatory role. During REM sleep, SLD neurons are may be activated by cholinergic neurons in the PPT/LDT, which help modulate projections from the medulla and MCH producing neurons to promote the REM sleep state, while monoamines and orexins help drive the brain towards a wake state (Scammell, Arrigoni, & Lipton, 2017).

SWTICHING BETWEEN SLEEP STAGES

The dynamic interactions between the neuron groups mentioned above coordinate to not only transition between sleep and wake, but also help transition multiple times between sleep states across the night. Krishnan et al. (2016) used biophysical models of the thalamocortical network to observe transitions between wake, NREM, and REM states within a sleep cycle to determine how different neuromodulators generate EEG rhythms. They found that transitional mechanisms are associated with the action of GABAergic and monoaminergic neural outputs. During NREM sleep, spindle power and slow oscillation power were negatively correlated in humans (positive correlation in animals), differences in which were explained by changing acetylcholine levels. Sleep spindles, which are generated by the thalamocortical interaction, have the highest prevalence during the NREM to REM sleep transition, while theta oscillations observed in the hippocampus have the highest prevalence during REM sleep (Weber, 2017).

However, the occurrence of spindles and slow oscillations do not explain what is driving sleep state transitions. As suggested by the presence of mutually inhibitory circuitry (e.g.

interaction between neurons of the SLD and vlPAG/LPT) regulating REM sleep mentioned above, the transition between NREM and REM sleep states is thought to be controlled by antagonistically coupled populations of REM sleep promoting and REM sleep suppressing neurons in the brainstem (Weber, 2017). One theory of switching between NREM and REM sleep involves a flip-flop switch driven by mutually inhibitory REM-OFF and REM-ON neuronal pathways in the mesopontine tegmentum. Each "side" of the switch contains GABAergic neurons that innervate and inhibit each other. Some REM-ON neurons project to the BF and regulate REM sleep EEG activity, while others project to the medulla and spinal cord to regulate muscle atonia during this state (Lu et al., 2006). The concept of a switch is further indicated by the presence of separate subpopulations of galanin-expressing GABAergic neurons in the dorsomedial hypothalamus (DMH) that have opposite effects on REM and NREM sleep. Galanin-expressing neurons in this region are either selectively activated (REM-ON) or selectively suppressed (REM-OFF) REM sleep (Chen et al., 2018). More specifically, the preoptic area contains REM-OFF neurons, which promote NREM sleep states and suppresses REM sleep, while raphe pallidus projecting neurons have the opposite effect (Chen et al., 2018).

Two models of mutually inhibitory interactions driving sleep transitions are presented here. The first model is the reciprocal interaction model in which the flip-flop switch is driven by two populations of neurons with antagonist firing patterns relative to the REM state. These populations consist of cholinergic neurons in the dorsal pons driving REM sleep (excitatory REM-ON neurons), and monoaminergic neurons in the LC and mDRN that inhibit REM sleep during wake and NREM sleep (REM-OFF neurons). In this model, the inhibition of REM-ON neurons slowly decays and then switches during sleep to self-inhibition of REM-OFF neurons (disinhibiting REM-ON neurons) (Weber, 2017).

This theory is considered in the context of homeostatic control of sleep, more generally referred to as sleep need. An increased need for sleep during wake is counteracted by prolonged sleep duration, particularly SWA during NREM sleep (Landolt et al., 2008). With respect to the homeostatic control of REM sleep, the timing of the next REM sleep episode is thought to depend on the duration of NREM sleep, not wake, since the last REM sleep episode, producing a consistent NREM-REM sleep cycle duration across the night (Weber, 2017). This puts forward the theory of the homeostatic pressure of REM sleep, in which the need for REM sleep drives the occurrence of REM sleep episodes. The duration of a REM sleep episode should be positively correlated with the length of the following NREM sleep episode because the longer a REM episode lasts, the more REM pressure is discharged, and it will therefore take longer to accumulate sufficient REM pressure to trigger another REM episode producing a longer NREM episode (Weber, 2017).

In contrast to the reciprocal interaction model in which REM pressure controls the timing of a REM sleep episode and state, the mutual inhibition model for the transition between NREM and REM sleep suggests that mutually inhibitory synaptic interactions in the brainstem, between REM-OFF and REM-ON neurons stabilizes sleep states. In this model, neurons are primarily GABAergic. REM-ON neurons, found in the midbrain, SLD in the pons, and in the medulla, project to REM-OFF neuronal regions including the vlPAG and mesencephalic reticular nucleus. Similarly, REM-OFF neurons in the vlPAG and DpMe project directly to areas populated with REM-ON neurons (including SLD) or indirectly via excitatory REM-ON neurons in the SLD. This creates a mutually inhibitory switch system for REM and NREM sleep states (Weber, 2017). The mutual inhibitory model is currently favored.

PHARMACOLOGICAL MANIPULATIOSN OF SLEEP

Understanding some of the potential mechanisms and neuromodulators involved in the regulation of sleep and wake states can help develop pharmacological methods of manipulating sleep. However, there are not many drugs that have been successfully used to drastically alter sleep states. Here I discuss various substances that affect sleep.

As mentioned above, serotonin and norepinephrine (from the DRN and LC) promote wake states. This is further supported by evidence demonstrating selective reuptake inhibitors, commonly given as antidepressants, decrease REM sleep. McCarthy et al. (2016) showed that antidepressants suppress REM sleep but are tolerated, and REM sleep homeostasis is unaffected. Acute treatment with paroxetine, citalopram, and imipramine (common antidepressants) inhibited REM sleep. However, only after imipramine, was REM deficits, prior to suppression of REM by antidepressants, recovered. SSRIs and dual uptake inhibitors not only cause a reduction in the overall amount of REM sleep and increase REM sleep latency, but more minor effects of these drugs include an increase in N1 sleep and increased waking during the night (Wilson, 2018). Similarly, monoamine oxidase inhibitors (MAOIs), such as phenelzine, also suppress REM sleep (Wilson, 2018).

In contrast to drugs that enhance monoamines, cholinergic agonists, such as carbachol, produce a state and behaviors similar to REM sleep after injection into the medical pontine tegmentum (Kubin, 2001). Carbachol has been frequently used to study and better understand mechanisms of REM sleep generation. Injections of carbachol in both rats and cats enhanced REM sleep, although a larger effect was observed in cats who also had no REM sleep latency after injection and longer REM sleep episodes (Kubin, 2001). Cholinergic neurons of the PPT/LDT may trigger or maintain REM sleep and may receive projections from the mPRG (Kubin, 2001). These neurons promote REM sleep activity and muscle atonia by activating SLD

neurons through cholinergic transmission, in which acetylcholine excites spinally projecting SLD neurons and increases input to these neurons (Weng et al., 2017). Injecting carbachol in the dorsomedial pons produces a REM like state including muscle atonia and cortical activation. The drug acts presynaptically by increasing glutamatergic excitatory postsynaptic currents (Weng et al., 2017). Carbachol was also found to directly excite neurons in the SLD by activating a sodium-calcium exchanger (Weng et al., 2017).

Drugs used to treat psychosis may affect a variety of receptors including inhibition of dopamine (D2), serotonin (5-HT1A and 5-HT2A), noradrenaline (alpha-1), histamine (H1), and cholinergic receptors. Drugs that antagonistically affect D2 and 5-HT2A decrease awakenings during sleep, prolonging the sleep period (Wilson, 2018). Typical and atypical antipsychotics decrease sleep onset latency and improve sleep maintenance in schizophrenia (Wilson, 2018). Drugs used to treat acute anxiety, such as benzodiazepine receptor agonists, modulate GABA(A) receptors to increase the effects of GABAergic transmission, which causes increases in sleep onset latency and awakening during sleep (Wilson, 2018).

