NEUROPSYCHOPHARMACOLOGY REVIEWS

Sleep as a translationally-relevant endpoint in studies of autism spectrum disorder (ASD)

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Sleep has numerous advantages for aligning clinical and preclinical (basic neuroscience) studies of neuropsychiatric illness. Sleep has high translational relevance, because the same endpoints can be studied in humans and laboratory animals. In addition, sleep experiments are conducive to continuous data collection over long periods (hours/days/weeks) and can be based on highly objective neurophysiological measures. Here, we provide a translationally-oriented review on what is currently known about sleep in the context of autism spectrum disorder (ASD), including ASD-related conditions, thought to have genetic, environmental, or mixed etiologies. In humans, ASD is frequently associated with comorbid medical conditions including sleep disorders. Animal models used in the study of ASD frequently recapitulate dysregulation of sleep and biological (diurnal, circadian) rhythms, suggesting common pathophysiologies across species. As our understanding of the neurobiology of ASD and sleep each become more refined, it is conceivable that sleep-derived metrics may offer more powerful biomarkers of altered neurophysiology in ASD than the behavioral tests currently used in humans or lab animals. As such, the study of sleep in animal models for ASD may enable fundamentally new insights on the condition and represent a basis for strategies that enable the development of more effective therapeutics.

INTRODUCTION

A recent estimate suggests that as many as 2.5% of children have an autism spectrum disorder (ASD) [1]. Despite the high prevalence of ASD, its cause is unknown in most individuals. There is a strong genetic contribution to ASD, with an estimated genetic heritability of between 50–80% [2, 3]. The remaining risk is thought to be related to environmental risk factors: as examples, prenatal exposure to hypoxia, inflammation, or maternal obesity all increase the risk of developing ASD [4] or may be related to other forms of non-heritable risk such as de novo genetic mutations [5]. While genetic and environmental factors are often studied in isolation, ASD is likely the result of a complex interplay of both. ASD is defined by core features including (1) dysregulated social behavior, (2) deficits in communication, and (3) the presence of restricted and repetitive behavior. Behavioral signs of ASD often emerge within the first couple years of life and can be reliably diagnosed by 24 months of age [6]. Prior to the diagnostic threshold there may be prognostic features of ASD in the first years of life related to the underlying biology including low attention to eyes [7], atypical cries [8], and potentially abnormal electrophysiological signatures in the brain [9]. By necessity, the diagnosis of ASD is made on the basis of these core behavioral features, since definitive biomarkers that can readily identify ASD are yet to be discovered. As a result, ASD is not a unitary condition, rather a set of converging symptoms that likely stem from multiple biological etiologies. While the diagnosis of ASD is based on core behavioral features, there is a growing appreciation that medical conditions including epilepsy, gastrointestinal disturbances, sensory abnormalities, and sleep

dysregulation are frequently comorbid and thus also represent important aspects of these conditions [10]. A better understanding of these comorbidities—including sleep dysregulation—may offer the opportunity to better understand and define the causes of ASD. Further, these comorbidities may also have potential as more objective and translationally-relevant endpoints in animal models used in the study of ASD, facilitating the development of effective interventions and therapeutics.

SLEEP PROBLEMS IN ASD

Prevalence of sleep problems

Here we provide a brief overview of findings related to sleep disturbances in ASD, complementing more detailed reviews focused on findings in humans [11–14]. Sleep disturbances are more common in ASD than in the general population. A recent study of 522 children with ASD, 228 with ASD and other developmental delays, and 703 from the general population demonstrated that sleep problems are twice as prevalent in children with ASD (odds ratio: 2.12) or ASD and developmental delay (odds ratio: 2.37) [15]. This may be independent of environmental factors: in a study of siblings, children with ASD have higher rates of sleep problems (47%) than their typically developing (TD) sibling (16%) [16]. Such findings are broadly consistent with prevalence studies, which estimate sleep problems in 40–80% of children with ASD compared to 25–40% in TD children [11, 13]. The range of these estimates reflects the difficulty in determining an accurate prevalence of sleep problems in ASD, as factors including age, cognitive ability, and biological

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Sleep as a translationally-relevant endpoint in studies of autism…
G Missig et al.

91

sex must be considered, all of which is further complicated by the lack of a clear consensus on the criteria and definitions of sleep disorders in pediatric populations [14]. Differences in methodology may also have an influence. Parental report is most commonly used to assess sleep in children and is the method of the majority of studies on children with ASD. However, actigraphy or polysomnography (PSG) studies—which offer more objective measures of sleep—are emerging and also suggest greater rates of sleep abnormalities in individuals with ASD compared to TD individuals [17]. Finally, considering that ASD is likely a cluster of disorders with multiple neurobiological etiologies, the prevalence and nature of sleep disorders may differ between these subtypes further adding variability. In spite of these complicating factors, there is a growing consensus that sleep disturbances are substantially more common in individuals with ASD.

Types of sleep problems
There is no singular form of sleep disruption in ASD. Symptoms of insomnia—difficulty initiating or maintaining sleep—are the most consistently reported sleep difficulty in ASD, including increases in sleep onset latency (time to fall asleep), poor sleep efficiency (proportion of time spent asleep while in bed), and less total sleep time [13]. Parental reports indicate that children with ASD are more likely to have bedtime resistance, increased awakenings, and decreases in the duration and continuity of sleep [18, 19]. Difficulties with sleep-onset insomnia (going to sleep) are more common than sleep-maintenance insomnia (staying asleep), although both frequently occur [11]. Parasomnias—undesirable physical or experiential events accompanying sleep—also occur at higher rates in ASD, according to some but not all studies [11, 20]. A PSG study of 23 children with ASD and 23 age-matched controls identified more frequent disorders of partial arousal, consistent with sleep terrors or confusional arousals, and more frequent spontaneous arousals [21]. Such findings are consistent with a growing number of studies in which actigraphy and PSG have been used to study sleep in individuals with ASD. While these studies are often restricted by smaller sample sizes, two recent meta-analyses have aggregated and summarized these findings [17, 22]. From studies involving actigraphy, an increase in sleep onset latency emerged as the sole significant parameter affected in ASD (5 studies, $n = 276$) [17]. From studies involving PSG, parameters significantly affected in ASD include a decrease in total sleep time (8 studies, $n = 247$), a reduction in sleep efficiency (7 studies, $n = 238$), an increase in sleep latency (7 studies, $n = 211$), an increase in wake time (7 studies, $n = 211$) and a decrease in REM sleep (9 studies, $n = 273$) [17]. Another meta-analysis of these PSG studies in ASD found significant differences in total sleep time (10 studies, mean decrease of 32.8 min in ASD), sleep latency (9 studies, mean increase of 10.9 min in ASD), and sleep efficiency (10 studies, mean decrease of 1.9%) [22]. Characterizing the nature of sleep problems in ASD has many of the same limitations as those seen with prevalence estimates, including small sample sizes, the heterogeneity of ASD, and differences in methodology. In addition, many of the commonly used survey instruments, such as the Children's Sleep Habits Questionnaire (CSHQ), are designed primarily as screening tools to identify the presence of sleep problems, and have less power to discriminate between types of sleep problems [23].

