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Angelman syndrome and melatonin: What can they teach us about sleep regulation

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Abstract

In 1965, Dr Harry Angelman reported a neurodevelopmental disorder affecting three unrelated children who had similar symptoms: brachycephaly, mental retardation, ataxia, seizures, protruding tongues, and remarkable paroxysms of laughter. Over the past 50 years, the disorder became Angelman's namesake and symptomology was expanded to include hyper-activity, stereotypies, and severe sleep disturbances. The sleep disorders in many Angelman syndrome (AS) patients are broadly characterized by difficulty falling and staying asleep at night. Some of these patients sleep less than 4 hours a night and, in most cases, do not make up this lost sleep during the day—leading to the speculation that AS patients may "need" less sleep. Most AS patients also have severely reduced levels of melatonin, a hormone produced by the pineal gland exclusively at night. This nightly pattern of melatonin production is thought to help synchronize internal circadian rhythms and promote nighttime sleep in humans and other diurnal species. It has been proposed that reduced melatonin levels contribute to the sleep problems in AS patients. Indeed, emerging evidence suggests melatonin replacement therapy can improve sleep in many AS patients. However, AS mice show sleep problems that are arguably similar to those in humans despite being on genetic backgrounds that do not make melatonin. This suggests the hypothesis that the change in nighttime melatonin may be a secondary factor rather than the root cause of the sleeping disorder. The goals of this review article are to revisit the sleep and melatonin findings in both AS patients and animal models of AS and discuss what AS may tell us about the underlying mechanisms of, and interplay between, melatonin and sleep.

KEYWORDS

Angelman syndrome, melatonin, sleep, *Ube3a*

1 | INTRODUCTION

Melatonin has become a very popular over-the-counter pharmaceutical and is frequently recommended by clinicians for adults and children with sleep disturbances.[1] Melatonin's role in the regulation of sleep and its frequent use as a somnogen have led to the investigation of melatonin as both a cause of, and treatment for, sleep disturbances in