In contrast, Wisden, Yu, & Franks (2017) reviewed sedative (suppress CNS and promote sleepiness) or hypnotic (induce sleep) medications for treating insomnia that work by enhancing inhibitory GABAergic transmission, as the diverse set of GABA(A) receptors may make good drug targets for enhancing/promoting sleep. Sleep promoting/active GABAergic neurons inhibit wake promoting neurons, and therefore enhancing the transmission of these neurons, using positive allosteric modulators (PAMs) of GABA(A) receptors enhances inhibition and promotes sleep (Wisden, Yu, & Franks, 2017). Most effective hypnotics, sedatives and anesthetics enhance GABA's effect at GABA(A) receptors (working with PAMs), and included a wide range of compounds with varied structures including some amobarvital (a barbiturate), zaleplon (a

pyrazolopyrimidine) and zopiclone (a cyclopyrrolone), benzodiazepines (e.g. diazepam, flurazepam, quazepam, temazepam and triazolam), and benzodiazepine agonists, such as zolpidem (an imidazopyridine) (Wisden, Yu, & Franks, 2017). Zolpidem (Ambien) induces sleep at the α2βγ2-type GABAA receptors, while other drugs act at GABA(A) receptors containing α2 and/or α3 subunits expressed in hypothalamic and brain stem areas (Wisden, Yu, & Franks, 2017). Mednick et al. (2013) used zolpidem (Ambien), a short acting GABA-A agonist, to manipulate daytime naps and found increased spindle density and decreased REM sleep. Those with naps with more spindles had better verbal memory but worse perceptual learning. Furthermore, Rasch et al. (2009) found that REM suppression by selective serotonin and norepinephrine inhibitors after training did not affect/impair consolidation of skills or word pairs in healthy men.

WHY WE NEED SLEEP

Learning and Memory Consolidation

Sleep may play a role in a variety of processes including, synaptic plasticity and memory function, emotional regulation, metabolic functions and energy balance, removal of metabolic waste, or cellular maintenance (Vyazovskiy, 2015). Many of these functions can be seen to have a recuperative or restorative purpose. Sleep has been shown to increase as a function of time spent awake and is thought to provide necessary restoration with respect to molecular, cellular, or network changes that occur during wake (Vyazovskiy, 2015). As a result, there has been further research into the homeostatic regulation of sleep by examining how sleep processes compensate for a sleep deficits.

The synaptic homeostasis hypothesis suggests that sleep is the price our brain plays for plasticity (Tononi & Cirelli, 2013). During wake periods, connections within the brain are

strengthened in response to learning, which has costs at the cellular and systems level due to a depletion in energy supplies. Spontaneous activity occurring during sleep renormalizes net synaptic strength and restores cellular homeostasis through activity-dependent down selection of synapses (Tononi & Cirelli, 2013). Neurons perform comprehensive sampling of synapses that result in competitive down selection, leaving some synapses less effective.

This role is further reflected in the shift towards the active system consolidation theory of sleep in which memory information replay is first selectively potentiated then globally downscaled (Lewis & Durrant, 2011). In general, it is thought that synapses are first selectively tagged for potentiation during NREM sleep, through replay in the context of overall downscaling, and then potentiated during subsequent REM sleep (Rasch & Born, 2013).

Sleep's role in memory consolidation has therefore become a huge avenue of research in an attempt to reveal whether sleep recovers behavioral performance or cognitive function. The first step is to try to understand the features of sleep in the context of memory consolidation. Memory consolidation refers to the transformation of internal and neurobiological representations of experiences through synaptic and cellular modifications of brain circuits that occurs through reoccurring reactivations during wake and sleep, resulting in the distribution and integration of new information (Dudai & Born, 2015). Specific patterns of neuromodulatory activity and electric field potential oscillations that differ throughout sleep states promote the necessary changes in memory representations to facilitate the consolidation of new memories (Rasch & Born, 2013). Both REM and NREM sleep states contribute to the consolidation and integration of new material. In general, consolidation is thought to begin during SWS, which involves the reactivation of recently encoded neuronal representations that are transformed for

long-term integration, and these transformed memories are then stabilized by REM sleep (Rasch & Born, 2013).

SWS during NREM sleep facilitates hippocampal-neocortical dialogue and information transfer. Consolidation of hippocampal memories relies on this dialogue, a crucial feature of which is the neuronal reactivation of new memories in the hippocampus during SWS that stimulates the redistribution of memory representations to neocortical networks (Marshall & Born, 2007). Repeated reactivation of these representations during SWS transforms episodic memory representations into long-term memories, redistributes them toward extra-hippocampal networks, and qualitatively changes them to decontextualize schema-like representations (Inostroza & Born, 2013).

A potential basic mechanism of hippocampal-dependent memories to neocortical sites involves the regulation of communication by NREM sleep EEG activity characterized by slow oscillations during SWS (<1 Hz) from the neocortex, spindles from the thalamus (12-15 Hz) that spread to cortical and hippocampal networks, and ripples in hippocampal networks at minimum cholinergic activity (Inostroza & Born, 2013). Sharp wave ripples and thalamic spindles mediate bottom-up transfer of reactivated memories in the hippocampus to extrahippocampal regions, while slow neocortical oscillations exert top-down control to synchronize hippocampal reactivations to their excitable phase (Inostroza & Born, 2013). Reactivated neuronal information reaches the neocortex during excitable up state of slow oscillation (Dudai & Born, 2015). Mak-McCully et al., (2016) examined the sequence of spindles in the thalamus and down-states of cortical activity during NREM sleep and found that cortical down-states lead to thalamic down-states resulting in the hyperpolarization of thalamic cells thus triggering spindles.

SWS downscaling of synaptic connections that were potentiated during prior wakefulness, for homeostatic purposes, is parallel with SWS support of the consolidation of hippocampal-dependent episodic memory consolidation which is linked to increases in synaptic connectivity (Huber & Born, 2014). The ability to form episodic memories during SWS is established during infancy and increases throughout childhood until puberty resulting in an imbalance in the regulation of synaptic connectivity during sleep favoring enhanced synaptic potentiation instead of downscaling (Huber & Born, 2014). During development there is mutual interaction between SWS slow oscillations and hippocampus-dependent memory consolidation resulting in enhanced system consolidation during sleep which promotes developmental increase in neocortical synaptic connectivity (Huber & Born, 2014). Therefore, sleep dependent system consolidation during development plays an essential role in establishing entire memory systems (Huber & Born, 2014).

Neural activity during REM sleep also plays an active role in memory consolidation. However, its contribution is still unclear, and findings are mixed. Recently, REM sleep seems to benefit specific memories that are not dependent on cortico-hippocampal circuitry, and is assumed to have a stabilization effect mediated via local synaptic consolidation (Dudai & Born, 2015) rather than continuing consolidation started in SWS. Local increases in plasticity during REM may be related to immediate gene activity at high cholinergic and theta activity to favor synaptic consolidation in the cortex (Rasch & Born, 2013). REM may also contribute to the reorganization of representations during system consolidation by disintegrating some parts of the hippocampal representations to loosen associative bonds (Dudai & Born, 2015). A study by Grosmark et al. (2012) examined another potential role of REM sleep and its role in reorganizing hippocampal excitability. They looked at firing rates of hippocampal CA1 neurons and found

that within NREM sleep episodes, firing rates gradually increased accompanied by a decrease in ripples, while firing rates rapidly decreased during a REM sleep episode. This sawtooth pattern resulted in overall downscaling of firing rates over the course of sleep in which rates decreased across the course of subsequent NREM while neuronal spiking of ripple episodes increased resulting in a net increase of neuronal synchrony. This decrease in firing rates and increase in synchrony was positively correlated with the power of theta activity during REM, suggesting REM regulates discharge firing rates and synchronization in the hippocampus (Grosmark et al., 2012).

CHARACTERIZING SLEEP ACROSS THE LIFESPAN

Typically Developing and Healthy Populations

Age accounts for most changes in sleep architecture across the lifespan. Four major age-related trends have been consistently demonstrated by polysomnographic studies: total sleep time (TST), sleep efficiency and SWS decrease with age, while wake after sleep onset (WASO) increases with age (Ohayon et al., 2004). However, findings on how the percentage of each stage of sleep changes with age remain mixed. Reviewing prominent polysomnographic studies, Ohayon et al. (2004) averaged various sleep values across multiple studies and found that TST, sleep efficiency, percentage of SWS, and percentage of REM sleep each showed significant decreases with age, while sleep latency, percentage of N1 sleep, percentage of N2 sleep, and WASO increase with age. In children and adolescents, percent SWS and REM sleep latency are negatively correlated with age, while percent N2 sleep increases with age. In adults, a large effect size was found for TST, sleep efficiency, percent SWS, and WASO when compared to age. Figure 1, taken from, Ohayon et al. (2004), shows relative changes in the amount of sleep stages, as well as changes in WASO and sleep latency across the lifespan.