A role for melatonin in ASD
Melatonin is an important regulator of the circadian sleep-wake rhythm, and there is evidence that dysregulation of this hormone might have a role in ASD-related sleep problems. Under normal physiological conditions in humans, plasma concentrations of melatonin follow a regular circadian pattern with low levels during the day and high levels at night [24]. The production of melatonin mainly occurs in the pineal gland, where release is entrained by ambient light via connections with the central circadian clock in the suprachiasmatic nucleus of the hypothalamus (SCN). The biosynthesis of melatonin occurs through an enzymatic process starting with the conversion of tryptophan to serotonin, which is then converted to melatonin through actions of two enzymes arylalkylamine-N-acetyltransferase (AA-NAT) and acetylserotonin O-methyltransferase (ASMT) [24]. Melatonin is of particular interest in ASD because of its role in neurodevelopment and sleep-wake rhythms, but also because abnormalities in the serotonin system are frequently identified in ASD [25]. The most consistent of these findings in ASD is a reduction in peripheral melatonin levels [26, 27]. The cause of this reduction in melatonin is thought to be an impairment of the serotonin-melatonin biosynthetic pathway, as there is evidence for reduced activity of the ASMT enzyme [27, 28]. Genetic variants within the promotor region of ASMT are more frequent in individuals with ASD and are associated with a decrease in the number of ASMT transcripts [27]. Consistent with these findings, it has been reported that 40% of those with ASD had abnormally high levels of serotonin in blood and 51% had abnormally low levels of melatonin, which would be expected if ASMT activity is reduced [28]. Additional evidence for a role for melatonin comes from the efficacy of supplemental melatonin in treating sleep problems in ASD, especially with improving sleep duration and sleep onset latency [29]. However, providing supplemental melatonin is not directly associated with changes in levels of melatonin, suggesting a more complex mechanism than simply replacing low levels of melatonin [30].

Relationships between sleep and behavioral dysregulation
The relationship between sleep dysregulation and behavioral dysregulation has received considerable attention in studies of ASD. In children and adolescents, sleep disturbances are associated with a wide variety of emotional and behavioral difficulties [31]. Numerous studies support the possibility that insufficient sleep exacerbates the behavioral symptoms of ASD [reviewed in ref. [13]]. In children with ASD, fewer hours of sleep per night predict greater severity of core ASD behaviors, such as deficits in social skills [32]. Additionally, higher levels of sleep problems are associated with higher levels of externalizing behavioral problems (e.g., aggression, impulsivity) and internalizing behavioral problems (e.g., withdrawal, anxiety, depression) [33]. Multivariate analyses suggest that sleep problems account for between 22–32% of the variance of behavioral problems in children with ASD [34]. While sleep problems are thought to worsen behavior, the inverse might also be true, with behavioral problems worsening sleep problems. Each of the core behavioral features of ASD may play roles: deficits in communication can interfere with social-related circadian cues, whereas difficulty understanding parental communication can interfere with putting children to bed, and repetitive and/or ritualistic behaviors can lead to difficulties with sleep when bedtime routine is disrupted [35]. Additionally, abnormal sensory functioning is common in ASD and over-responsivity to environmental stimuli and difficulties in regulating arousal can lead to problems with sleep-onset and insomnia [36]. In a population of hospitalized children with ASD, higher scores on the Aberrant Behavior Checklist-Community scale (ABC-C) (Irritability, Stereotypy, and Hyperactivity subscales) at admission were correlated with significantly fewer minutes slept during the last five nights of hospitalization [37]. These findings have led to the hypothesis that sleep and behavioral dysregulation may have a bidirectional relationship in ASD (Fig. 1, Condition B.). While this is a prevalent hypothesis, the vast majority of support is based on cross-sectional studies in which the relationship between sleep and behavior is only captured at one point in time, making both directionality and causality difficult to establish. A recent longitudinal study of sleep in 5151 children with ASD has challenged the assumption of a bidirectional relationship between sleep and

Sleep as a translationally-relevant endpoint in studies of autism…
G Missig et al.

92
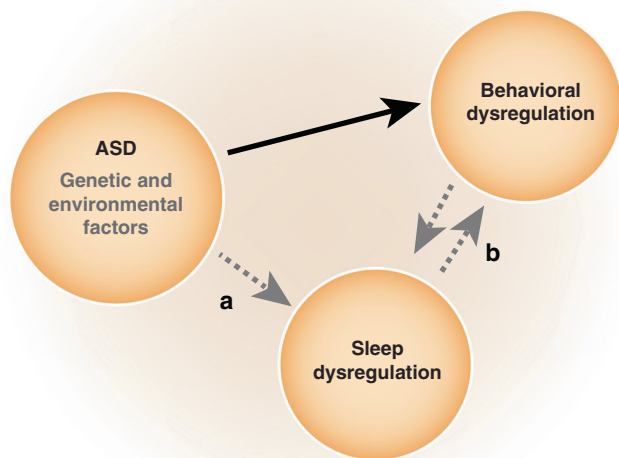
Fig. 1 Schematic of hypothesized etiology of sleep dysregulation in ASD. Sleep dysregulation may stem directly from the underlying pathology of ASD (**a**) and/or indirectly as the consequence of a bidirectional relationship with behavioral dysregulation (**b**)

autistic behavior [38]. Evaluating sleep and behavioral problems in children with ASD between 1.5 and 9 years of age revealed that even though higher levels of sleep problems are more likely to be associated with higher autistic traits, neither precedes or predicts the other over time [38]. These findings raise the possibility that sleep dysregulation may instead be a separate construct directly related to the underlying etiology of ASD (Fig. 1, Condition A).

The cause of sleep problems in ASD
The cause of sleep problems in ASD is still unknown. Mazzone, Postorino [35] have proposed three possible etiological explanations for sleep problems in ASD. Possibility 1 is that sleep problems arise as a direct consequence of the underlying biology and genetics of ASD. This explanation would suggest that underlying changes in the neural process and circuits controlling the sleep-wake cycle is disrupted in ASD, for example, physiological changes in the melatonin, GABAergic circuits, or overall synaptic balance. Possibility 2 is that sleep problems arise from the clinical phenotype and core symptoms of ASD. As discussed above, several of the core behavioral features of ASD communication and social deficits and the presence of repetitive/stereotyped behaviors could directly lead to difficulties with sleep onset and insomnia. Possibility 3 is that sleep problems reflect a co-occurring condition that is completely independent from ASD and are related to psychiatric comorbidities. Numerous common psychiatric comorbidities of ASD such as attention deficit hyperactivity disorder (ADHD) or anxiety and mood disorders are also associated with sleep difficulties and as such sleep may be an intermediate phenotype of these conditions. Given the complex and heterogenous nature of ASD, the etiology is likely to be multifactorial and a single explanation is unlikely to be true for all individuals with ASD. Regardless, understanding the etiology of sleep problems in ASD is crucial as it can have direct implications for care and treatment. The growing understanding of the neurobiology of both ASD and sleep fueled by discoveries in animal models may present opportunities to begin to address these questions.

A ROLE FOR ANIMAL MODELS IN THE STUDY OF ASD
Types of animal models for ASD
Most fundamentally, animal models for ASD can be categorized as focusing on genetic (G) factors, environmental (E) factors, or G × E factors. Genetic models involve deletions or mutations that are associated with ASD—most commonly mice—but also in rats, drosophila, zebrafish, and recently non-human primates [39]. They often imitate monogenic syndromic ASD, which are forms of ASD due to chromosomal abnormalities or mutations that affect a single gene. These are powerful models due to being able to directly model a known cause for select forms of ASD (e.g., Fragile X syndrome), but are limited in scope as such conditions represent a small portion (~5%) of ASD prevalence [40]. Other genetic models alter the function of ASD associated risk genes or introduce identified copy number variations (CNVs) identified in humans with ASD, or use various inbred mice that exhibit behaviors resembling ASD. Explanations for the genetic basis of ASD are becoming increasingly complex, with more recent suggestions that the majority of genetic risk for ASD may be the result of numerous common genetic variants [2]. Each of these genes individually tend to have small effects, but impacts can be synergistic when combined. Unfortunately, modeling such a complex genetic landscape in laboratory animals is currently not possible and represents a substantial hurdle for genetic models used to study ASD.

