Sleep and Development in Genetically Tractable Model Organisms

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ABSTRACT Sleep is widely recognized as essential, but without a clear singular function. Inadequate sleep impairs cognition, metabolism, immune function, and many other processes. Work in genetic model systems has greatly expanded our understanding of basic sleep neurobiology as well as introduced new concepts for why we sleep. Among these is an idea with its roots in human work nearly 50 years old: sleep in early life is crucial for normal brain maturation. Nearly all known species that sleep do so more while immature, and this increased sleep coincides with a period of exuberant synaptogenesis and massive neural circuit remodeling. Adequate sleep also appears critical for normal neurodevelopmental progression. This article describes recent findings regarding molecular and circuit mechanisms of sleep, with a focus on development and the insights garnered from models amenable to detailed genetic analyses.

KEYWORDS sleep; development; invertebrate sleep; ontogeny; *Caenorhabditis elegans*; *Drosophila melanogaster*; *Danio rerio*

DISORDERS disrupting sleep during childhood and adolescence undermine physical health as well as emotional, cognitive, and social development. Therefore, despite caveats associated with any single line of evidence, sleep is widely believed to play a crucial role in the developing brain (Owens 2014). Despite great interest and progress, the interplay between sleep and development is not well understood. In their seminal article, Roffwarg *et al.* (1966) demonstrated in humans that the total amount of daily sleep and the percentage of rapid eye movement (REM) sleep peak in infancy and decrease with age (Kleitman and Engelmann 1953; Parmelee *et al.* 1961; Roffwarg *et al.* 1966; Hoppenbrouwers and Sterman 1975; Awoust and Levi 1984). This maturation of sleep characteristics as an organism develops from embryo to mature adult is referred to as sleep ontogeny or ontogenetic sleep changes. Similar trends, where the amount of total sleep, or vertebrate REM sleep, is highest early in development, have been observed in mammals, fish, birds, insects, and nematodes (Jouvet-Mounier *et al.* 1970; McGinty *et al.* 1977; Szymczak

1987; Shaw *et al.* 2000; Kirov and Moyanova 2002; Paredes *et al.* 2006; Raizen *et al.* 2008; Hasan *et al.* 2012; Todd *et al.* 2012; Sorribes 2013). These findings led to the hypothesis that during early life, REM sleep and invertebrate sleep may play an important role in the development of the nervous system, by establishing a period of globally heightened plasticity and/or providing endogenous, specialized activity in certain neural circuits (O'Donovan 1999; Mohs and Blumberg 2008, 2010; Blumberg *et al.* 2013; Corner 2013). Such specialized activity could, for instance, promote appropriate neurodevelopment through feedback by coupling function (such as recurring limb movement) to the refinement of neural connections. Consistent with this notion, nonlethal deprivation can cause long-lasting neurological and behavioral defects in vertebrates and invertebrates alike (Halbower *et al.* 2006; Jan *et al.* 2010; Kayser *et al.* 2014). Yet a mechanistic grasp is lacking with regard to how sleep ontogeny is controlled and why inadequate sleep during development is particularly deleterious.

How can relatively simple models inform such broad questions? The universality of sleep in the animal kingdom has been a matter of long-standing debate, but it is now widely accepted that zebrafish, fruit flies, and nematodes sleep (Table 1; Cirelli and Tononi 2008). As discussed below, sleep is developmentally regulated across these species, suggesting deep conservation of ontogenetic sleep changes and providing an opportunity to leverage the powerful genetic approaches in

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Table 1 General and developmental sleep features across phylogeny