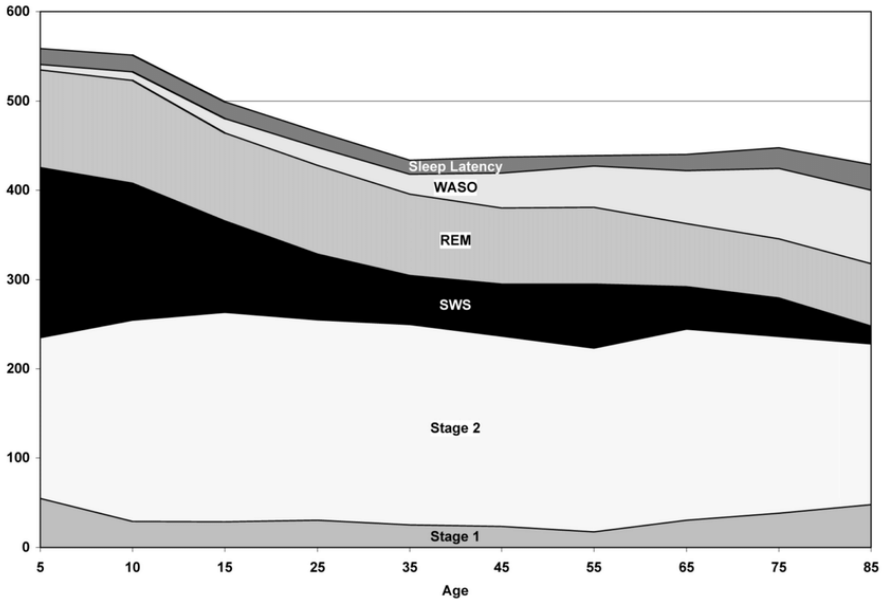

Figure 1: Taken from Ohayon et al. (2004). Changes in sleep architecture as a function of increasing age.

Coble et al. (1984) looked at overnight EEG recordings in 87 normal, healthy children ranging in age from 6-15 years. Total sleep demonstrated a gradual linear decline with age, declining from approximately 9 hours of sleep in 6 and 7 year-olds, to 7 hours of sleep per night in 14 and 15 year-olds. The percent N2 sleep was observed to increase linearly across each age group as the percent delta sleep (SWS) declined, which was accounted for by a significant decrease in N4 sleep. No significant changes in REM sleep were found as a function of age or gender. However, REM sleep latency and the number of REM sleep episodes showed a nonsignificant decrease with increasing age, but the percent of REM sleep across the night remained relatively constant. When looking at changes in sleep cycles during the night, REM sleep episode length appeared to increase across the night from cycle to cycle.

Montgomery Downs et al. (2005) report normative values of various sleep variables recreated in table 1. These values include findings from their own overnight polysomnographic recordings of 542 typical school aged children.

Table 1: Polysomnographic Values and Cyclicity of Sleep for Pediatric Normative Literature (Montgomery-Downs et al., 2005)

STUDY	N	Age Range (years)	TST (hours)	Sleep Efficiency	Sleep Latency (min)	REM Latency (min)	%WAKE	%N1	%N2	%N3	%N4	%SWS	%REM	# Sleep Cycles
Montgomergy-Downs et al. (2005)	153	3.2-5.9	7.9 (.7)	90 (7.0)	24.1 (25.6)	87.8 (41.2)	9.4 (7.3)	5.2 (2.0)	36.0 (6.6)	5.9 (2.3)	20.4 (4.7)	16.9 (4.1)	21.4 (4.9)	6

Montgomergy-Downs et al. (2005)	388	6.0-8.6	7.9 (.7)	89.3 (7.5)	23.0 (25.3)	132.0 (57.7)	8.1 (7.1)	5.0 (2.9)	41.8 (8.3)	4.7 (2.0)	17.9 (5.7)	14.4 (4.2)	19.6 (4.3)	5
Coble et al. (1984)	9	6.0-7.0	9.1 (.9)	94.5 (2.9)	18.4 (9.0)	142.3 (38.7)	8.2 (9.0)	7.7 (3.4)	47.2 (5.4)	6.3 (2.1)	17.6 (3.0)	24.0 (3.9)	20.7 (3.9)	5
Uliel et al. (2004)	70	1.0-15.0	6.5 (1.2)	90.8 (6.5)	---	---	---	4.1 (4.1)	48.9 (9.7)	---	---	25.2 (9.1)	17.4 (5.7)	---
Wong et al. (2004)	11	3.0-8.0	7.0 (.8)	89 (7)	---	---	---	5 (3)	49 (7)	---	---	27 (8)	20 (4)	6
Stores et al. (1998)	13	5.0-7.0	9.8 (1.4)	98.1 (2.2)	16.9 (9.1)	116.0 (53.7)	5.1 (10.6)	4.2 (4)	19.2 (6.8	---	---	58.5 (10.5)	18.2 (4.3	---

Ohayon et al. (2017) further presents sleep variable values that indicate good sleep qualities across nine age points: newborn (0-3 months), infant (4-11 months), toddler (1-2 years), preschooler (3-5 years), school aged (6- 13 years), teenager (14-17 years), young adult (18-25 years), adult (26-64 years), and older adult (≥65 years). Across all ages, good sleep quality can be indicated by sleep latency less than or equal to 15 minutes, one or less awakenings during the night, less than or equal to 20 minutes of wake after sleep onset (WASO), sleep efficiency greater than or equal to 85% (sleep efficiency less than 74% is not sufficient for good sleep, less than 64% in teens). In contrast, poor sleep quality is suggested by sleep latencies between 45-60 minutes (greater than 60 minutes in elderly), four or more awakenings during the night (3 or more in teens), greater than or equal to 51 minutes of WASO (greater/equal to 41 minutes for school aged children, young adults, and adults), or sleep efficiencies less than 74% (less than 64% in young adults) across most age groups.

In newborns, REM sleep activity greater than or equal to 41% indicates good sleep quality while less 20% suggests poor sleep. In adults, the percentage of REM sleep should be between 21-30% to indicate good sleep, while REM sleep greater than 40% indicates poor sleep in young adults, adults (toddlers, preschoolers, teens less than 10% REM not good). For school aged children, teens, young adults, and adults, if the percentage of N1 sleep is less than or equal to 5%, then good sleep quality is indicated, while N1 sleep greater than 20% is not good for toddlers pre-schoolers, school-aged children, teens, young adults, and adults (greater than 26%

for older adults). Across all groups, N2 sleep greater than 81% does not indicate good sleep. School aged children and teens need 20-25% of N3 sleep for good quality (adults need 16-20%), while infants toddlers and preschoolers, and school aged children with less than 10% (teens, young adults, adults less than 5%) of N3 sleep may not have good sleep quality. Lastly, for school aged children and adults, taking no naps (for teens one or less naps) during the day may indicate good sleep quality, while four or more naps suggests poor sleep quality across all ages (even 2 or more naps in school aged children and 3 or more in teens and young adults may indicate poor sleep). Nap duration for teens should be 20 minutes or less to suggest good sleep quality, while naps taken by young adults, adults, and older adults, longer than 100 minutes suggest poor sleep quality (teens longer than 120 minutes) doesn't indicate good quality (Ohayon et al., 2017).

Feinberg & Floyd (1979) was one of the first and only papers to look at and describe sleep cycles and cycle duration across the night in an attempt to better explain systematic trends across the night across the lifespan. Nighttime sleep was recorded in 125 typical subjects ranging in age from 4-96 years for 3-5 consecutive nights. Participants were divided into groups for analysis based on age: below 16 years (children), 16-65 years (adults), older than 65 years (elderly). Data from 105 of the participants was analyzed to characterize trends across the night for sleep periods and sleep cycles. A NREM sleep cycle was measured from the onset of stage 2 of a NREM sleep episode to the onset of stage 2 of the following NREM sleep episode. This period also encompassed a REM sleep period. Results of participants who completed four or five complete NREM sleep cycles for each age group was adapted for this paper and is recreated in tables 2 and 3. Adults and elderly groups demonstrated a curvilinear umbrella shaped trend across the night in which the first and fourth NREM sleep cycle durations were lower than the

second and third NREM sleep cycles which had similar durations. This pattern was observed to differ in children in which the first NREM sleep cycle is much longer in duration than any other sleep cycle. This is thought to reflect the lengthy first NREM sleep period seen in younger individuals. In all groups, if a fifth NREM sleep cycle was present, it was much shorter in duration than any of the four previous sleep cycles. This may be due to the continued decline in the NREM sleep period late in the night while the duration of the REM sleep period remains mostly constant.