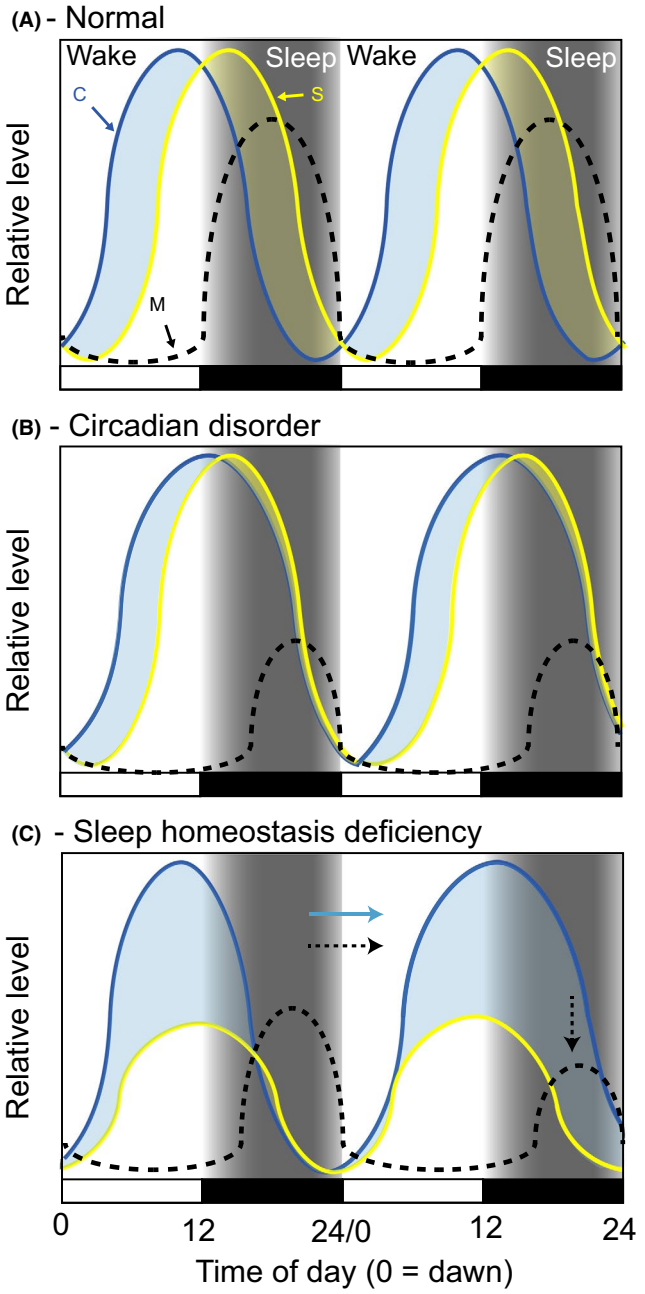

FIGURE 1 Two-process model depictions of how sleep might be altered in Angelman syndrome. In these simplified models, Process C (C; circadian clock) provides drive for wakefulness and Process S (S; sleep homoeostasis) builds pressure to sleep. At any time of day, whichever drive is higher dictates sleep state (light blue shading = wake, yellow shading = sleep). Under normal conditions (A) Wake drive (blue curve), driven by Process C, dominates during the day keeping the subject awake; sleep drive (yellow curve) is building simultaneously, just at a slower rate. At night, melatonin (M) is produced, the circadian clock stops providing wake drive, and the accumulation of sleep drive causes the subject to fall asleep; sleep causes sleep drive to dissipate. In Angelman syndrome, sleep disorders could be caused by changes to circadian control (B), sleep homeostatic control (C), or both (not shown). Data suggest that AS patients may have a long circadian period and/or a delayed and suppressed nighttime melatonin rhythm, as depicted by Process C and melatonin curves (B). If Process S is normal, then Process C may compete against it during the night, delaying and suppressing both sleep and melatonin production. Alternatively, data also suggest the sleep homeostatic process is blunted (C) and may accumulate less efficiently. In this scenario, overall sleep drive is reduced, leading to decreases in sleep. In either B or C, not sleeping may also lead to an increase of light exposure at night (ie, turning on lights or TV) that may further contribute to circadian-like deficits in sleep regulation (indicated by the arrows in C)

neurological disorders. This review is intended to highlight the relationship between melatonin, sleep, and Angelman syndrome (AS).

2 | SLEEP AND MELATONIN

The need for sleep is universal; all animals do it (for a review see[2]). And while the answers to the question "why sleep?" are hotly debated, it is clear that sleep is very tightly regulated. The consensus view of sleep regulation, the two-process model, proposes that sleep is regulated by the interaction of two processes that separately control timing and amount of sleep (see Figure 1). Although several variations of the two-process model have been proposed,[3,4] the basic tenets described here remain consistent.

The circadian clock (Process C) is responsible for regulating sleep/wake to the appropriate time of day, by driving a daily rhythm in a sleep-wake threshold or set point by which sleep or wake is initiated (depending on time of day).[5] Behavioral circadian rhythms are controlled by intracellular clocks localized to the 20 000 neurons within suprachiasmatic nucleus (SCN) of the hypothalamus. This timekeeping mechanism is a coupled set of transcriptional negative feedback loops that ultimately drive rhythms in firing rates of SCN neurons.[6] SCN neurons project through the ventral and dorsal subparaventricular zone (SPZ) to the dorsal medial hypothalamus (DMH) to regulate sleep/wake and other rhythmic outputs[7] like melatonin synthesis.

Sleep is also regulated by a homeostatic process (Process S) that is responsible for "sleep pressure" and the regulation of total daily sleep amount. Sleep pressure (ie, sleep drive, sleep load) is homeostatically regulated and accumulates with increasing duration of wakefulness: The longer the duration of wakefulness, the higher the drive to sleep and the longer and deeper the subsequent sleep episode. When sleep pressure reaches the upper threshold set by the circadian clock (Process C), sleep is initiated. Most organisms require a species-specific duration of sleep; in humans this is ~7 h/d.[8] Not reaching this minimum threshold leads to the feelings and symptoms of sleepiness, including neurocognitive impairment.[9] Despite popular anecdotes, empirical evidence has repeatedly shown

that one cannot "learn" or "train" themselves to need less sleep.[10] Once asleep, accumulated sleep pressure dissipates in a duration-specific manner (the more you sleep, the more sleep pressure is reduced). When sleep pressure reaches the lower limit set by the circadian clock (Process C), wake is initiated (Figure 1A). While we have a good understanding of circadian clock mechanisms (see below), and extensive information of the neural circuitry promoting both sleep and arousal, the nature of the sleep homeostatic process is a major gap in our understanding of how sleep is regulated.[5,7,11-13]

Projections from the SCN ultimately send time of day information to the pineal gland to regulate melatonin production. Melatonin is involved in regulating multiple physiological processes in mammals[14] including the sleep-wake cycle.[15,16] The pineal gland produces melatonin exclusively during the night; light at night quickly shuts down melatonin synthesis. In this way, plasma melatonin levels transduce information about light/dark cycle as well as the presence of light at night.[17] The Dim Light Melatonin Onset/Offset (DLMO on/off) is among the most accurate measures for assessing the circadian pacemaker in human subjects.[18] The robustness or amplitude of the melatonin rhythm is directly related to overall strength and health of the circadian system, and flattened nighttime melatonin production signifies a potential problem in the underlying circadian clock.[19] When combined, melatonin onset and amplitude are commonly used to provide key insights into the timing and functioning of the circadian system in humans and other mammals.[18]

Melatonin administration can also help induce sleep, but it seems to do this at least in part by resetting the circadian clock. Evidence from rodent studies suggests that the actions of melatonin in the SCN are mediated primarily by two G-protein coupled melatonin receptors, MT1 and MT2. Melatonin feeds back into the SCN to modulate circadian clock timing as a "nighttime" signal (as opposed to just a "dark" environment).[20,21]