In contrast, environmental models mimic identified risk factors for ASD during early development. Two of the most commonly used models are maternal exposure to valproic acid (e.g., refs [41–43]), which is based on the identified risk of valproate during fetal development and ASD, and maternal immune activation, which is based on epidemiological findings of viral and bacterial infection during pregnancy increasing the offspring's risk of developing ASD (e.g., refs [44, 45]). A variety of other environmental risk factors have also been examined in the context of ASD such as exposure to diesel exhaust, maternal autoantibodies, and hypoxia [46–48]. A limitation of these approaches is that these epidemiological risk factors are often not unique to ASD; for instance, maternal inflammation has been associated with a wide range of psychiatric conditions including schizophrenia, bipolar disorder, and ADHD [49–52]. Furthermore, whereas certain genetic models reflect direct causal relationships between genes and illness that have been established in humans, in environmental models the causal links in humans are based on an associational studies, making assessment of construct validity (i.e., how accurately the model reflects the underlying biology) more complicated.

Uncovering the relationship between ASD and sleep
Animals models offer unique insights into the relationship between sleep dysregulation and ASD. Use of animal models enables exquisite control over numerous potentially confounding variables while utilizing objective measures of sleep (electroencephalography [EEG], electromyography [EMG], and actigraphy) that are analogous to human measurements. Whereas studies in humans are challenging because of the broad inclusiveness of the ASD diagnosis, animal models enable the study sleep in relation to known or hypothesized etiologies and pathophysiologies. By their very nature, animal models eliminate extraneous environmental factors that often complicate and/or confound studies in humans. For instance, impaired social ability in ASD might make parental interactions more difficult and complicate bedtime behaviors, whereas in animal models such concerns are mitigated as sleep can be studied in isolated subjects. Considering that there are many ways in which the core ASD behavioral phenotype (deficits in social behavior and communication and repetitive/stereotyped behaviors) might directly contribute to sleep dysregulation, animal models can offer key evidence for uncovering the relationship between the dysregulation of behavior and sleep in ASD. Findings

Sleep as a translationally-relevant endpoint in studies of autism…
G Missig et al.

93

of sleep dysregulation in animal models more directly support that sleep difficulties emerge as a construct (core feature) of ASD (Fig. 1, Condition A) rather than as a secondary consequence of behavioral dysregulation (Fig. 1, Condition B).

Sleep as a translational endpoint for understanding ASD
Studying sleep may provide opportunities to improve the animal models for ASD by enhancing their translational value. Modelling neuropsychiatric disorders in animals has been a significant challenge due to both the complexity of these conditions and the unique human attributes of many of the symptoms, as well as a lack of objective endpoints that are homologous across species. These problems are especially salient within the study of ASD, considering that recently there have been several prominent failures of drugs for Fragile X syndrome that were based heavily upon their initial and promising efficacy in genetic animal models of this condition [53]. Broad failures of drug candidates that were based on promising findings in animals models to translate to the human condition have contributed to a massive divestment of industry development of neuropsychiatric drugs and led to fundamental questions of the utility of animal models in studying psychiatric conditions [54]. Animal models for ASD often use classical behavioral tests related to core ASD symptoms [55]. For example, social behaviors are often assessed by examining the duration with which a mouse interacts with a novel age- and sex-matched partner mouse. Communication-related behaviors are often inferred from changes in the quantity of ultrasonic vocalizations. To test repetitive or stereotyped behaviors, the degree of self-grooming or performance on a rotarod (which can be enhanced) are often used as endpoints. However, these classical behavior tests have significant limitations. First, these tests are based on a face validity that is ultimately derived from anthropomorphic evidence as they require inferences of the cognitive and emotional states of an animal in a given situation. Second, they often only capture brief "snapshots" of time, with most tests occurring only over 5–10 min during a brief (and often arbitrary) portion of the day, missing any abnormalities that are not occurring during this period. Finally, they often lack tests or endpoints that are directly analogous in humans, adding more complexity to bench-to-bedside translation. There is increasing interest in using measures that overcome these limitations [56]. In this regard, studying sleep/wake physiology may have numerous advantages. Sleep is thought to be a critical biological process and is based on neurophysiological states that can be readily determined in both animals and humans using the same types of objective measures. Additionally, sleep and actigraphy studies are able to examine function over longer time frames, from days to weeks, instead of minutes in classical behavioral studies. Prospectively, the emergence of digital technologies and devices (e.g. fitness trackers, smartphones) are making studies of sleep/wake physiology more easily accessible and may provide new opportunities to align basic and clinical research [57].

Considerations in using animal models for sleep studies
To effectively study sleep in laboratory animals, one must consider the known similarities and differences of sleep across species. Most—if not all—vertebrate animals display the characteristic signs of sleep and there is evidence that sleep may serve an essential biological function [58, 59]. Many of the molecular mechanisms that subserve sleep are conserved between diverse species, from flies to humans [60]. Sleep can be identified through behavioral, physiological, and electrophysiological measures. In mammals and birds, sleep/wake states are often determined from EEG and EMG recordings. Sleep can be divided into rapid-eye-movement (REM) sleep (also referred to as paradoxical sleep [PS]) or non-rapid-eye-movement (NREM) sleep (also referred to as slow-wave sleep [SWS]). NREM sleep is characterized by low-frequency, high amplitude delta EEG oscillations, low muscle tone,

and sleep spindles. In humans, NREM can be further divided into different stages. REM sleep is characterized by pronounced theta oscillations and a complete loss of muscle tone (muscle atonia). Wake is characterized by muscle activity and higher frequency EEG oscillations. EEG features can vary across species; for example, sleep involves only a single brain hemisphere in some species, whereas others lack the characteristic features of REM sleep [61]. Sleep can also be determined behaviorally, characterized by a quiescence of motor activity, elevated thresholds for arousal, and through homeostatic need [60]. There is variation in the daily duration, cyclical organization, and diurnal timing of sleep among different species and even different strains of inbred laboratory mice [62]. In contrast to humans, mice and rats predominantly sleep during the light phase and are awake during the dark phase. Sleep in humans is normally consolidated and monophasic (one daily sleep/wake cycle), whereas sleep in mice and rats is more fragmented and polyphasic (multiple daily sleep/wake cycles) [61]. As a result, sleep in animal models must always be considered in the context of the species being studied. However, despite these differences, the common and conserved features of sleep and the ways in which its architecture is defined across species makes it an endpoint of great potential relevance for translational studies.

Considerations in using animal models for ASD
Animal models are increasingly being generated to assess underlying mechanisms of ASD. However, numerous technical and interpretive issues may limit the reproducibility of findings in these models. With genetic models, the ways in which a particular mutation is created and maintained can be critical for accurate characterization of how a gene relates to a particular phenotype [reviewed in refs [63–65]]. An unfortunately common issue relates to use of mixed genetic backgrounds, where a mutation is produced in one strain of mouse and bred to a second strain. The mixed genetic background of the progeny may inadvertently influence the phenotype if not properly controlled, as the chromosomal regions linked or flanked by the target gene will contain more of the genomic materials from the strain of the mutant founder. Several strategies to reduce this bias include using congenic mice in which backcrossing for 10 generations or more is performed, using co-isogenic mice in which the cells used to generate the mutant mouse originate from the same strain, or using an F1 hybrid in which the first generation cross is evaluated [64]. Additionally, it is becoming clear that environmental factors can have a strong influence on phenotypes of rodent models, such as rearing environment or the caging system used and should be carefully controlled and reported [66].