Table 2: Mean Cycle Durations across the Night (wake excluded) (Feinberg & Floyd 1979)

Mean Ages (Ranges in Parentheses)		N	Mean NREM Sleep Cycle Duration (min) (SDs in Parentheses)				
			Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
Children	10.6 (4.4-15.7)	36	120.2 (37.4)	96.8 (11.7)	98.0 (15.7)	90.3 (12.9	---
	9.9 (4.4-15.7)	27	102.6 (39.1)	89.7 (14.7)	94.5 (15.5)	90.6(12.3)	81.9 (14.2)
Adults	27.7 (16.2-64.8)	52	78.4 (20.3)	92.5 (15.2)	96.2 (16.3)	87.0 (17.4)	---
	26.4 (18.5-64.8)	22	69.8 (17.2)	81.7 (13.8)	86.5 (18.7)	85.6 (17.1)	76.6 (15.2)
Elderly	77.3 (67.4-95.8)	14	71.2 (17.7)	84.4 (20.1)	97.6 (21.2)	85.7 (16.9)	---
	76.1 (67.4-95.8)	8	62.3 (19.9)	81.7 (7.6)	85.0 (14.7)	78.6 (14.6)	63.5 (9.1)

Table 3: Mean Cycle Durations across the Night (wake included) (Feinberg & Floyd 1979)

Mean Ages (Ranges in Parentheses)		N	Mean NREM Sleep Cycle Duration (min) (SDs in Parentheses)				
			Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
Children	10.6 (4.4-15.7)	36	120.8 (37.6)	98.1 (13.0)	99.2 (16.0)	94.3 (16.4)	---
	9.9 (4.4-15.7)	27	103.6 (40.0)	89.8 (14.7)	95.6 (16.6)	93.1 (13.6)	83.0 (14.9)
Adults	27.7 (16.2-64.8)	52	80.3 (20.5)	94.6 (15.8)	98.4 (16.3)	89.6 (17.3)	---
	26.4 (18.5-64.8)	22	70.7 (17.3)	83.7 (14.9)	87.1 (18.8)	87.3 (16.7)	80.2 (15.9)
Elderly	77.3 (67.4-95.8)	14	75.5 (21.6)	96.9 (24.5)	112.4 (26.7)	101.3 (23.1)	---
	76.1 (67.4-95.8)	8	69.5 (29.9)	87.9 (11.4)	95.1 (27.4)	85.5 (18.0)	79.4 (16.3)

Recently, a similar study longitudinally analyzing sleep cycles in young children was conducted by Lopp et al. (2017). The sleep of 8 healthy children were recorded at 3 time points (2 years, 3 years, and 5 years). No developmental change in TST, total NREM sleep, or total REM sleep was observed across each age time point. However, when examining the mean duration of a NREM-REM sleep cycle, they observed a nonsignificant trend for increasing cycle duration with age. Mean NREM sleep episode duration also increased with age and a significant difference was found between 2 years and 5 years. Mean REM sleep episode duration showed no significant change across age time points. Most participants (75%) completed a 7th NREM-REM

sleep cycle at each age period, while only a subset completed an 8th or 9th cycle (1 participant at 1 age point completed a 10th cycle, no 9th cycles were completed at 5 years). When looking at the number of cycles, a nonsignificant trend was found indicating that the number of sleep cycles is decreased at 5 years compared to 2 and 3 years. A 9th NREM sleep episode is also less likely to occur at 5 years compared to 2 and 3 years. They also looked at the percentage of each cycle spent in NREM sleep as a function of cycle number across the night and found that the percentage of NREM sleep in a sleep cycle decreased from the first to the second cycle and from cycles 2 through 7 there was a nonsignificant trend indicating a decrease in percentage of NREM sleep from cycle to cycle (significant between 3rd and 4th cycle at 2 years; 5th to 6th cycle at 2 years; 2nd to 3rd cycle at 5 years).

Sleep in Special Populations

Alzheimer's Disease

Advancement in age is accompanied by decreases in sleep, particularly a decline in NREM SWS sleep, as well as increasing time spent awake at night and fragmented sleep (Mander et al., 2016). While changes in sleep are part of normal aging, the presence of dementia further disrupts sleep patterns (Peter-Derex et al., 2015), and individuals with mild cognitive impairment or Alzheimer's disease (AD) have exaggerated changes in sleep structure and often the onset, nature and severity of impairments are accelerated (Mander et al., 2016). Sleep disorders are highly prevalent in individuals with AD. Symptoms usually present during severe cognitive decline but may be observed at earlier stages of the disease, and while the disturbances are similar to those reported by normal aging adults, they are more severe (Peter-Derex et al., 2015). Patients with AD have more disrupted/fragmented nighttime sleep, increases in WASO (as the disease progresses), increases in sleep latency, and decreases in total sleep time, as well

as potential inversions of the sleep-wake cycle (Peter-Derex et al., 2015). However, REM sleep in those with AD appears to be reduced while it typically remains constant during normal aging (related to dysfunction of Ach neurotransmission) (Peter-Derex et al., 2015).

Low amplitude slow wave activity is common in both sleep and wake states in patients with AD, which makes it difficult to differentiate NREM stages. However, most studies have determined a decrease in SWS and alterations in spindles and K complexes in these patients. Changes in sleep patterns may be due to degeneration of neural pathways regulating sleep and wake (Peter-Derex et al., 2015). Furthermore, amyloid β (Aβ), is believed to play a significant role in the development of sleep disturbances in the preclinical and clinical phases of AD. There is an established relationship between sleep and Aβ, in which disturbed sleep and increased wake leads to increased Aβ production and decreased Aβ clearance (Holth, Patel, & Holtzman, 2016). Aβ levels change with the sleep-wake cycle, rising during wakeful and decreasing during sleep (Holth, Patel, & Holtzman, 2016). However, this This fluctuation is lost with Aβ deposition, likely due to its sequestration into amyloid plaques (Holth, Patel, & Holtzman, 2016).

Parkinson's Disease

The prevalence of sleep disturbances in Parkinson's disease (PD) range approximately from 25-98% (Roychowdhury & Forsyth, 2012), and sleep problems are twice as common in patients with PD compared to typical controls. Similar to AD, sleep disturbances can arise during the early stages of PD and may even predate the onset of the disease. Some common sleep problems in patients with PD include restless legs syndrome (RLS), excessive daytime sleepiness (EDS), and REM sleep behavior disorder (RBD) (Roychowdhury & Forsyth, 2012). Individuals with PD showed reduced TST and sleep efficiency, sleep arousals and fragmentation, and circadian variations (Roychowdhury, & Forsyth, 2012). Sleep disturbances in PD may be associated with

specific cognitive difficulties, such as working memory and working memory consolidation, rather than global patterns of cognitive dysfunction (Gunn et al., 2014).

Down Syndrome

Sleep disturbances are highly prevalent in populations with neurodevelopmental disabilities (NDD), contributing to neurological, medical and psychiatric comorbidities, along with learning and behavioral problems (Angriman et al., 2015). Primary sleep complaints in populations with NDD include difficulty settling at night, nigh awakenings, and fragmented sleep (Angriman et al., 2015). Many studies have demonstrated that children with Down syndrome (DS) have sleep problems. The most common sleep disturbance is obstructive sleep apnea (OSA). The frequency of respiratory/sleep apneas as a co-occuring medical condition within DS was found to be 77% (Capone et al., 2018). Overall, 31-54% of children with Down syndrome (DS) have reported sleep problems which include insomnia, obstructive sleep apnea (OSA), and daytime sleepiness (Esbensen & Schwichtenberg, 2016). Furthermore, Lukowski & Milojevich (2017) found that children with DS went to bed at the same time each night less frequently when compared to typically developing children, often did not fall asleep within 20 minutes after going to bed, were more likely to snore loudly during sleep, obtained less sleep at night, and were more likely to appear tired and fall asleep during daytime activities. Actigraphy and PSG measurements of sleep in children with DS showed more fractured sleep, longer time spent in bed, lower sleep efficiency, less time in REM, and more movement compared to typically-developing children (Esbensen & Schwichtenberg, 2016). Children with DS demonstrated a reduction in percent REM sleep and a decreased R index that positively correlated with low IQ (Diomedi et al., 1999). Sleep disordered breathing (in children with DS),

was also found to be linked with poorer performance on tests of cognitive flexibility and lower verbal IQ (Esbensen & Schwichtenberg, 2016).