When taken at night, melatonin and melatonin receptor agonists (ie, Agomelatin, Ramelteon, Tasimelteon)[22] shift the circadian system toward night, improving sleep latency. Exogenous melatonin is not affected by light at night and, therefore, can be used to offset the effects of light exposure at night. Because derivative compounds of melatonin appear to shift the circadian clock, they are effective in treating some Circadian Rhythm Sleep Disorders (CRSDs), such as Non-24-Hour Sleep-Wake Disorder.[23] This, combined with the fact that CRSDs are often accompanied by an alteration in the melatonin rhythm, suggests that melatonin may act directly as a "somnolescent" and regulate sleep. However, melatonin and its derivative compounds are not efficacious for treating primary (ie, noncircadian) sleep disorders[24]; this argues against actions as a somnolescent independent of the circadian clock. This may explain why melatonin therapy has mixed results in treating the sleep disorders associated with Angelman syndrome, as discussed below.

3 | ANGELMAN SYNDROME

AS is a rare but severe neurodevelopmental disorder with a prevalence of 1 in 12 000-20 000. Its broad complex presentation is characterized by severe developmental delay and speech impairment resulting in the use of few if any words; disordered balance and/or movement, ranging from ataxia and unsteadiness, to quick jerky movements; and a "behavioral uniqueness" described as a happy, easily excitable demeanor accompanied by hypermotoric behaviors (typically hand-flapping/waving motions).[25-28] More than 80% of individuals with AS display microencephaly, abnormal electroencephalography (EEG), and epilepsy.[29,30] An estimated 70%-80% of those with AS also exhibit sleep disturbances,[31-33] described in detail below.

AS is caused by a lack of *ubiquitin protein E3A ligase gene* (*UBE3A*) expression in the brain. Both *UBE3A* alleles are expressed in peripheral cells and tissues. But in the vast majority of neurons, the paternal *UBE3A* allele is imprinted, preventing its expression. Thus, the maternally inherited allele is responsible for the expression of UBE3A protein in neurons. The imprinting mechanism is not fully understood, though it appears to be due to expression of a large, non-coding, antisense transcript (LNCAT) that selectively blocks expression of the paternal allele, which has been nicely reviewed elsewhere.[34,35] There are a few regions in the brain where *UBE3A* expression is biallelic. Interestingly, the SCN is the site with the highest concentration of neurons that express UBE3A from the paternal allele, at least in mice. UBE3A levels are reduced by only ~50% in the SCN of a mouse model of AS compared to wild type controls,[36,37] whereas UBE3A is absent most everywhere else.[37,38] On the surface, this would suggest a relatively intact circadian rhythmicity in AS; however, this is not the case and circadian impairments, which are discussed later, do exist.

An absence of maternal *UBE3A* expression can be caused by four different molecular mechanisms.[39-44] The most common cause (70%-80%) is a chromosomal deletion from 15q11-q13 on the maternal allele, containing the *UBE3A* gene.[45] This deletion usually occurs as a de novo event but it can be due to structural rearrangement in the mother.[46] Mutations within the *UBE3A* gene account for 5%-10% of AS cases,[47] with the remaining cases due to paternal uniparental disomy (UPD) of chromosome 15 (1%-2%)[48] or defects in the imprinting mechanism (1%-3%).[49] The severity of some aspects of AS correlates with the underlying genetic anomaly. For example, 15q11-q13 deletions tend to cause more severe phenotypes compared to other mechanisms, suggesting potential contribution of nearby genes in AS. However,

mutations that only affect the maternal *UBE3A* allele cause all of the core AS disabilities suggesting this is the primary underlying cause of AS.

Mouse lines in which maternal UBE3A is disrupted provide additional evidence of a causal role of UBE3A in AS. Mice with a maternal deletion of *Ube3a* (*Ube3a*$^{m-/p+}$ mice) have phenotypes that resemble many of the clinical symptoms of AS, including motor deficits, impaired spatial learning, seizures, and deficiencies in synaptic plasticity. *Ube3a*$^{m-/p+}$ mice have an imprinting mechanism—homologous to humans—that silences the paternal *Ube3a* allele in neurons. Thus, *Ube3a*$^{m-/p+}$ mice are widely used as a preclinical model to understand how UBE3A loss leads to AS-like pathologies and to evaluate potential treatments. Mice, however, are nocturnal, and AS-like behavioral phenotypes can vary by genetic background, strain, and age. All of these factors should be considered when using *Ube3a*$^{m-/p+}$ mice as an animal model for AS.[50]

4 | SLEEP DISORDERS IN ANGELMAN SYNDROME

The widespread sleep disturbances reported in AS are based on two broad categories of evidence: (a) survey-based studies relying on the reports of parents and caregivers and (b) polysomnographic (PSG) studies based on EEG data. Both evidence categories provide important information, with surveys providing subjective information regarding mood, sleep patterns, sleep habits and behavioral consequences, whereas PSG provides objective metrics of sleep quantity, quality and potential diagnostic criteria. In addition to clinical studies, animal models also provide detailed sleep metrics and analysis that are not available in the human literature. Overall, these data converge to indicate that AS is associated with a debilitating sleep disorder that appears to have its roots in the altered accumulation of sleep pressure.