Incorporating sex differences
Identifying and understanding sex differences have recently gained considerable interest, driven by changing policies by funding agencies, an increasing awareness of their impact, and a growing consensus that continuing to neglect sex differences will be detrimental to brain- and health-related sciences [67]. ASD is a heavily sex-biased disease with a 4:1 male to female sex ratio in prevalence [68]. ASD may manifest differently within females, and there is a hypothesis that females may be protected against some symptoms of ASD—the so-called "female protective effect" [69, 70]. Alternatively, the "extreme male brain theory of autism" proposes that there are differences in male and female brains, and in ASD there is an extreme or hypermasculinized version of the male brain [71]. As such, sex differences have been influential in the conceptualization of ASD and may provide insights into the underlying mechanisms of ASD. Sex differences also exist within sleep. While large-scale studies are sparse, there are reports that women have greater total sleep time and have a higher percentage of slow-wave activity both at baseline and during recovery sleep following sleep deprivation [72]. Paradoxically, women also have higher rates of a wide range of sleep problems

Sleep as a translationally-relevant endpoint in studies of autism...
G Missig et al.

94
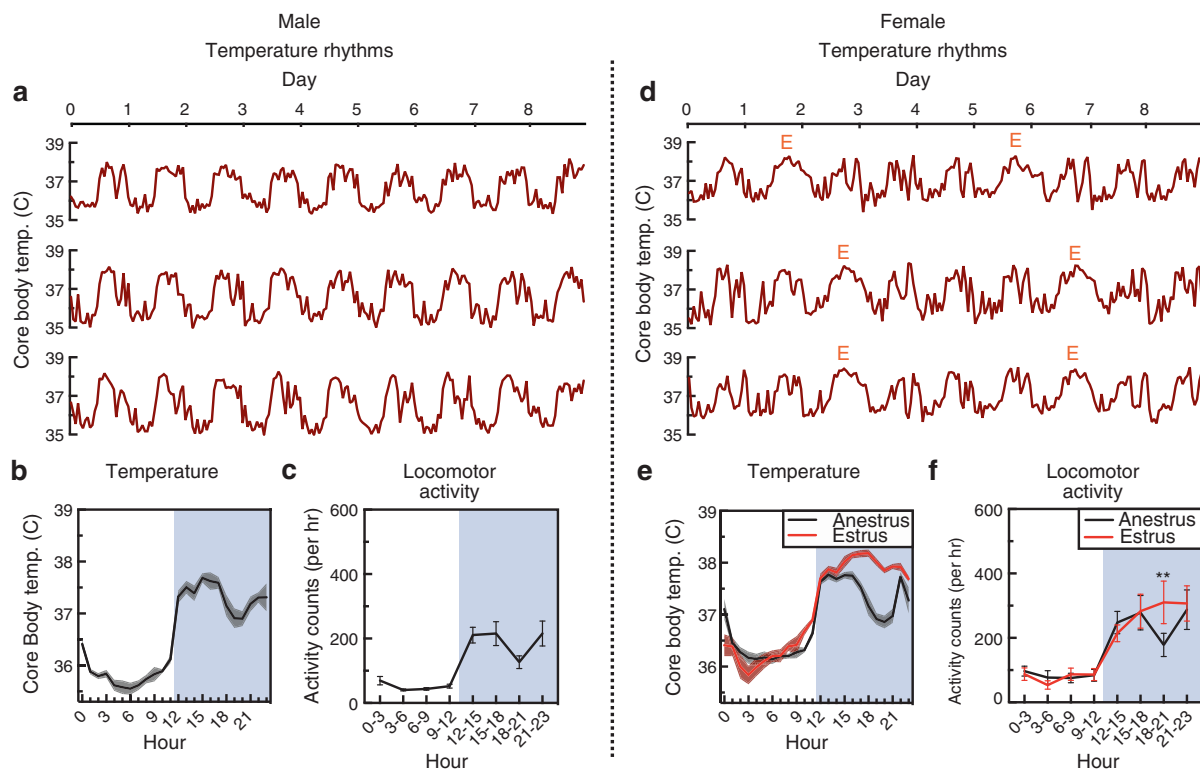
Fig. 2 Sex differences and influence of body temperature and locomotor activity. Males have regular monophasic temperature oscillations, seen on example recordings from eight days (**a**), and averaged temperatures (**b**), that correspond to rhythms of locomotor activity (**c**). Due to the estrous cycle females have a four-day pattern of polyphasic oscillations during anestrus and a monophasic peak (E) that readily distinguishes the night of estrus (**d**, **e**). Locomotor activity is also selectively increased in estrus during the middle of the dark phase (**f**). Data from 5–6 C57BL/6J mice. **$p < 0.01$

including poorer overall sleep quality, disrupted sleep, and insomnia [72]. Studies in rodents also indicate sex differences in sleep and suggest a crucial role for gonadal hormones. At baseline, female mice spend more time awake and decreased NREM compared to male mice, but following sleep deprivation show a more robust NREM recovery response [73]. The majority of these differences disappear following gonadectomy [73]. Furthermore, administration of estradiol alone or in combination with progesterone to rats promotes arousal by consolidating wake and fragmenting sleep in the dark phase, but not light phase [74]. These results are consistent with a well-established effect of the estrous cycle on sleep and activity, which in rats and mice follows a 4–5-day cycle consisting of multiple hormonal and behavioral phenomenon that often—but not always—coincide. The estrous cycle can be observed in the regular pattern of body temperature oscillations in female mice [45, 75]. Whereas male mice have consistent body temperature oscillations, female mice show a polyphasic profile (multiple peaks) during most of the cycle, but show a pronounced monophasic profile (one peak) during the middle of the dark phase at the start of estrus [75] (Fig. 2). Coinciding with this peak is a selective increase in locomotor activity 6–9 h following start of the dark phase. Both the increase in temperature and locomotor activity are dependent on gonadal hormones, and are lost following ovariectomy [75]. While readily modulated by environmental factors, typically this period corresponds to behavioral estrus (spontaneous activity and sexual receptivity) that is followed shortly by the start of ovulation in the same night [76]. Elevations in locomotor activity, body temperature, and sexual receptivity are inherent to the term "estrus", which is derived from the Greek word for "gadfly" or "frenzy"; indeed, a colloquial (and increasingly anachronistic) euphemism for this period is "in heat" [77]. Coinciding with these alterations,

during the dark phase of proestrus rats show significantly less NREM and REM sleep and spend greater time awake [78, 79]. Whether or not humans with a menstrual cycle have a corresponding behavioral estrus period is debated [80]; thorough investigation of sex differences may require examination of potential differences both across time-of-day and stage of cycle. In a mouse model of prenatal immune system activation, we recently identified a phenotype that is "silent" in females except within the small portion of the dark phase where the activity is increased during estrus [45]. While identifying sex-related differences in sleep behavior may require complex experimental designs, understanding the factors that contribute to them may enable unique new insights on the pathophysiology of ASD.