Looking specifically at children with DS with OSA, Levanon, Tarasiuk, and Tal (1999) compared sleep of 27 children with DS with OSA to 13 typical children with primary snoring. They found that the respiratory disturbance index was significantly higher and sleep was significantly fragmented in children with DS. However, sleep fragmentation, manifested by arousals and awakenings, were only partially related to OSA. Children with DS also had a higher number of awakenings, higher percentage of jerks, and a greater number of apnea associated arousals. The median length of episodes of stage 2 NREM sleep was 27% shorter in DS, and the number of shifts from deeper to lighter stages of NREM sleep was 30% greater in DS. Analysis of sleep architecture in children with DS was further analyzed by Nisbet et al. (2015) using polysomnography. They performed a retrospective study to examine the sleep characteristics of 130 children with DS (aged 0-17.8 years) compared to 130 matched controls. They found that children with DS had lower sleep efficiency and higher percentage of SWS at ages 2-17.9 years compared to the controls. Children with DS over 2 years of age also had a reduction in REM sleep and increased SWS (independent of OSA).

SLEEP ENHANCES LEARNING AND MEMORY IN CHILDREN AND ADULTS

The amount of either REM or NREM sleep may also have important implications for learning and memory. While the exact mechanisms underlying memory consolidation during sleep are not completely understood, sleep has been shown to be beneficial for learning and memory, particularly during development. Backhaus et al. (2006) looked specifically at the importance of sleep to enhance declarative memory consolidation in children. Memory of learned word pairs was examined in 27 children (aged between 9 and 12 years) in either a Sleep-

Wake condition, in which word pairs were learned in the evening and delayed recall was tested the next morning and following evening after a period of daytime waking, or a Wake-Sleep condition, in which word pairs were learned in the morning and delayed recall was tested the same evening after a daytime wake period and the following morning after nighttime sleep. Retention was significantly increased in both groups only after an interval of sleep either immediately after learning (Sleep-Wake) or one that followed a daytime wake period (Wake-Sleep). This supports the active role of sleep in declarative memory consolidation in children. However, it is not only nighttime sleep that has been shown to be beneficial in children. A study by Kurdziel, Duclos, & Spencer (2013) provides evidence that classroom naps support learning in preschool children by enhancing memories acquired prior to sleep. They measured performance on a visuospatial task over a nap and equivalent interval of wake in 40 pre-school children (within-subject). Delayed recall measurements showed significantly higher performance in following a nap. The negative effects of nap deprivation on memory consolidation could not be recovered during subsequent nighttime sleep.

Due to sleep's role in learning and memory, persistent sleep problems, resulting in sleep deficits, may disrupt cognitive development and contribute to cognitive dysfunction. As mentioned above, individuals with DS have significant sleep deficits, and these deficits impact learning and memory. Ashworth et al. (2015), examined the effects of sleep on declarative memory consolidation in children with neurodevelopmental disorders. School-aged typically developing children, and children with DS or Williams syndrome (WS) were tested on a novel "animal names" task to examine the effects of sleep-dependent learning. After a learning phase, participants were tested on the animal names and then re-tested after wake and sleep intervals (after 12 and 24 hours). Half of the participants were trained in the morning and re-tested after a

wake interval then again after a sleep interval (Wake-Sleep condition). The other half were trained in the evening then re-tested after a sleep interval and then a wake interval (Sleep- Wake condition). The typically developing group in both conditions showed significant improvement (remembered more words) following the sleep period, but no significant change in score was found after the wake interval. The DS group showed no significant improvement after sleep or wake periods, however, there was a non-significant trend of improvement for those in the Wake-Sleep condition after the sleep period, and a non-significant decline in performance for the Sleep-Wake condition across the intervals. The WS group showed significant improvement following the first retention period, either after sleep for the Sleep-Wake condition or after wake for Wake-Sleep condition.

While many sleep studies focus on the role and importance of SWS for learning and memory in typical adults and children, a recent study by Spano et al. (2018), highlights the differential role of REM sleep during a nap in typically developing children and children with DS. 25 children with DS and 24 typically developing controls were tested on a novel object-label pairing task in three within-subject conditions: five-minute delay, after a nap (sleep condition) or after wake. Polysomnography recordings were collected during the sleep condition. Naps were found to benefit performance in typically developing children. Memory retention of the object label was also positively correlated with the percent time spent in REM sleep during the nap in typically developing children. However, children with DS retained less when tested after a nap and had a better performance after the wake interval. Furthermore, children with DS with reduced REM sleep demonstrated impaired learning only after a nap. These findings suggest that naps may not be beneficial for learning in all populations and that REM sleep may play an active role in verbal learning.

Further research is needed to assess the role of REM sleep in memory consolidation and learning, particularly in special populations that have sleep problems and REM deficits. In adults with DS, the risk for sleep problems was increased by the risk of dementia (Esbensen & Schwichtenberg, 2016). Older individuals with DS and decreased alpha EEG background have been shown to have dementia, fewer visuospatial skills, decreased attention span, larger third ventricles, and global decrease in cerebral glucose utilization in comparison to aged matched individuals with DS with normal alpha activity (Devinsky et al., 1990).

CHARACTERIZING SLEEP CYCLES IN CHILDREN AND ADOLESCENTS WITH DS

Methods

For this paper, ambulatory polysomnography recordings and cognitive measurements of a community-based sample of 43 children and young adults (mean age 11.9 years; range 7.0-22.6 years) with DS were used to further characterize sleep in this population. The IQ of participants was calculated from the Kaufman Brief Intelligence Test-2. Data was taken from recordings conducted for Breslin et al., (2014), and further scored for NREM-REM sleep cycle duration and characteristics. Participants were divided into five separate age groups to analyze changes in sleep architecture across development.

EEG activity was recorded in 30 second epochs and sleep stages were scored by a resident polysomnographic technologist. NREM-REM sleep cycles were scored based on criteria presented in Lopp et al. (2017) and Feinberg & Floyd (1979). A NREM-REM sleep cycle was defined to include 1 NREM sleep episode and 1 REM sleep episode. A NREM-REM sleep cycle was considered complete only if both a NREM and REM sleep episode were present. A cycle began with the onset of NREM sleep (the first cycle was measured at the onset of N2 sleep) and

ended with the last epoch of the REM sleep episode. If wake occurred between the end of a REM sleep episode and the start of the next NREM episode, it was included in the next sleep cycle. Wake periods of less than 30 minutes were included in the sleep cycle.

Results

Total Sleep Across the Night

Total sleep times of each stage of sleep are summarized for reference in table 4. Total durations of each state and stage of sleep were averaged across all participants and within each age group. The mean total wake time across all participants was 102 minutes, which is much higher than the typical amount of wake time during sleep. Mean wake durations during sleep across the first four age groups appear consistent, while a sharp increase in wake time was seen for the oldest group. TST appears to decrease with age from 8-9 years, to 10-11 years, to 12-13 years, but there was an unexpected increase in duration for the 14-15 year-olds, and then a large decrease in TST for participants older than 16 years. Similarly, total NREM sleep begins to decrease after 8-9 years, with a large decrease in duration after 16 years. While total N2 sleep follows this same trend, it is offset by an opposite trend in total N1 sleep which appears to increase slightly between the age ranges highlighted. Therefore, the decrease in total NREM sleep is most likely due to a general decrease in total SWS (total N3 remains relatively constant while total N4 sleep decreases with age). Total REM sleep remained relatively constant across all age groups.