The first consensus guidelines for the diagnostic criteria of AS included sleep disturbances as an associated feature of the disorder[51]; this was later updated in the guidelines to include abnormal sleep-wake cycles and a diminished need for sleep.[52] The latter feature, a diminished need for sleep, is of high interest, as it appears AS patients do not suffer the negative consequences of sleep deprivation. Currently, "sleep need" cannot be objectively measured, and its biological basis represents a critical knowledge gap in sleep research. Indeed, evidence for altered sleep need is rare in the literature and mostly based on evidence from natural short sleepers; individuals reporting significantly less daily sleep than the population average with seemingly little effect on health and performance.[53,54] Thus, the study of sleep in AS may provide important information on the nature of sleep need and related health problems, as well as uncover novel sleep-regulatory mechanisms.

EEG studies provide a potential means for AS diagnosis, and when combined with other PSG measures they can provide objective analysis of sleep in AS patients. Studies focused on AS diagnosis have found widespread EEG disturbances in these patients.[29,55-61] Two of the most commonly reported abnormalities in the EEG are intervals of high-amplitude slow waves (in the EEG delta/theta band) and interictal epileptiform spike-wave discharges.[55,62] The degree to which these commonly reported abnormalities are present during sleep is not clear. This is because, although these recordings were made during both wake and spontaneous sleep,[29,56,59,60,62] the prevalence of anomalies during specific sleep/wake states have not been reported. State-specific analysis is critical for determining the degree to which EEG anomalies are related to the sleep disturbances in AS; several studies have used PSG to accomplish this goal.

PSG uses EEG and additional measures (eg, electrooculography, electromyography) to objectively assess sleep and wakefulness with a level of precision far superior to behavioral analysis. PSG studies of AS patients provide direct evidence that multiple sleep parameters indicative of decreased sleep efficiency exist. AS patients have nearly twice the number of transitions between sleep states, a fourfold increase in the frequency of awakenings (ie, sleep is fragmented), and a 50% reduction in the deepest stages of NREM sleep—all indications that AS patients have reduced sleep quality and sleep efficiency during the night.[63,64]

Recently, differences in the number and duration of NREM sleep spindles were also reported.[65] Approximately half the number of sleep spindles of a significantly decreased length were found in the EEG of children with AS. This spindle activity in the 11-16 Hz range occurs during NREM sleep and is associated with memory consolidation,[65] suggesting a potential direct relationship between poor sleep quality and memory difficulties in AS patients. Despite the aforementioned deficits in NREM sleep quality, there are no indications that the total amount of NREM sleep is reduced in AS patients. This is somewhat at odds with subjective reports from parents/caregivers regarding overall amount of sleep. Nevertheless, though the degree to which AS patients sleep less may be unclear, there is no question that the quality of their sleep is significantly impaired. Studies report a significant decrease in rapid-eye movement (REM) sleep in AS patients compared to healthy controls—especially in patients younger than 8 years old.[63,64] This could be directly due to poor NREM sleep in AS patients, as they may not be efficiently progressing through the 3 stages of NREM in order to reach REM sleep. Thus, the emerging picture from objective PSG studies is

consistent with caregiver reports that AS patients sleep poorly during the night. However, the subtle inconsistencies in overall sleep time make it difficult to predict the possible underlying causes of this disturbed sleep.

5 | ORIGIN OF THE SLEEP PHENOTYPES IN AS

5.1 | Evidence of circadian dysfunction

One potential cause of the sleep disturbances observed in AS (and other neurodevelopmental disabilities) is an inability to synchronize the sleep-wake cycle with the light-dark cycle, resulting in abnormal melatonin secretion and, consequently, CRSDs.[66,67] Indeed, emerging evidence from studies in model organisms[36,68-70] provides some support for this hypothesis (more on this below). Although few studies have examined the melatonin secretion profile of AS patients, the available data indicate that it varies broadly between individuals with a tendency toward reduced levels and/or altered timing of nighttime melatonin secretion. Additionally, melatonin administration in the early evening has been shown to consistently improve sleep outcomes in individuals with AS.

Some of the first evidence indicating that AS patients exhibit altered nighttime melatonin levels came from studies that compared nighttime melatonin secretion patterns in AS children to published reports of healthy children. A study of 13 AS children demonstrated that both the average hourly rate of production across the 24-hour period and peak nighttime levels of melatonin production were highly variable between individuals.[71] In a majority of these children, peak nighttime melatonin levels were noticeably lower compared to a published report of unaffected children of a similar age.[72] In addition, the nighttime surge in melatonin secretion was significantly delayed in 3 AS children (relative to habitual bedtime and sleep onset). Two of these children exhibited prolonged morning sleepiness that was correlated with low sleep quality and quantity. A separate study of 8 AS patients with idiopathic insomnia (4-9 yo, N = 5; 12-20 yo, N = 3) also reported low levels of endogenous melatonin.[73] In this case, sampling times were restricted to 5-11 PM, making it impossible to distinguish between low melatonin per se vs a delayed onset in nighttime melatonin secretion (see below).