SLEEP IN ANIMAL MODELS FOR ASD
Environmental models for ASD
Prenatal valproic acid (VPA) exposure. Valproate is a medication used for the treatment of epilepsy and bipolar disorder. However, it has been discovered that prenatal (in utero) exposure to valproate significantly increases the risk of developing ASD [81]. The mechanism of these effects is not understood, but may be related to general toxicities of the drug or its actions as a histone deacetylase inhibitor. In rodent (rat or mouse) studies, prenatal valproic acid (VPA) exposure induces numerous sex-dependent behavioral abnormalities later in life that resemble core features of ASD [41–43]. Prenatal VPA exposure also induces sleep dysregulation. For example, rats treated prenatally with VPA exhibit frequent arousals during the light/sleep phase, as measured by increased levels of locomotor activity and increased bouts of feeding [82]. Consistent with these findings, another report indicated that prenatal VPA-treated rats spend more time in wake

Sleep as a translationally-relevant endpoint in studies of autism…
G Missig et al.

95

and less in NREM sleep during the light phase [83]. These changes coincide with an increase in high-frequency EEG spectral power during wake and REM sleep, and a decrease in theta power across all vigilance states [83]. Two potential mechanisms have been proposed for these alterations in sleep. First, prenatal VPA treatment increases basal levels of serotonin in the brain [82], which is consistent with findings of dysfunction in the serotonin-melatonin biosynthetic pathway in ASD. Second, prenatal VPA decreases cortical levels of GAD65 and GAD67—markers of GABAergic synapses—suggesting potential disruption of GABAergic signaling that might lead to imbalances in overall inhibitory tone [83]. These possibilities are not mutually exclusive: either mechanism, or both, could be contributing to the effects of VPA on sleep.

Prenatal immune activation. There is accumulating evidence for a potential immune involvement in ASD [84]. There are epidemiological associations between various forms of inflammation during pregnancy—in the form of bacterial or viral infection, or a parental autoimmune disorder—with an increased risk for the offspring to develop ASD [49, 85, 86]. Prenatal (or maternal) immune activation models have been developed where immune-stimulating agents are administered to animals (mice, rats, monkeys) during pregnancy to assess the consequences to neurodevelopment of the offspring. Across these species, maternal immune activation can lead to disruptions in behaviors that resemble aspects of both ASD and schizophrenia [87, 88]. Two studies have included evaluations of sleep in these models. In one study, lipopolysaccharides (LPS) administered near the end of gestation in rats led to no significant changes in sleep at baseline or following an acute stressor [89]. In contrast, a study from our group examined sleep in a "two-hit" immune activation model in mice, where polyinosinic-polycytidylic acid (Poly I:C) is administered during mid-gestation and a second "hit" (LPS) is administered on postnatal day 9 (roughly equivalent to the end of the third trimester in humans based on neurodevelopmental milestones) [90]. This regimen produces offspring with reductions in social behavior and increases in anxiety-like behavior, changes in synaptic strength of prefrontal to amygdala neural circuits and a persistent increase in microglia and neuroinflammatory markers in the brain [44]. The combination of prenatal and early postnatal immune activation also altered sleep with a selective increase in SWS, a shift in EEG spectral power, and spontaneous epileptiform activity (spike-wave discharges) [90]. Effects on sleep were strongest in mice that received the combined treatment; however, qualitatively similar effects were seen in mice that received only the postnatal (second) "hit", whereas prenatal Poly I:C had no effects. These studies raise the possibility that either the timing of the immune system activation and/or the specific receptor target of LPS (in this case, toll-like receptor-4 [TLR4]) may play critical roles in regulating the effects on sleep. Many of these findings resemble the effects in the first several hours following acute administration of cytokines or LPS either peripherally or directly into the brain of rodents [91, 92]. As such, alterations in sleep in this model may reflect a state of elevated inflammatory signaling in the brain. The two different findings with prenatal Poly I:C and postnatal LPS are not surprising as animal models of perinatal immune activation have often had variable results. Emerging research is suggesting that environmental factors, the gut microbiome, the strain and vendor of mice, and the timing and type of immune activation used can all lead to highly divergent phenotypes [45, 66, 93].

Genetic models for ASD
Fragile X syndrome (FXS). Fragile X syndrome (FXS) is the most common heritable cause of intellectual disability and one of the most thoroughly studied of the monogenic syndromes associated with ASD [94]. In FXS, the fragile X mental retardation gene (*FMR1*)

is silenced, which leads to nonfunctional fragile X mental retardation protein (FMRP), an RNA-binding protein involved in the development of synaptic connections [95]. There are reports of sleep dysfunction in FXS, with parental reports indicating that 32% of children with the condition have sleep difficulties [96]. Additionally, a PSG study indicates reduced time in bed, lower sleep efficiency, and decreased NREM sleep [97]. Sleep and circadian disturbances are found in multiple models of FXS. Deletions of the *Drosophila* homolog to *FMR1 (dFmr1)* leads to arrhythmic locomotor activity in conditions of constant darkness (DD), but not under normal light:dark conditions (LD), whereas overexpression of *dFmr1* leads to a lengthened circadian period implicating the d*Fmr1* gene in regulation of circadian rhythms [98, 99]. Furthermore, d*Fmr1* directly regulates daily sleep need with a loss of *dFmr1* increasing sleep duration and overexpression of *dFmr1* decreasing sleep duration [100]. In mammalian species, models are more complicated as two paralogs to *Fmr1* exist—*Fxr1* and *Fxr2*—that can compensate for loss of *Fmr1*. In mice, loss of either *Fmr1* or *Fxr2* decreases activity rhythms in DD conditions only, but combined deletion of *Fmr1* and *Fxr2* decreases activity rhythms in DD and LD conditions [101]. Interestingly, sleep disruption may follow a developmental time-course, as *Fmr1* deletion leads to significant decreases in sleep during the light phase at later stages in development (postnatal day 70 and 180) but not at early stages (postnatal day 21), and sleep loss is worsened by incorporating the heterozygous deletion of *Fxr2* [102]. While the mechanisms of these effects are unknown, the *Fmr1* gene may directly modulate the cortical networks that regulate sleep. In vivo Ca^{2+} imaging in mice lacking *Fmr1* revealed hypersynchronous neural activity in cerebral cortex, with increased neuronal involvement during the neuronal "up states" that occur every 1–2 s and are characteristic of SWS and quiet wakefulness [103]. Dysregulation of melatonin secretion is another potential mechanism, as chronic melatonin treatment is capable of reducing the learning deficits and enhanced anxiety-like behaviors of mice lacking *Fmr1* [104].

Rett syndrome (RTT). Rett syndrome (RTT) is an X-linked neurological disorder caused by mutations in the *MECP2* gene encoding for methyl-CpG-binding protein 2 (MeCP2) [105]. RTT syndrome occurs almost exclusively in females, and individuals with RTT often meet criteria for ASD based on behavioral features during childhood [106]. RTT was historically classified as an ASD, but was removed from *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (DSM 5) due to its known etiology reclassifying it as a medical condition [107]. Sleep problems occur in more than 80% of patients with RTT, with daytime napping, night-time laughing, teeth grinding, night screaming, and seizures being the most common problems [108, 109]. Animal models of RTT involve different strategies to alter MeCP2 expression, such as conditional deletion, cell-type-specific deletion, or introduction of various mutations in *Mecp2*, which can all recreate many of the behavioral ASD-like features [110–112]. Sleep disruption is found in many of these models. *Mecp2*-deficient mice have substantially reduced locomotor activity levels, highly fragmented wakefulness, and alterations in the timing but not quantity of sleep [113–115]. Additionally, the frequency of hippocampal theta rhythms is decreased and there is spontaneous abnormal rhythmic EEG cortical activity in these mice [116]. The advent of new gene-editing technologies (e.g., CRISPR-Cas9) has enabled the development of genetic models of syndromic ASD in non-human primates [39]. Expressing loss-of-function mutations within the human MeCP2 gene in cynomolgus monkeys creates a model that strongly resembles RTT on multiple behavioral and physiological measures [117]. For example, eye-tracking of *MeCP2*-deficient monkeys suggests that they show less interest in emotionally charged monkey faces, but prefer neutral monkey faces as compared to wildtype monkeys. This model also exhibits altered

Sleep as a translationally-relevant endpoint in studies of autism…
G Missig et al.