Table 4: Total Sleep Time Across the Night

Mean Age, years (Range)	N	TST (min)	Total NREM (min)	Total REM (min)	Total N1 (min)	Total N2 (min)	Total N3 (min)	Total N4 (min)	Total SWS (min)	Total Wake (min)
11.9 (7.0-22.6)	43	484.6	388.7	96.2	43.2	234.2	26.7	84.6	111.3	102
7.5 (7.0-7.9)	6	512.33	417.08	95.42	40.33	249.25	19	108.5	127.42	31.83

8.9 (8-9.9)	11	516	426.36	89.64	31.55	243.68	24.86	126.27	151.14	35.5
11.1 (10.4-11.6)	7	498.6	402.7	95.9	38.5	258.6	33.2	72.4	105.7	41.5
12.7 (12.1-13.4)	7	468.93	360.64	109.7	41.29	229.29	27.64	62.42	90.07	39
15.0 (14.8-15.7)	6	495.1	383.2	111.9	44	240.2	30.4	68.6	99	43.8
18.1 (16.3-22.6)	7	405.71	323.07	82.65	70	182.57	26	44.5	70.5	90.43

Table 4: Total sleep time, total NREM and REM sleep, and total time spent in each stage of sleep across the night for children with DS separated by age. Data supplied by Breslin et al. (2014).

Table 5 summarizes sleep efficiency, sleep latencies, and sleep stage percentages across the night. Sleep efficiency remains relatively constant across age groups with a larger decrease seen in children older than 16 years. Sleep latency appears to increase slightly with increasing age. REM latency decreases across the first four age groups before increasing at 14-15 years, and then decreasing slightly again at >16 years. Percentages of each sleep stage follows similar trends as sleep stage duration observed in table 4. %N1 increases slightly with age while %N2 remains relatively constant across each age group. %SWS decreases slightly with age, while %REM sleep increases slightly with age.

The values in table 5 were roughly compared to the normative sleep values of typically developing children in table 1. Participants with DS aged 7-9 years (7 years and 8-9 years, table 5) were compared to typical children aged 6-8 years Montgomery-Downs et al., 2005). Total sleep time for children ages 7-9 appears greater by approximately 40 minutes. For this age group, %N1 (slightly), %N2 (a lot), and %N4 sleep appear to be increased in those with DS, while time spent in %N3 sleep is relatively the same (slightly decreased in 7-year-olds). There is a slight decrease in the amount of %REM sleep and an increase in %SWS for those with DS. Time spent awake during the night also appears to be similar between those with DS and typical children. However, the most significant difference observed between those with DS and typical children is a large increase in REM sleep latency in children with DS.

Table 5: Sleep Efficiency, Latencies, and Stage Percentages (Breslin et al. 2014)										
Mean Age, years (Range)	N	Sleep Efficiency	Sleep Latency (min)	REM Latency	%N1	%N2	%N3	%N4	%SWS	%REM
11.9 (7.0-22.6)	43	85.3	34.4	187.7 9 (n=42)	9.2	48.2	6	17	23	19.6
7.5 (7.0-7.9)	6	89.6	26.92	224.92	7.82	48.05	3.77	23.22	26.92	17.25
8.9 (8-9.9)	11	88.45	24.32	209.18	6.19	47.36	4.75	24.45	29.21	17.25
11.1 (10.4-11.6)	7	86.2	33.9	191.8 (n=6)	8.2	52.3	7.2	14	21.3	18.7
12.7 (12.1-13.4)	7	84.94	44.43	132	8.87	48.4	6.74	12.66	19.4	23.59
15.0 (14.8-15.7)	6	85.14	40.9	207.7	9.08	48.66	6.52	13.42	19.84	22.36
18.1 (16.3-22.6)	7	76.23	42.36	159.86	16.79	45.2	7.59	10.09	17.67	20.34

Table 5: Summary of sleep efficiency, sleep latencies, and percentage spent in each sleep stage across the night for each age group of children with DS. Data supplied by Breslin et al. (2014).

NREM-REM Sleep Cycles

Individuals with DS appear to follow similar sleep cycle trends as demonstrated by typically developing children in the studies mentioned above. The mean number of NREM-REM sleep cycles per night, averaged across all individuals with at least 2 sleep cycles was 4.02 cycles. The mean duration of a NREM sleep episode for all participants, including instances of wake, was 98.02 minutes (88.15 minutes excluding wake). The mean duration of a REM sleep episode was found to be 26.16 minutes (25.16 minutes excluding wake). The mean sleep cycle number and episode durations for each separate age group are summarized in table 6.

Table 6: Mean Sleep Cycle Number, Duration, and Episode Duration								
Mean Age, years (Range)	N	# Sleep Cycles	Mean NREM Episode (min) (+W)	Mean REM Episode (min) (+W)	Mean NREM-REM Cycle Duration (min) (+W)	Mean NREM Episode (min) (-W)	Mean REM Episode (min) (-W)	Mean NREM-REM Cycle Duration (min) (-W)
11.9 (7.0-22.6)	42	4.02	98.02	26.44	133.56	88.15	25.16	121.26
7.5 (7.0-7.9)	6	4.17	96.54	26.12	138.17	89.65	25.45	129.6
8.9 (8-9.9)	11	4	106.61	24.22	139.47	99.33	23.06	130.39
11.1 (10.4-11.6)	7	4.33 (n=6)	96.43 (n=6)	26.23 (n=6)	126.56 (n=6)	88.28 (n=6)	25.35 (n=6)	117.2 (n=6)
12.7 (12.1-13.4)	7	4.57	83.8	25.43	110.86	76.44	24.02	101.92
15.0 (14.8-15.7)	6	3.4	109.43	36.31	159.8	101	33.07	146.78

18.1 (16.3-22.6)	7	3.57	93.22	24.29	130.28	71.74	23.55	104.38

Table 6: Mean NREM-REM sleep cycle number, cycle duration, and NREM and REM sleep episode durations across a night of sleep for children with DS separated by age. Data was supplied by Breslin, et al. (2014).

When broken down by age groups, the number of NREM-REM sleep cycles did not appear to show significant differences across age groups. However, the mean number of cycles was slightly lower in the two oldest age groups. Mean duration of a NREM-REM sleep cycle did appear to decrease slightly with age between 8-9 year-olds, 10-11 year-olds, and 12-13 year-olds (Figure 2A). This is most likely due to a similarly observed trend in which the duration of the NREM episode also decreased with age across these groups (Figure 2B). In contrast, REM sleep episode duration appeared to remain constant across the age groups (Figure 2C). There was an unexpected increase in mean cycle and mean NREM episode duration for the 14-15 year-olds, and a similar, though not as large, increase for mean durations in those older than 16 years.