More recent investigations compare melatonin secretion profiles between AS patients and unaffected age-matched controls within the same study. A direct comparison of 15 AS patients (including children, adolescents, and young adults) and age-matched controls revealed that individuals with AS exhibit significantly lower nighttime melatonin levels.[66] Surprisingly, total sleep time and ratio of nocturnal to total sleep time did not differ from controls. AS patients with a diagnosed CRSD, however, exhibited less total sleep than AS patients without CRSD. Patients with CRSD also had a lower percentage of nocturnal sleep to total sleep compared to both AS patients without CRSD and unaffected age-matched controls. AS patients with CRSD also exhibited a significantly smaller nighttime peak in melatonin secretion compared to AS patients without CRSD. AS patients with free-running type and irregular sleep-wake type CRSDs exhibited low serum levels of melatonin throughout the 24-hour day with virtually no nighttime surge in melatonin. Peak melatonin levels in AS patients with delayed sleep phase type CRSD were similar to controls, but the peak was significantly delayed.

Another study,[74] in which AS children were directly compared to both non-AS/epilepsy and non-AS/nonepilepsy age-matched controls, found differences in the pattern, but not levels, of melatonin secretion in AS patients. Although these AS children—all of whom were diagnosed with a CRSD—had melatonin levels similar to levels reported in the literature for both age-matched control groups and healthy children,[72] their individual melatonin secretion curves were highly variable. The duration of nighttime melatonin secretion was elongated, and both the phase and the offset of nighttime melatonin secretion were significantly delayed in AS children. Rather than the typical bell-shaped curve, these children exhibited a "triangular" melatonin secretion profile that may explain the sleep onset insomnia and sleep maintenance problems so common to AS.

The shortage of studies, small sample sizes, high individual variability and differences in patient characteristics make it difficult to draw conclusions from most of these human studies. The fact that most AS patients take anti-seizure medications containing sodium valproate—known to suppress plasma melatonin levels[75]—further complicates interpretation of these data. Braam et al,[73] reported no differences in melatonin levels between AS children taking and not taking sodium valproate; furthermore, Takaesu et al[66] found no relationship between daily dose of sodium valproate and peak melatonin levels in AS patients. These data indicate that reduced melatonin production is a characteristic of AS and not a result of anti-seizure medication per se. On the whole, it appears that in AS patients nighttime melatonin levels are low and/or its pattern disturbed.

Melatonin is commonly used by caregivers and routinely recommended by physicians as a treatment for sleep disruption in AS patients.[1,73] Despite the limited number and inconsistent methodologies of investigations into the effectiveness of melatonin in AS children, the available data consistently indicate that melatonin administration improves sleep latency, efficiency, duration, and nighttime awakenings. In an open label study, a physiological dose of melatonin (0.3 mg) just before bedtime decreased sleep latency, reduced motor activity during sleep, and increased sleep duration in children with AS.[71] Parents of these children reported improved sleep consolidation

and quality. This treatment also resulted in a 2- to 3-hour phase advance in the onset of melatonin secretion in children that otherwise exhibited a significant phase delay in nighttime melatonin secretion.[71] In a double-blind, randomized placebo-controlled trial, evening melatonin treatment (<6 yo: 2.5 mg at 6 PM; ≥6 yo: 5 mg at 7p) advanced sleep onset, decreased sleep latency, reduced nighttime awakening, and increased total sleep time in children with AS.[73] Parents indicated that their children were less sleepy and more attentive during the day, with easier to manage behavior. In a case study of a 9-year-old boy with AS that exhibited prolonged mid-night awakenings, treatment with sustained-release melatonin (3 mg) 30 minutes before bedtime improved sleep efficiency, decreased nighttime arousals, and increased total sleep time.[76] Furthermore, melatonin treatment (1mg between 6 and 7 PM) increased the percentage of nocturnal sleep in four of six individuals with AS (mostly children) that also exhibited a recognized CRSD.[66]

All of these studies indicate that improving nighttime melatonin can improve sleep and appears to be doing it by realigning AS patients' circadian timing with the day-night schedule. This suggests that perhaps the sleep disorders in AS patients could be due to a defect in the circadian system—either with its overall functionality or in how it is aligned with the day-night cycle. The data also suggest that if this is indeed the case, the effects on the circadian system vary quite broadly from patient to patient. It is not yet clear if CRSDs fully explain all of the idiosyncratic sleeping disorders in AS patients. However, the data do suggest that melatonin can improve sleep in those with CRSDs and those that exhibit reduced melatonin levels, suggesting that circadian dysfunction is a leading contributor to the sleep disturbances described in this patient population.