Criteria		Human	Rodent	Fish	Fly	Worm
Sleep (general)	Behavioral quiescence	Y	Y	Y	Y	Y
	Increased arousal threshold	Y	Y	Y	Y	Y
	Circadian control of sleep onset	Y	Y	Y	Y	N ^a
	Homeostatic sleep rebound	Y	Y	N	Y	Y
	Postural change	Y	Y	Y	Y	Y
	Physiological correlates	Y	Y	?	Y	Y
	Impaired performance after sleep deprivation	Y	Y	?	Y	Y ^b
Sleep (ontogeny)	Developmental control of sleep onset/amount	Y	Y	Y	Y	Y
	Reduced sleep depth with developmental progression	Y	Y	?	Y	?
	Structural (brain) abnormalities with sleep loss	Y	Y	?	Y	?
	Adult behavioral sequelae of juvenile sleep loss	Y	Y	?	Y	Y ^b
	Correlated timing of early sleep with major brain remodeling	Y	Y	Y	Y	Y
	Larval sleep	NA	NA	Y	?	Y
	Embryonic sleep	Y	Y	?	?	?

Most features of sleep during development and in adulthood are conserved across a wide variety of species. Negative sequelae of sleep loss during mature and immature states are likewise highly conserved. Y, yes; N, no; NA, not applicable.

^a Developmentally timed sleep is controlled by *lin-42*, the worm homolog of the clock gene *per*.

^b D. Biron, unpublished observations.

these systems to answer questions at the intersection of sleep and development. Here, we review the connections between sleep and appropriate development of nervous systems with a focus on genetic model organisms. Recent findings in fish, flies, and nematodes join and expand on studies of mammalian sleep in immature states. Taken together, this work suggests a fundamental role for sleep in patterning of the brain and sculpting neural circuits throughout development.

Negative Impacts of Disrupted Sleep During Mammalian Development

One of the strongest pieces of evidence to suggest a privileged role for sleep during development is the unique consequence of disturbed sleep within this period. Testing this idea has proved difficult in humans and other mammals. Most work in humans has taken advantage of a common and naturally occurring sleep disruptor, obstructive sleep apnea (OSA). OSA in adults and children alike results in numerous complications, including cognitive dysfunction (Findley *et al.* 1986; Gozal 1998; Beebe *et al.* 2003; Archbold *et al.* 2004). Indeed in children, sleep-disordered breathing has been associated with reduced cognitive function and general intelligence, as well as neuroimaging-based evidence for prefrontal cortical abnormalities (Schechter 2002; Gottlieb *et al.* 2004; O'Brien *et al.* 2004; Halbower *et al.* 2006); severity of apnea also correlates with worsened neuropsychological deficits (O'Brien *et al.* 2004). A caveat to utilizing OSA as a means to study the impact of sleep fragmentation is the difficulty in distinguishing the effect of sleep loss from that of poor oxygenation or even obesity, which could independently impair developmental processes. However, snoring with sleep fragmentation in the absence of abnormal oxygenation also negatively affects multiple cognitive measures (Gottlieb *et al.* 2004), lending credence to the hypothesis that sleep itself is of primary importance.

If sleep has a special role in brain development, disturbing sleep processes in children should have a more severe impact

than in adults, although it is challenging to obtain direct evidence for this idea in humans. Suggestively, while attentional and cognitive measures are impaired in both, a correlation between OSA and general intelligence has been observed only in children (O'Brien *et al.* 2004; Alchanatis *et al.* 2005; Gozal 2008). Thus, sleep fragmentation during a heightened period of brain maturation might cause more profound and long-lasting impairments. However, most studies use premorbid IQ as a control between OSA and non-OSA populations and new onset of OSA in adults is difficult to predict and study.

Work in mammalian models has reached similar conclusions to those in humans, although also with constraints (Frank 2011). REM sleep deprivation in rodents with pharmacologic agents (such as tricyclic antidepressants, which inhibit serotonin–norepinephrine reuptake) during the perinatal period results in myriad adult deficits, such as increased anxiety and social behaviors, as well as abnormal sleep itself (Mirmiran *et al.* 1981, 1983a). Can REM sleep disturbances be dissociated from persistent alterations in monoaminergic signaling during a sensitive developmental period? REM sleep deprivation by distinct pharmacologic agents seems to cause inconsistent impairments (Frank and Heller 1997), and more specific REM inhibiting agents such as selective serotonin reuptake inhibitors do not result in adult sleep impairments (in contrast to tricyclic antidepressants) (Farabollini *et al.* 1988). Notably, gentle mechanical sleep deprivation likewise does not recapitulate tricyclic antidepressant exposure (Mirmiran *et al.* 1983b).