96

sleep patterns. Using video recording and experimenter rating, *MeCP2*-deficient monkeys spend significantly more time awake and have more fragmented sleep, showing more bouts of wake and sleep in comparison to wildtype monkeys [117]. Studies in drosophila also demonstrate that cell-type-specific changes in *MeCP2* expression can affect sleep. Expression of human *MeCP2* in astrocytes leads to a reduction in total sleep time, whereas expression in octopamine (homolog of mammalian noradrenaline) neurons causes phase-specific sleep deficits, increases in sleep latency, and sleep fragmentation [118, 119]. Mechanistically, MeCP2—which functionally is involved in DNA methylation—can directly modulate the central circadian system. MeCP2 is expressed in the SCN, where it becomes phosphorylated following light stimulation [120] and transcriptionally activates the circadian clock genes *Per1* and *Per2* [121]. SCN neurons of *Mecp2*-deficient mice have reduced spontaneous firing rates and decreased rhythmic expression of PER2 [113, 114].

Prader-Willi syndrome (PWS). Prader-Willi syndrome (PWS)—together with Angelman syndrome (AS) (see below)—is a disorder caused by imprinting deficits in chromosomal region 15q11-q13. The 15q11-q13 region contains (1) a set of non-imprinted genes, (2) imprinted genes that are paternally expressed only including the bistronic *SNURF-SNRPN* gene, (3) preferentially maternally expressed genes, including the E3 ubiquitin ligase (*UBE3A*) gene, and (4) non-imprinted genes with paternally biased expression [122]. AS is due to loss of expression from the maternally inherited portion of this region, with alterations in *UBE3A* being critical, whereas PWS is due to loss expression of paternally inherited genes in this region. PWS is associated with excessive appetite with a gradual development of obesity, short stature, intellectual disability, and behavioral problems [122]. In those with PWS due to uniparental disomy, an estimated 35% met criteria for ASD, and in those with PWS due to deletion, 18% met criteria for ASD [123]. In an elegant cross-species comparison, Lassi, Priano [124] examined the role of the PWS gene candidate small nucleolar ribonucleic acid-116 (SNORD116) comparing sleep in paternal heterozygous deletion of *Snord116* in mice, with sleep in two human PWS analogous cases characterized by deletions of *SNORD116*. Mutant mice had increased REM and decreased NREM, with an increase in the number of REM sleep episodes, and specific increases in EEG theta power during REM. The individuals with PWS with SNORD116 deletions had remarkably similar changes with increased REM and decreased NREM, an increased number of REM sleep episodes, REM sleep intrusions, and a selective increase in theta power during REM [124].

Angelman syndrome (AS). Angelman syndrome (AS) is a due to the disruption of the maternally inherited *UBE3A* gene. AS typically presents with motor dysfunction, severe intellectual disability, seizures, significant language impairment, happy demeanor, and is commonly comorbid with ASD [125]. Besides the loss-of-function in AS, multiple mutations of *UBE3A* are associated with ASD including duplications, triplications, and other gain-of-function mutations [126]. Sleep problems are common in AS with reductions in total sleep time, increases in sleep onset latency, disrupted sleep architecture, and reductions in REM sleep [127, 128]. Maternal-specific deletion of *Ube3a* in mice increases the number, but not duration, of SWS and wake episodes, decreases the number and duration of REM episodes, and leads to characteristic paroxysmal EEG discharges [129]. Furthermore, loss of maternal *Ube3a* drastically disrupts sleep homeostasis. Following 4 h of sleep restriction, these mutant mice fail to show a rebound increase in SWS duration or an increase in delta power, as well as a smaller increase in REM sleep [129, 130]. In one study, maternal *Ube3a* deletion altered circadian rhythms, causing disruptions in circadian locomotor activity patterns in free-running conditions and in response to entrainment, as well as

circadian changes in metabolic homeostasis and functional circadian periodicity that are restored in neurons by unsilencing of the paternal *Ube3a* gene [131]. However, in another study circadian rhythms were mostly intact, although maternal *Ube3a* deletion eliminated the siesta (timed rest period) during the dark phase, which may be the consequence of an inability to accumulate sleep pressure [130]. The mechanisms for sleep dysregulation in AS could be diverse, since UBE3A is a ubiquitin ligase involved in targeting a wide range of proteins for degradation. UBE3A is important in synaptic plasticity, and deficits in *Ube3a* impairs experience-dependent maturation of cortical circuits and Ube3a ubiquitination of the protein Arc is critical for synaptic plasticity [132, 133].

Tuberous sclerosis complex (TSC). Tuberous sclerosis complex (TSC) is multiple organ system disorder caused by a loss of function in one of two genes: *TSC1* or *TSC2*. The clinical features of the disease include tumors in multiple organs, seizures, cognitive disability, and ASD [134]. TSC1 and TSC2 form a protein complex that acts as an inhibitor of the mechanistic target of rapamycin (mTOR), a key cell growth and regulation pathway that regulates the translation of thousands of genes [135]. Disruptions of sleep and circadian rhythm are frequently reported in TSC patients including early arousals, sleep-maintenance insomnia, and fragmented sleep architecture especially in the presence of seizures [136, 137]. In *Drosophila*, heterozygous loss of homologs to *TSC1* or *TSC2* results in significantly weaker circadian rhythms and longer circadian periods [138]. Recently, a conditional deletion of TSC1/2 mouse model revealed that a direct interaction of the TSC protein complex with the central clock component BMAL1 could be the mechanism explaining these circadian abnormalities [139]. Deletion of TSC1 or TSC2 in post-mitotic neurons led to abnormalities in circadian rhythms with a loss of rhythmicity and shorter circadian periods under DD conditions, but were capable of entrainment on LD conditions. The mTOR inhibitor rapamycin—which is currently under clinical trials for treatment of TSC—was able to normalize the shortened period phenotype. Importantly, mTOR can regulate the proteostasis of BMAL1, and BMAL1 function is increased when *Tsc* gene function is lost. By genetically lowering BMAL1 expression, the abnormal circadian phenotype of *Tsc2* deficient mice can be rescued [139].

Smith–Magenis syndrome (SMS) and Potocki–Lupski syndrome (PTLS). Smith–Magenis syndrome (SMS) and Potocki–Lupski syndrome (PTLS) are neurodevelopmental conditions caused by either deletion of chromosomal region 17p11.2 (in SMS) or duplication of 17p11.2 (in PTLS) [140]. While not typically considered ASDs, both frequently involve ASD behavioral features, with ~90% of individuals with SMS and ~80% of individuals with PTLS meeting the criteria for ASD [141, 142]. Aberrant function of the *Retinoic Acid Inducible 1* (*RAI1*) gene, which is within the genomic interval for both SMS and PTLS, seems critical to both of these disorders: point-mutations within *RAI1* in SMS recapitulate almost all of the phenotype of larger deletions [143] and *RAI1* is the smallest overlapping genomic region in PTLS duplications [144]. SMS and to a lesser extent PTLS are characterized by severe sleep disruptions and circadian abnormalities [141, 145]. Strikingly, the circadian rhythm of melatonin secretion is essentially inverted in SMS [146]. Mouse models of these conditions recapitulate many of the phenotypes of these disorders, including changes in body weight (increased in SMS and decreased in PTLS), altered activity (hypoactivity in SMS and hyperactivity in PTLS), learning and memory deficits, disrupted social behavior, and abnormal circadian period [142, 147]. *Rai1* deletion in specific neuronal populations is sufficient to recapitulate learning deficits and obesity characteristic of the human conditions [148]. Together, these disorders represent an example of two neurodevelopmental disorders that have severe disruptions in sleep and circadian

Sleep as a translationally-relevant endpoint in studies of autism…
G Missig et al.