The cause-effect relationship between circadian dysfunction and sleep disorders is not yet clear. While it is possible that circadian dysfunction (Process C) could be the root cause of the sleeping disorders, it is also possible that a defect in sleep homeostasis (Process S) is the root cause of the sleeping disorders in AS, including CRSDs. Insomnia can lead to circadian misalignment even though the circadian system is functioning normally, simply by increasing an individual's exposure to light at night (watching TV, reading, playing on a cell phone, etc) while struggling to go to sleep. This would cause the circadian system to shift out of alignment with the day-night cycle (Figure 1C). While melatonin therapy does improve sleep in many AS patients, it is not clear that it restores sleep back to normal levels, durations, and consolidation. Thus, more work is needed to understand the etiology of the sleep disorders in AS, improve the treatment of these sleep disorders, and potentially uncover new aspects of sleep regulation in general.

In order to determine whether the sleep disorders in AS are a possible consequence of a defect in Process C, several groups have begun examining interactions of UBE3A with core circadian clock proteins, as well as examining circadian rhythmicity in AS model Drosophila and mice. The first evidence that circadian dysfunction may be involved comes from findings that removing UBE3A protein in fruit flies disrupts circadian patterns of locomotor activity[68,69]; however, effects on the molecular circadian clockwork have not yet been reported for this species. In human cells, the UBE3A protein (also known as E6-AP) interacts with and regulates the stability of the BMAL1 protein—a protein essential for mammalian clock function.[69] UBE3A is an E3 ubiquitin ligase that targets substrate proteins for proteasomal degradation.[77] Thus, the hypothesis was that UBE3A loss dysregulated BMAL1 protein levels by impairing its normal degradation. Indeed, depleting UBE3A using RNAi in cell-based circadian clock models results in a slight lengthening of circadian periods,[36,69] providing support for the circadian-based sleep disorder hypothesis.

Our group as well as Carl Johnson's group at Vanderbilt independently extended these studies to the same in vivo mouse model, and largely came up with conflicting results.[36,70] The AS model mice used in these studies all inherited a null *Ube3a* allele from their mothers (ie, maternally deleted *Ube3a* or *Ube3a*$^{m-/p+}$), and show phenotypes very similar to those described in humans, including sleep deficits.[78-80] Of note here, all of the mice used in these studies are on the C56Bl6 background, a strain that does not produce detectable amounts of melatonin. The Vanderbilt group found that these mice, as well as another model with a larger deletion, displayed slightly longer circadian periods (~20-30 minutes) and realigned more quickly to phase advances in the light cycle,[70] consistent with observations in AS patients described above. However, our group, using nearly identical assays, did not find these, or any, differences in circadian function in *Ube3a*$^{m-/p+}$ mice.[36] Although subtle differences in experimental design and/or animal environment likely accounts for the discrepancy in the data, the difference in findings between groups suggests that any potential circadian deficiencies are not robust.

Quite surprisingly, we found that UBE3A is still present, albeit at reduced levels, in SCN neurons of *Ube3a*$^{m-/p+}$ mice,[37] perhaps explaining why we do not find alterations in circadian behavior.[36] This relaxation in the imprinting of the paternal *Ube3a* allele is specific to the SCN, as UBE3A is absent from nearly all other brain regions in these mice.[36-38] Altogether, although UBE3A may regulate BMAL1 stability and cellular clock function, it is not clear that its loss in AS simply results in changes in Process C that explain the severe sleep disorders described for these patients.

5.2 | Evidence of deficits in sleep homeostasis

As previously discussed, sleep disturbances in AS patients—twice the number of sleep-state transitions, fourfold increase