Other experiments have sought to disturb regions of the brain involved in pontine–geniculate–occipital wave generation, which is a core feature of REM sleep and thought to be critical for memory consolidation during sleep in adults. Lesions that eliminate pontine–geniculate–occipital waves in neonate kittens result in aberrant development of the lateral geniculate nucleus (Davenne and Adrien 1984). These results support the notion that REM sleep in early life could be crucial for normal brain patterning, although nonspecific

or non-sleep-dependent effects of the lesion are difficult to rule out. In line with these findings, REM sleep deprivation in adolescent mice delays the critical period of visual cortical development, suggesting REM sleep disturbances somehow impinge upon brain maturation (Shaffery *et al.* 2002; Blumberg *et al.* 2013). Compelling evidence for an active role of sleep in brain sensorimotor development has also come from rodent pups. Like human infants, rat pups spend a large amount of time in REM/active sleep, characterized by muscle atonia with sudden bursts of muscle twitches and resultant limb jerks. Blumberg *et al.* (2013) have shown that this twitching is highly structured and that sleep-dependent twitches may provide a source of patterned neural activity with a role in sensory map development. Whether sleep disruptions during specific developmental times permanently disturb map establishment remains unknown.

A different approach seeks to exploit differences between wild-derived inbred strains to identify genetic factors that regulate sleep and wakefulness. For instance, inbred mouse strains are generally nocturnal but can exhibit phenotypic differences in total amounts of sleep, distributions of sleep throughout the day, and consolidation of sleep periods. These can be correlated with differences in whole brain monoamine contents, which are under elaborate genetic control (Hiyoshi *et al.* 2014). However, this approach has not been widely applied to ontogenetic changes in sleep. Moreover, some of the caveats in interpreting the results of pharmacological perturbations amid the complexity of mammalian brain development and sleep ontogeny may still apply to genetic studies.

Finally, sleep plays an important role in synaptic plasticity (Abel *et al.* 2013), which has been most closely studied leveraging a form of visual-cortical plasticity that is highest in early development (Frank 2011). This body of work perhaps speaks more broadly to a role for sleep in consolidating changes in synaptic strength rather than a specific or limited function in development, *per se* (Frank 2015). In sum, experiments in humans and mammalian models highlight how a mechanistic link between ontogenetic sleep changes and brain development has remained tantalizing but elusive and present an opportunity to leverage powerful genetic models to facilitate progress.

Worm Sleep

The nematode *Caenorhabditis elegans* sleeps during lethargus, a 2- to 3-hr long developmental stage that precedes ecdysis at the termination of each larval stage (Cassada and Russell 1975; Raizen *et al.* 2008). Lethargus is notably characterized by behavioral quiescence—a cessation of feeding and episodic suspension of head and body motion (Cassada and Russell 1975; Singh and Sulston 1978; Raizen *et al.* 2008), with distinct posture (Figure 1) (Schwarz *et al.* 2012; Tramm *et al.* 2014). During lethargus, responses to external stimuli are reduced or delayed, and deprivation of quiescence by mechano- or optogenetic stimulation is followed by rebound sleep (Raizen *et al.* 2008; Cho and Sternberg 2014; Nagy

Figure 1 Postural/positional changes during sleep (right) and wakefulness (left) in model organisms. Worms assume a hockey-stick posture (Tramm *et al.* 2014). Flies have been observed to show a head “droop” and preference to sleep near food (Hendricks *et al.* 2000). Zebrafish exhibit two postures: floating with head droop or remaining horizontal near the bottom of the tank (Zhdanova *et al.* 2001).

et al. 2014). Weaker disruption to the architecture of quiescence also evokes homeostatic compensation, suggesting a deep complexity to worm sleep (Driver *et al.* 2013; Iwanir *et al.* 2013; Nagy *et al.* 2014).