97

rhythms that are also present with ASD, raising the possibility of common pathophysiologies.

Timothy syndrome. Timothy syndrome is a neurodevelopmental condition caused by mutations in the *CACNAC1C* gene encoding for Ca$_v$1.2, that lead to multiorgan dysfunction, including lethal heart arrhythmias, congenital heart disease, immune deficiency, intermittent hypoglycemia, cognitive abnormalities, and ASD in ~80% of cases [149]. The most common mutation, a G406R missense mutation, alters the negative feedback mechanisms of voltage and calcium-dependent inactivation in L-type calcium channels [150]. As yet, sleep has not been in evaluated in an animal model that fully recapitulates the type of alteration in L-type channels of this mutation. Nonetheless, heterozygous deletion of *Cacnac1c* in mice suggest its involvement, as this results in normal total sleep amounts per day but more sleep in the dark period, a reduction in REM sleep recovery following sleep deprivation or restraint stress, and decreased EEG gamma power compared to wildtype mice [151].

Cortical dysplasia-focal epilepsy syndrome (CDFE). First described in an Amish family in 2006, Cortical dysplasia-focal epilepsy syndrome (CDFE) is characterized by epileptic seizures, language regression, hyperactivity, aggressive behavior, and intellectually disability, of which ~66% of cases meet the criteria for ASD [152]. In this family a recessive mutation was identified within the gene connactin associated protein-like 2 (*CNTNAP2*) also known as (*CASPR2*), that encodes for a protein within the neurexin family [152, 153]. Numerous groups have now demonstrated that common and rare variants in *CNTNAP2* conferred risk to both ASD and ASD-related endophenotypes including language delay [153, 154]. In mice, deletion of *Cntnap2* produces an ASD-like phenotype, including deficits in social interaction, communication-like behavior, and repetitive behaviors-which are mitigated by risperidone treatment—as well as hyperactivity and epileptic seizures [155]. Abnormal sleep-wake physiology was recently described following the deletion of *Cntnap2* in both mice and rats, although there were important species differences [156]. In mice, deletion of *Cntnap2* induces absence seizure-like events, decreases in activity, and wake fragmentation. In rats, deletion of *Cntnap2* induces severe motor seizures, increases in activity, and a consolidation of wakefulness. In both species, *Cntnap2* deletion reduces spectral power in the alpha range during wake.

16p11.2 hemideletion. Chromosomal copy number variations (CNVs) are associated with ASD [157], including hemideletion of the chromosomal region 16p11.2, which greatly increases the risk for both ASD and ADHD [158]. Mice with 16p11.2 hemideletion have numerous behavioral phenotypes reminiscent of the human condition, with alterations in brain structure coinciding with cognitive and communication-related behavioral deficits [159, 160]. Angelakos, Watson [161] characterized sleep and circadian alterations in mice with 16p11.2 hemideletion. In both male and female mice, 16p11.2 hemideletion leads to robust hyperactivity, with no overt changes in circadian rhythm. In males, but not females time awake is increased while NREM is decreased. Furthermore, male 16p11.2 hemideletion mice have a greater proportion of prolonged bouts of wakefulness [161].

Neuroligins (NLG). Neuroligins (NLG) are a family of cell adhesion molecules found on the surface of the postsynaptic membrane that facilitate the connection of neurons at synapses [162]. Some studies have identified rare single gene variations of NLGs in ASD [163, 164], whereas other studies failed to demonstrate an association [165]. There are five identified members of the neuroligin family in humans and four in rodents [166]. The distribution of the NLGs is isoform specific, with NLG1 being found predominantly at excitatory synapses, NLG2 exclusively on inhibitory synapses, and NLG3 at both excitatory and inhibitory synapses [166]. *Nlg1* deletion in mice causes deficits in spatial learning and memory, along with repetitive, stereotyped grooming behavior—a potentially autism-related abnormality [167]. Several of the NLGs may be involved in sleep-wake regulation. *Nlg1* deletion in mice decreases wake and increases NREM sleep [168]. Additionally, sleep deprivation can either increase or decrease expression of *Nlg1* depending on the specific variant [168]. *Nlg4* deletion in drosophila leads to longer sleep onset and less total sleep time and impairs GABAergic synaptic transmission [169]. The NLG3^{R341C} point mutation is found in rare cases in ASD [163]. Introducing the Nlg3^{R451C} mutation into mice leads to impaired social interaction, enhanced spatial learning abilities, and an increase in inhibitory synaptic transmission, effects that do not occur following complete deletion of *Nlg3* [170]. Nlg3^{R451C} mice have a normal duration and distribution of sleep states, but have differences in their EEG power spectrum, with increases in sigma and beta power during wake and decreases in delta power during NREM sleep [171].

Phosphatase and tensin homolog (PTEN). Cowden Syndrome and several related disorders caused by mutations in phosphatase and tensin homolog (*PTEN*) are associated with multiple tumor-like growths, macroencephaly, and neurobehavioral features that often meet criteria for ASD [172, 173]. Conditional deletions of *Pten* in the CNS of mice can mimic behavioral traits of several of the phenotypes of ASD [174, 175]. Results suggesting circadian abnormalities in these mice have been mixed and may be dependent on the type of deletion. For example, conditional deletion of *Pten* in cortical and hippocampal neurons results in spontaneous epileptiform activity and a constant, lengthened circadian free-running period [176]. However, while haploinsufficiency of *Pten* reproduces many of the behavioral features of ASD in mice, including sex-specific changes in locomotor activity, no changes in the length of the free-running period have been observed [175].

SYNTHESIS AND FUTURE DIRECTIONS

There is now substantial evidence for a diverse array of sleep abnormalities in animal models for ASD. In general, animal models for ASD exhibit some form of alteration in sleep or circadian rhythm (Table 1). The diversity of these changes is not surprising given the heterogeneity of ASD, but in the few instances when specific models are compared to the human condition of the same etiology, the types of alterations observed are consistent across species. These findings support the concept that sleep dysfunction is inherent to many forms of ASD—a frequently co-occuring medical condition rather than a diagnostic criterion. Additionally, findings in animal models suggest that sleep problems derive from the underlying neurobiological pathophysiology of ASD, as opposed to being a non-specific (secondary) biproduct of behavioral problems.

One intriguing possibility is that sleep dysregulation in ASD may reflect imbalances in synaptic homeostasis. In ASD, a dysregulation in synaptic homeostasis has been proposed as one of the central pathophysiological mechanisms [177]. Many of the monogenic syndromes can be considered "developmental synaptopathies" and converge on processes critical to synaptic signaling, plasticity, and development [178]. As examples, FXS, TSC, and AS are all characterized as disorders of transcriptional regulation, local translation, and protein quality control [178]. Many of the risk genes for ASD identified in genome-wide association studies are involved in synaptic plasticity, synthesis, or degradation, and transcriptome analysis from post-mortem brain tissue from ASD suggest a dysregulation cluster of synaptic and neurotransmitter signaling genes [177, 179]. Concurrently, there is substantial evidence that sleep may be crucial for synaptic

Sleep as a translationally-relevant endpoint in studies of autism...
G Missig et al.