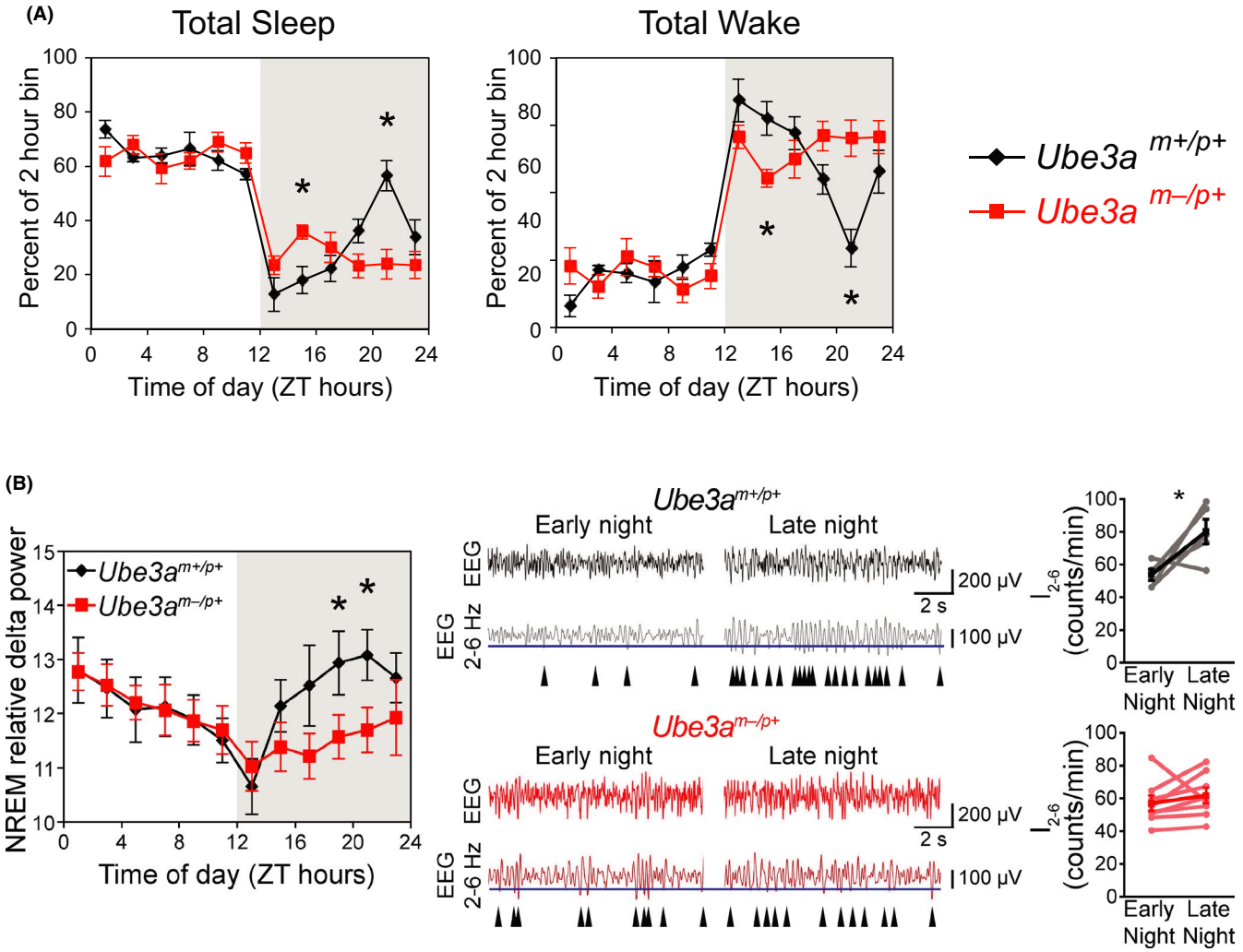

FIGURE 2 Sleep patterns are disrupted in AS model mice. A, Total sleep or wake in wild type (*Ube3a*$^{m+/p+}$) or AS (*Ube3a*$^{m-/p+}$) mice recorded over an uninterrupted 24-hour period in 12-hour light/dark cycle. Data are shown as percentage of each 2-hour block spent asleep or awake within each mouse, averaged within genotypes (mean ± SEM; n = 6 wild type or 8 AS mice). Note the difference in sleep/wake amounts between genotypes across the night (indicated by the gray shading). B, AS model mice exhibit reduced nighttime sleep-pressure accumulation. Left—NREM sleep intensity, as measured by delta power, is significantly reduced in *Ube3a*$^{m-/p+}$ mice during the latter portion of the night. *$P < .05$ between genotypes, n = 6-8 mice. Right—The increase in the wave incidence (I2—6) across the night (wake period) is abolished in *Ube3a*$^{m-/p+}$ mice. Representative EEG recordings (black and red) are shown above band-pass-filtered versions (gray and dark red) of the same EEG recording. The incidence of peaks in the upper 30% by amplitude (blue line) in the filtered signal was counted in epochs scored as waking are indicated by arrowheads. I2—6 has been previously validated as a measure of sleep-pressure accumulation in awake mice.[81] I2—6 significantly increased across the active period in wild type (*$P < .05$) but not *Ube3a*$^{m-/p+}$ mice. All data are replotted from Ehlen et al[36]

in awakenings and a halving of time in the deepest stages of NREM sleep—indicate reduced sleep efficiency.[63,64] A defect in the homeostatic mechanism(s) regulating sleep (Process S) is a potential cause of this reduced sleep efficiency (see Figure 1C). Indeed, our group has uncovered evidence in the mouse model that sleep homeostasis, rather than the circadian clock, may be a major underlying source of the sleep deficits in AS.[36]

As noted above, the maternally deleted *Ube3a* mouse model (*Ube3a*$^{m-/p+}$) exhibits imprinting of the paternal allele and most, if not all, of the core features of AS, including sleep disorders.[78-80] The sleep features in this AS mouse model include an increased number of transitions between sleep states, decreased depth of NREM sleep, and a trend for decreased REM amount during undisturbed sleep[80]—traits reminiscent of humans.