The developmental nature of lethargus inherently emphasizes a conserved role for sleep in maturation, but its coupling to molting is a key difference from adult sleep (Singh and Sulston 1978). This naturally leads to the suggestion that mechanical restriction is the primary cause of lethargus quiescence; however, such an assertion is inconsistent with several lines of evidence, most notably that the onset of quiescence is abrupt, that quiescence is rapidly reversible, and that brief periods of normal locomotion are interspersed with quiescent bouts (Raizen *et al.* 2008; Nelson and Raizen 2013; Nagy *et al.* 2013; Tramm *et al.* 2014). Cessation of locomotion and feeding can also be induced in adult *C. elegans* by satiety, stress, or genetic manipulations. Examples of the latter include heat-shock-induced activation of epidermal growth factor signaling (Van Buskirk and Sternberg 2007), mutations affecting cGMP, cAMP, or TGF- β signaling (Raizen *et al.* 2008; You *et al.* 2008; Iwanir *et al.* 2013), specific rescue of a peptidergic or insulin/insulin-like signaling pathway (Driver *et al.* 2013; Hill *et al.* 2014; Nagy *et al.* 2014; Nelson *et al.* 2014), or optogenetic activation of an avoidance response-promoting neuron (Cho and Sternberg 2014). Such findings potentially decouple worm sleep from molting. Moreover, a recent study suggests that the regulation of stress-induced quiescence is distinct from that of developmentally timed sleep (Trojanowski *et al.* 2015).

What function might lethargus quiescence serve? The molt is a major biosynthetic event (Singh and Sulston 1978;

Nelson and Raizen 2013), so the involvement of sleep in this process lends credence to a hypothesized role for sleep in macromolecular biosynthesis (Mackiewicz *et al.* 2007; Nelson and Raizen 2013). In addition, large-scale synaptic formation and rearrangement in *C. elegans* occur during development. Eighty neurons (mostly motoneurons) of the 302 in the adult hermaphrodite develop postembryonically; moreover, synaptogenesis maintains synaptic density through the fivefold increase in body length during larval development (White *et al.* 1986). Interestingly, many synaptic formation and rearrangement events are temporally correlated with periods of lethargus (White *et al.* 1986). For instance, the dorsal GABAergic motor neurons (DD) remodel their synaptic pattern (from ventral to dorsal) during late first larval stage and its corresponding lethargus period (White *et al.* 1978; Walthall *et al.* 1993; Hallam and Jin 1998); migration of axons and synaptic formation in the egg-laying circuit likewise occur during this period (Shen *et al.* 2004). Therefore, lethargus provides the opportunity to study the link between sleep and development in this simple organism.

How similar is lethargus to sleep in mammals and other organisms? Genetically, deep cross-species conservation is found between lethargus, fruit fly sleep, and mammalian sleep (Singh *et al.* 2014). The *C. elegans* homolog of the circadian clock protein Period is required for clocking larval development and thus the timing of lethargus—its messenger RNA (mRNA) levels track the molting cycle (Jeon *et al.* 1999; Tennessen *et al.* 2006; Monsalve *et al.* 2011). Multiple additional conserved signaling pathways exhibit functional similarities in mammalian, insect, and nematode sleep, including epidermal growth factor signaling, protein kinase A (PKA) and G (PKG) activity, dopamine signaling, and pigment dispersing factor (PDF) signaling (Graves *et al.* 2003; Kramer *et al.* 2003; Snodgrass-Belt *et al.* 2005; Van Buskirk and Sternberg 2007; Foltenyi *et al.* 2007; Raizen *et al.* 2008; Langmesser *et al.* 2009; Choi *et al.* 2013, 2015; Iwanir *et al.* 2013; Singh *et al.* 2014).