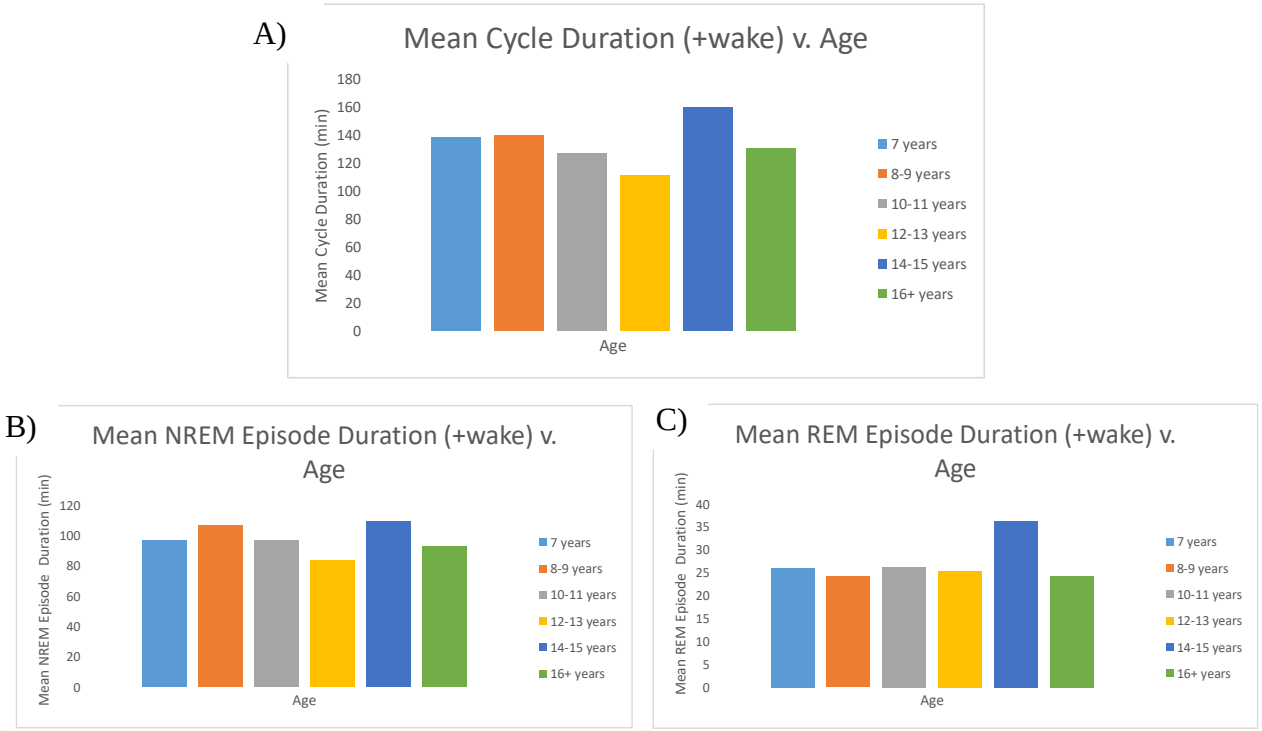

Figure 2: A comparative look at sleep cycle (A), NREM sleep episode (B), and REM sleep episode (C) duration across the night for children with DS separated by age. Wake was included in total duration times.

Table 7 further breaks down the night of sleep into individual NREM-REM sleep cycles. While the mean number of cycles was around 4 per night, participants experienced a range of 1-6 sleep cycles (one individual had only one sleep cycle and was excluded from table 6; only one individual had 6 sleep cycles). Most participants had at least 3 NREM-REM sleep cycles before a large decrease in the number of cycles was found between 3 and 4 cycles, and again between 4 and 5 cycles.

Table 7	Mean NREM-REM Sleep Cycles (n=43)	NREM-REM Sleep Cycle 1 (n=43)	NREM-REM Sleep Cycle 2 (n=42)	NREM-REM Sleep Cycle 3 (n=40)	NREM-REM Sleep Cycle 4 (n=29)	NREM-REM Sleep Cycle 5 (n=15)	NREM-REM Sleep Cycle 6 (n=1)
Mean Duration (min)	130.45	204.72	117.99	107.50	85.97	94.20	43.50
Mean NREM Duration (min)	76.22	172.36	79.38	67.71	54.36	54	29.50
Mean REM Duration (min)	22.67	15.84	25.83	29.48	24.97	31.90	8.00
Mean Wake Duration (min)	10.01	16.52	12.77	10.31	6.64	8.30	5.50
Mean %NREM	62.92	83.66	66.87	62.61	62.92	57.69	68.60
Mean %REM	28.70	8.48	23.02	27.91	28.7	34.25	18.60
Mean %Wake	8.36	7.86	10.11	9.48	8.36	8.06	12.79
Mean %N1	10.82	4.86	8.41	6.50	10.82	6.05	5.81
Mean %N2	45.28	38.49	46.94	46.26	45.28	46.57	62.79
Mean %N3	2.78	14.15	4.62	3.55	2.78	2.23	0
Mean %N4	4.05	26.16	6.89	6.30	4.05	2.82	0

Table 7: Average NREM-REM sleep cycle durations and percent of each sleep stage in each cycle across the night for children with DS separated by age.

The first NREM-REM sleep cycle is the longest in duration, most likely due to a lengthy first NREM sleep episode. The decrease in NREM sleep form the first to the second cycle is most likely due to a decrease in SWS. Percents of N1 and N2 are similar from cycle 1 to cycle 2 (increase slightly) while percents of N3 and N4 experience a large decrease between these first two sleep cycles. After the first NREM-REM sleep cycle, there is a steep drop-off in total sleep cycle duration. Subsequent sleep cycles appear to be similar in length (consistent durations for cycles 2-5). However, the percentage of NREM sleep in each cycle appears to decline slightly across cycles 2-5. In contrast, the percentage of REM sleep, which was very low in the first cycle, appears to increase slightly across cycles 2-5. The durations of each NREM or REM sleep

episode within a sleep cycle also appear to follow similar trends, in which NREM episode duration appears to decrease across the night, and REM episode duration increases slightly.

Overall changes in NREM-REM sleep cycle patterns across the night are depicted in figure 3. The duration of a NREM sleep episode in a sleep cycle is lengthy for the first cycle before sharply decreasing for subsequent cycles. Cycles 2-5 appear to have similar durations of NREM sleep, while the 6th cycle again decreases slightly in NREM length. REM sleep episode duration from cycle to cycle is much more consistent across the night. REM episode duration increases slightly between the first cycles, and then decreases in length during the 6th cycle. The decrease in state duration is most likely due to the overall decrease in the duration of the 6th sleep cycle. Sleep state trends are more apparent when looking at the percentage of NREM or REM sleep in sleep cycles across the night (Figure 3B). Between the first two cycles there is a sharp decrease in percent NREM sleep and a sharp increase in percent REM sleep. These patterns continue much more gradually between cycles 2-5, before reversing in the last cycle.

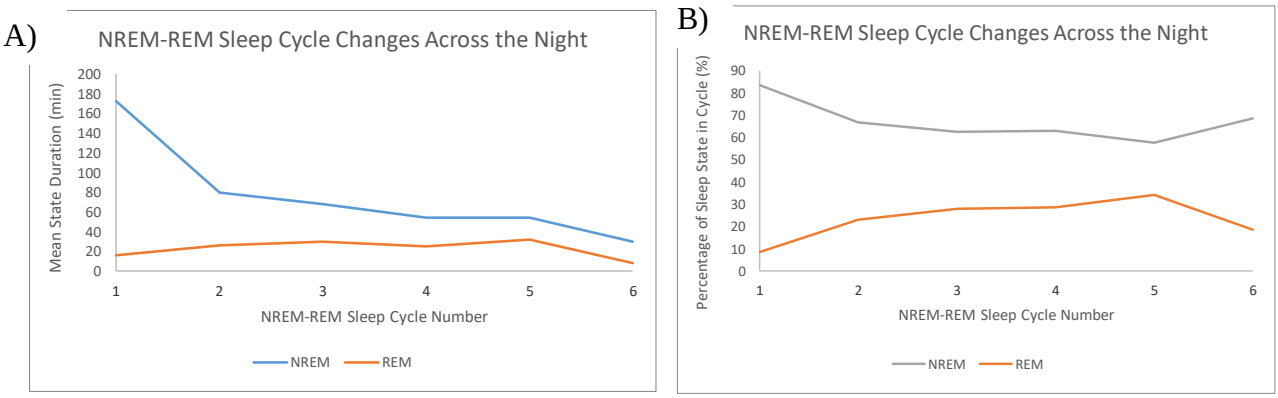

Figure 3: NREM-REM sleep cycle changes across the night for children with DS. A) Average changes in duration of both NREM and REM sleep states within a cycle throughout the night. B) Average changes in the percentage of NREM or REM sleep within each sleep cycle throughout the night.

These overall trends are very similar to those found in typically developing children and adults (Feinberg & Floyd, 1979). Tables 8 and 9 attempt to characterize sleep cycle trends better by taking a closer look at participants who completed at least 4 or 5 sleep cycles. As observed

above and by Feinberg & Floyd (1979) in typical children and adults who completed 4 NREM-REM sleep cycles, the first sleep cycle duration is much longer, sleep cycles 2 and 3 are of similar duration, and cycle 4 drops off in length (Figure 4A). However, when participants experienced a 5th NREM-REM sleep cycle, the duration of the fourth and fifth cycles remain consistent with the duration of cycles 2 and 3, and a decrease in cycle duration is not apparent. (Figure 4B).

Table 8: Mean NREM-REM sleep cycle Durations, Excluding Wake							
Mean Ages (Ranges in Parentheses)		N	Mean NREM-REM Sleep Cycle Duration (min) (SDs in Parentheses)				
			Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
Children	11.3 (7.0-18.2)	29	169.66	103.05	97.60	79.33	---
	10.4 (7.0-17.2)	15	159.17	94.23	88.67	85.77	85.90

Table 8: NREM-REM sleep cycle durations throughout the night, excluding wake, averaged across all participants with DS who completed at least four or five sleep cycles.