The physiological basis of the homeostatic mechanism regulating sleep remains unknown; therefore, EEG and behavioral measures of sleep pressure are used to identify changes in this homeostatic mechanism. This homeostatic process is responsible for the increases in sleep amount and EEG power in low frequency ranges (ie, slow-wave power a.k.a NREM delta power, 0.5-4 Hz) during NREM sleep and increases in the number of higher frequency EEG peaks during waking

("wave incidence"), especially prolonged durations of waking.[81,82] NREM delta power is among the most reliable measures of Process S, as it builds with wake, dissipates with sleep, and increases in response to sleep deprivation. In this way, it is a widely accepted as an objective indicator of sleep pressure. Wave incidence provides similar information on changes in sleep pressure, except that it is measured during wake, when sleep pressure is actively accumulating.

We serendipitously discovered that mice inheriting a null allele of the *Ube3a* gene from their mothers (*Ube3a*$^{m-/p+}$ mice) have pronounced deficits in NREM delta power and subsequent NREM sleep patterns.[36] Mice are nocturnal, with high amounts of wakefulness to start the night that devolve into a late-night "siesta" (ie, nap) before an increase in wake again before lights on. During this time, normal mice build wave incidence and NREM delta power that peaks coincident with the timing of the siesta, suggesting a potential causal relationship (though it should be noted that in normal mice, the precise timing and presence of siesta from day to day can be variable). AS model mice, in contrast, show little changes in wave incidence and display a much slower increase in NREM delta power during the night, with the latter peaking at lights on. These mice also lack a mid-to-late night siesta (Figure 2). Recently, elevated delta power (2-4 Hz) was reported in both children with AS and a mouse model of AS.[29] This elevated delta power was found to be an effective biomarker of AS that is present in both sleep and waking; however, based on our findings, this reported overall elevation does not impact changes in delta power (0.5-4 Hz) related to the duration of sleep and wakefulness. Overall, these findings are consistent with reduced accumulation of sleep pressure and suggestive of a deficit in sleep homeostasis.

To test this hypothesis more directly, we forcibly deprived mice of sleep during the early daytime—when mice are sleeping most intensely, a common paradigm used to probe sleep homeostasis. Indeed, AS mice displayed blunted changes in wave incidence and NREM delta power accumulation, as well as less recovery sleep after sleep deprivation.[36] Taken together, our findings demonstrate that neuronal loss of UBE3A in mice eliminates the homeostatic sleep responses to both ad libitum wakefulness and forced wakefulness; thus, indicating a deficit in the accumulation of sleep pressure.

Overall, the sleep studies in mice provide direct evidence that UBE3A-loss reduces sleep pressure. However, direct comparisons to humans with AS are complicated by differences in nocturnality/diurnality, experimental paradigm (ie, forced sleep deprivation), and by the fact that most clinical sleep studies focused on times when AS patients typically sleep. Furthermore, when studied in a controlled environment, animal models are not subject to the disruptive nighttime influences of light. Nevertheless, this animal model demonstrates that undisturbed, spontaneous sleep is altered by neuronal loss of UBE3A in ways that are at least superficially similar to AS patients. Both display similar NREM sleep characteristics, an increase in the number of transitions between sleep states, and decreased depth of NREM sleep. Both effects are indicative of reduced sleep need. Detailed analysis of EEG waveforms from AS patients is needed to confirm that markers of sleep pressure are reduced similarly in both mice and humans. These studies in humans are technically challenging but are critically important as they have the potential to confirm a core mechanism for sleep disturbances associated with UBE3A-related developmental disorders.

6 | CONCLUSION AND FINAL CONSIDERATIONS

In Figure 1, we depict two possible causes of sleep problems in AS that are both suggested by the data and consistent with the two-process model.[11] Sleep in AS patients has thus far been most successfully improved by nighttime melatonin therapy, suggesting that some aspects/portions of the sleep disorders may be due to changes in circadian timing. In mouse models, however, neuronal loss of UBE3A does not produce robust circadian deficits but results in marked disruption in sleep homeostasis. So, the truth may lie somewhere in-between. Mouse models, because of the varied genetic tools available (ie, floxed *Ube3a* alleles, melatonin deficient/proficient strains, strains with very diverse sleep characteristics), are going to be extremely valuable for identifying the fundamental relationships between AS and circadian and sleep dysfunction. One intriguing possibility is that UBE3A loss impairs clock function in a brain region outside of the SCN to cause some of these effects. It is also possible that there are interactions between clocks/SCN and an unknown site responsible for maintaining sleep homeostasis. In that regard, although its loss causes a wide array of phenotypes, *UBE3A* appears to be a "sleep gene." Determining how and where in the brain it functions to regulate sleep could prove pivotal in understanding the mechanism(s) underlying sleep homeostasis regardless of the exact mechanism that is impaired by neuronal loss of UBE3A.

AUTHOR CONTRIBUTIONS

DB, DLH, AA, JCE, and JPD performed research and wrote the paper. All authors have reviewed and approved the final manuscript.

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