98

Table 1. Summary of studies of sleep in animal models for ASD

Study	Relevant human condition	Manipulation	Species	Sleep and circadian-related phenotypes
Tsujino et al, 2007	ASD	Prenatal VPA	Rat	More frequent arousals during the light phase
Cusmano and Mong 2014	ASD	Prenatal VPA	Rat	Increased wake and decreased NREM during the light phase
Zager et al. 2014	ASD and SCZ	Prenatal Immune Activation	Rat	No changes in sleep at baseline or following stressor
Missig et al. 2018		Perinatal Immune Activation	Mouse	Increased SWS and epileptiform activity
Dockendorff et al. 2002	Fragile X syndrome	dFMR1 loss-of-function	Drosophila	Arrhythmic circadian activity and erratic locomotor activity
Inoue et al. 2002		dFMR1 loss-of-function	Drosophila	Arrhythmic circadian activity in DD
Zhang et al 2008		FMR1 and FXR2 deletion	Mouse	Shortened circadian period in DD and decreased rhythmicity in LD
Bushey et al. 2009		dFMR1 deletion	Drosophila	Deletion of dFMR1 increased- and overexpression decreased sleep time
Wither et al. 2012	Rett syndrome	MeCP2 deletion	Mouse	Disrupted circadian body temperature and activity rhythms
Tsuchiya et al. 2015		MeCP2 deletion	Mouse	Decreased circadian rhythms in DD and altered central clock gene expression
Li et al. 2015		MeCP2 deletion	Mouse	Fragmented sleep, decreased strength and precision of circadian rhythms
Chen et al. 2017		MeCP2 deletion	Cynomolgus Monkey	Increased wake and fragmented sleep
Lassi et al. 2016	Prader-Willi syndrome	SNORD116 deletion	Mouse	Increased REM and decreased NREM duration, and increased REM episodes
Colas et al. 2005	Angelman syndrome	Ube3a deletion	Mouse	Increased number of SWS and wake episodes
Shi et al. 2015		Ube3a deletion	Mouse	Weakened circadian rhythms and disrupted metabolic homeostasis
Ehlen et al. 2015		Ube3a deletion	Mouse	Blunted recovery following sleep deprivation
Zheng and Sehgal 2010	Tuberous Sclerosis Complex	TSC1/TSC2 deletion	Drosophila	Weaker circadian rhythms and longer circadian period
Lipton et al. 2017		TSC1/TSC2 deletion in neurons	Mouse	Loss of rhythmicity and shorter circadian period in DD
Kumar et al. 2015	Timothy syndrome	Cacna1c (Cav1.2) deletion	Mouse	Reduced REM sleep recovery
Thomas et al. 2017	CDFE	Cntnap2 deletion	Mouse and Rat	Rats: hyperactivity, increased and consolidated wake and REM, Mice: hypoactivity and fragmented wake
Angelakos et al. 2016	ASD and ADHD	16p11.2 −/+deletion	Mouse	Hyperactivity, increased and consolidated wake in males
El Helou et al. 2013	ASD	NLG1 deletion	Mouse	Decreased wake and increased NREM
Li et al. 2013	ASD	NLG4 deletion	Drosophila	Delayed sleep onset and decreased sleep time

ADHD attention deficit hyperactivity disorder, *ASD* autism spectrum disorder, *CDFE* cortical dysplasia-focal epilepsy syndrome, *DD* dark/dark conditions or constant darkness, *LD* light/dark conditions, *NREM* non-rapid eye movement sleep, *REM* rapid eye movement sleep, *SCZ* schizophrenia, *SWS* slow-wave sleep

Sleep as a translationally-relevant endpoint in studies of autism...
G Missig et al.

99

homeostasis [180]. The highly influential sleep homeostasis hypothesis (SHY) proposes that the function of sleep is to regulate synaptic strength [181]. SHY suggests that during wake there is a net increase in overall synaptic strength, and that sleep globally downscales synaptic strength to restore cellular homeostasis [182]. While phenomena potentially contrary to SHY have been observed, such as synaptic strengthening during sleep, the crucial role of sleep in synaptic homeostasis has consistently been supported [183, 184]. Together, this may suggest that in ASD an intrinsic imbalance in synaptic homeostasis may manifest in abnormalities in sleep. This may suggest that altered sleep would be a direct consequence of an underlying pathophysiology of ASD. As such, measurements of sleep would provide an accessible neurophysiological metric of impairment in ASD that may be an ideal target in mechanistic studies aiming to treat or reverse the condition.

While a substantial body of work indicates that sleep is disrupted in ASD and in animal models for ASD, how this relationship changes over development has been far less studied. Sleep is highly developmentally regulated, changing dramatically during brain development, and the coincident maturation of sleep and synaptic physiology has been speculated to play a role in sleep disruption in neurodevelopmental disorders [reviewed in ref.: [185]]. In one of the few longitudinal studies, children with ASD followed a different trajectory of sleep problems compared to TD children. In TD children, sleep problems tended to decrease over time, whereas in children with ASD sleep problems worsened [38]. Additionally, alterations in sleep may have predictive value for the development of ASD. In one study infants with more sleep problems by 12 months had an increase in ASD symptoms a year later [186]. Future studies are needed to understand how the relationship between sleep and ASD evolves over the course of development and to determine whether altered sleep could be a factor that contributes to the pathophysiology ASD. Parallel studies and consistent results in animal models would increase confidence in the translational relevance of sleep-related endpoints. Furthermore, while not discussed in detail in this review, robust electrophysiological differences often exist in humans with ASD and animal models for ASD, including changes in baseline levels of spectral power and the presence of epileptiform activity [90, 187–189]. While these types of physiological measures have features that could make them valuable as biomarkers of ASD, alterations in baselines can complicate the interpretation of any putative changes in sleep architecture. Additionally, the majority of studies to date have focused on establishing that sleep differences exist while thus far offering limited mechanistic insights. With the advent of new methods (e.g., wireless EEG) in neuroscience there have been considerable advances in the understanding of the underlying neural circuits regulating sleep/ wake physiology [190, 191] under increasingly ethologically-relevant conditions (e.g., freely moving/untethered) and time periods (e.g., weeks to months). How these specific neural circuits are affected in ASD is still unknown, but many of the technologies now exist that have the ability to uncover the mechanisms governing sleep disruptions in ASD.

CONCLUSIONS
Sleep is commonly disrupted in ASD. Animal models of ASD frequently recapitulate these sleep disruptions, suggesting that altered sleep/wake physiology appear to be well preserved across species. Studies in laboratory animals also support the interpretation that sleep disruptions likely derive directly from the underlying pathophysiology of ASD, as opposed to reflecting non-specific signs of more generalized behavioral problems. Beyond these implications, examining sleep may have particular utility in animal models for ASD in translating to humans. Sleep has several advantages compared to classical behavioral approaches,

including being highly objective, having common definitions and analogous metrics that can be measured across species, and being conducive to collection of continuous data sets over long periods of time. As theories of ASD pathophysiology and the function of sleep become more refined and focused on key roles for synaptic homeostasis, sleep may offer a more direct measure of altered neurophysiology in ASD compared to traditional behavioral tests in humans or lab animals. As such, the study of sleep in animal models for ASD may enable fundamentally new insights on the condition and strategies for more effective therapeutics.

ADDITIONAL INFORMATION

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Sleep as a translationally-relevant endpoint in studies of autism…
G Missig et al.

100

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G Missig et al.

101

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G Missig et al.

102

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Sleep as a translationally-relevant endpoint in studies of autism...
G Missig et al.

103

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