Table 9: Mean NREM-REM sleep cycle Durations, Including Wake							
Mean Ages (Ranges in Parentheses)		N	Mean NREM-REM Sleep Cycle Duration (min) (SDs in Parentheses)				
			Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
Children	11.3 (7.0-18.2)	29	183.28	115.76	107.90	85.97	---
	10.4 (7.0-17.2)	15	168.1	102.93	96.60	93.60	94.20

Table 9: NREM-REM sleep cycle durations throughout the night, including wake, averaged across all participants with DS who completed at least four or five sleep cycles.

The values in table 8 and 9 were roughly compared to the sleep cycle durations, found by Feinberg & Flyod (1979), of typically developing children and adults (presented in tables 2 and 3). Comparisons were made between groups labeled as children since the mean ages were comparable. For both healthy individuals and participants with DS, the first NREM-REM sleep cycle duration was less if 5 sleep cycles occurred during a night of sleep compared to four sleep cycles. For healthy individuals, not much difference in each NREM-sleep cycle duration was observed between wake inclusion and exclusion measures (a couple minutes). In contrast, individuals with DS showed substantial differences in cycle duration when wake was included (approximately 8-15 minutes longer). In general, the mean duration of each separate cycle for those with four sleep cycles and including wake, was much greater in those with DS compared to

typical children, particularly when comparing the first NREM-REM sleep cycles (182.28 in DS, 120.8 for typical for four cycles/168.1 minutes compared to 103.6 for 5 cycles). However, for individuals with DS, the duration of the fourth cycle was slightly less compared to healthy controls (85.97 DS, 94.2 TD). Individuals with DS with five sleep cycles had longer first and second cycles while the third and fourth cycles were of similar duration to TD. Interestingly, the fifth sleep cycle was longer in duration (94.2 DS; 83.0 TD), unlike the drop-off in cycle duration observed in TD for the fifth sleep cycle.

Similar patterns were observed when comparing cycle durations excluding wake. The first two cycles had longer durations while the fourth cycle was shorter in DS for those with both four or five sleep cycles compared to TD. The third cycle was about the same duration in DS for those with four cycles, while its duration was slightly decreased for those with five cycles compared to typically developing children. The fifth cycle in DS was slightly longer, but not as much when comparing durations including wake.

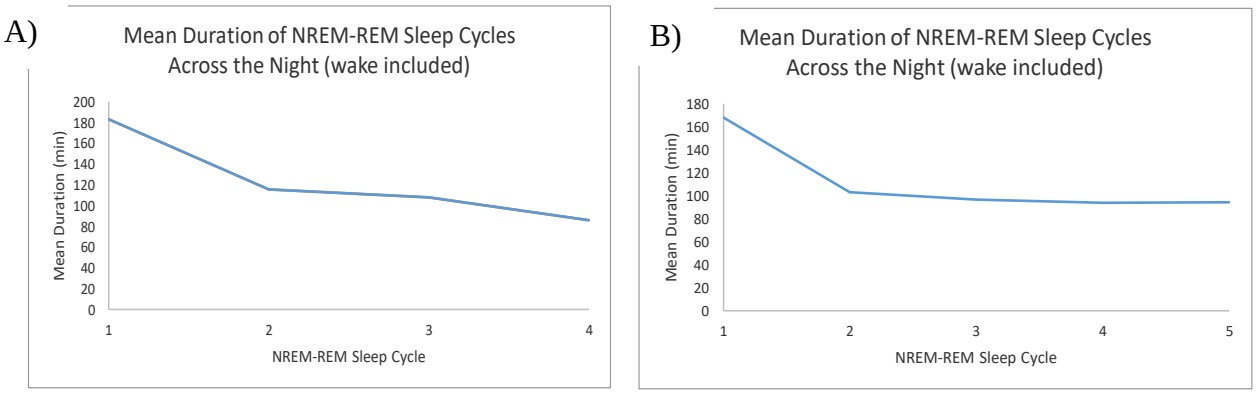

Figure 4: NREM-REM sleep cycle duration trends across the night for children with DS, including wake. A) Average changes in cycle duration for participants who completed at least four sleep cycles. B) Average changes in cycle duration for participants who completed at least five sleep cycles.

Although, REM latency was greatly increased in children with DS, it does not appear to be related to changes in RDI scores, the percent of SWS in the first NREM sleep episode, or to the amount of wake during the first NREM sleep episode. No correlations were found between

REM sleep latency and RDI scores, between REM sleep latency and percent SWS in NREM1, between REM sleep latency and the number of awakenings of wake duration in NREM1.

DISCUSSION

When examining sleep characteristics, most studies look at sleep architecture in the context of the whole night, and variables analyzing each sleep state are compared as percentages of total night of sleep. This overlooks many details regarding possible points of differentiation in sleep outcomes. Therefore, when looking at populations with sleep deficits, it is unclear where in the night these deficits originate. A night of sleep involves alternation between two distinct states with very different behavioral and cortical activity. Therefore, it is also important to understand and characterize the transitions in and out of these states, especially since each state may play its own unique role in learning and memory. Understanding the structure of the night of sleep itself may give further insight into how (mechanisms) and why sleep is altered.

Most sleep deficits in children with DS are related to the whole night of sleep (e.g. more fragmented sleep, decreased sleep efficiency, decreased N2 sleep, and decreased REM sleep). Similar trends across the night were found in our sample of children with DS. However, the biggest difference in sleep between these participants and normative sleep values for typically developing children, was a significant increase in REM sleep latency.

This phenomenon was made more apparent when analyzing individual NREM-REM sleep cycles. Participants with DS had approximately the same number of NREM-REM sleep cycles throughout the night, and the mean duration of each cycle, while slightly decreased in those with DS, is relatively equal (with the exception of the first cycle) compared to typical children. In DS, the first NREM-REM sleep cycle is significantly longer, primarily due to a very

long first NREM sleep episode. It is this long first NREM sleep episode that results in the increase in REM sleep latency.

It is therefore important to understand what might be causing (what is impaired) this delay in REM sleep at the beginning of the night. There were no correlations found between REM sleep latency and percent SWS in the first NREM sleep episode, number of awakenings/wake duration in the first NREM episode, or with RDI apnea scores. Therefore, it is most likely that the difference in REM latency involves impairment in the NREM to REM transition. It is possible that individuals with DS could have impairment in the mutual inhibitory flip-flop switch that drives the transition from NREM sleep to REM sleep. However, this transition does not appear to be impaired later in the night as individuals with DS have the same number of cycles and similar cycle duration for later cycles. Therefore, something may be delaying the initial switch, prolonging the inhibition of SLD GABAergic (REM-ON) preventing their reciprocal inhibition of vlPAG/LPT GABAergic neurons (REM-OFF), but after this initial switch, normal alternation patterns in the inhibition of these neurons are assumed. Another possible site of mechanistic impairment, may be in the monoaminergic and cholinergic modulators. These neurotransmitters selectively activate neurons in REM sleep promoting and suppressing regions. Potential impairment to cholinergic projections promoting REM sleep may contribute to the REM delay in children with DS. Overall, more research is needed to determine the effects of manipulating these populations of neurons to determine what affects/controls the first NREM sleep episode.

From a behavioral perspective, the increase in REM sleep latency may explain why children with DS with reduced REM sleep demonstrated impaired learning after a nap compared to a period of wake (Spano, et al., 2018). A nap may not be a long enough sleep period to

compensate for a long delay in REM sleep and allow the NREM-REM sleep cycle to be normalized for the remainder of sleep. In contrast, a full night of sleep contains enough NREM-REM sleep cycles to reach a more regular mean sleep cycle duration, allowing a learning benefit from sleep to occur.

Moving forward, further examination of NREM-REM sleep cycles across both nighttime sleep and naps is needed to better characterize sleep in special populations. This will help provide a better understanding of where sleep deficits are most impacted. Lastly, further neurochemical manipulations should be completed while simultaneously looking for effects in sleep cycle architecture not just percentages of sleep stages across the night.

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