Circuit mechanisms of *C. elegans* sleep and arousal likewise reveal parallels to those in flies, fish, and mammals. The nematode sleep circuitry includes multiple sensory circuits and classes of interneurons that display sleep-specific changes in activity and/or functional correlations (Schwarz *et al.* 2011; Choi *et al.* 2013, 2015; Iwanir *et al.* 2013; Cho and Sternberg 2014). Glutamate and PDF neuropeptides both mediate excitatory outputs from sensory neurons to promote arousal (Choi *et al.* 2013, 2015), consistent with pharmacological manipulations of glutamate in mammals (Juhász *et al.* 1990; Alam and Mallick 2008; Li *et al.* 2011) and the function of neuropeptides in vertebrates (orexin/hypocretin) and flies (PDF) (Sutcliffe and de Lecea 2002; Prober *et al.* 2006; Parisky *et al.* 2008; Choi *et al.* 2015). As both molecular and circuit mechanisms can be conserved across phyla, *C. elegans* lethargus can provide insight into core functions and regulation of sleep (Nelson and Raizen 2013; Singh *et al.* 2014).

Fly Sleep

Drosophila melanogaster, the fruit fly, was the first simple genetic system in which sleep was described (Hendricks *et al.* 2000; Shaw *et al.* 2000). Unbiased genetic screens have revealed numerous sleep-regulatory genes, pointing toward a deeper understanding of the genetics of sleep (Sehgal and Mignot 2011). Flies exhibit most behavioral features of sleep found in mammals, including a prolonged period of behavioral quiescence with increased arousal threshold, regulation by the circadian timing system, and homeostatic rebound in response to sleep loss (Hendricks *et al.* 2000; Shaw *et al.* 2000). In addition, sleep in flies as in mammals is suppressed with drugs that are wake promoting to humans, specifically caffeine and modafinil (Hendricks *et al.* 2000, 2001; Shaw *et al.* 2000). Remarkably, *Drosophila* recapitulate mammalian changes to sleep throughout their life span: young flies demonstrate increased sleep amount and depth, while old flies exhibit more fragmented sleep (Shaw *et al.* 2000; Koh *et al.* 2006; Seugnet *et al.* 2011a; Kayser *et al.* 2014). *Drosophila* life span is ~60–80 days, and the features distinguishing juvenile sleep from that of the mature adult occur only during the first 3–5 days after eclosion (Seugnet *et al.* 2011a; Kayser *et al.* 2014). During this juvenile period, flies are far more somnolent than adult flies: they sleep more during the day, are more difficult to arouse from sleep at night, and are notably resistant to sleep deprivation (Kayser *et al.* 2014).

Conserved ontogenetic sleep changes between flies and mammals have presented an opportunity to leverage the powerful genetic toolkit of the fly toward understanding the mechanisms and purpose of juvenile sleep. Although developmental changes to sleep quality/quantity are conserved across multiple species, mechanisms controlling such changes remain underexplored. Recently, work in the fly has demonstrated that a small group of dopamine neurons undergo developmentally regulated changes in activity that likely control sleep ontogeny (Kayser *et al.* 2014). These few neurons project to and inhibit a sleep-promoting center in the fly brain, the dorsal fan-shaped body (Liu *et al.* 2012; Ueno *et al.* 2012). In young flies, the dopaminergic neurons are less active, allowing increased activity of the dorsal fan-shaped body (dFSB) and increased sleep; with maturity, dopaminergic activity increases, dFSB activity is reduced, and mature sleep quantity and quality are achieved (Kayser *et al.* 2014). Dopamine serves a wake-promoting function in mammals as well, so this basic developmental paradigm may represent a conserved logic for control of sleep ontogeny (Figure 2).

Fish Sleep

Like roundworms and fruit flies, zebrafish (*Danio rerio*) also sleep. Fish show brief periods during which they stop swimming and are immobile, with increased arousal threshold; these behaviors occur nearly exclusively during the night (zebrafish are diurnal) (Zhdanova *et al.* 2001; Yokogawa *et al.* 2007; Appelbaum *et al.* 2009). Recent work leveraging

Figure 2 Sleep ontogeny changes across phylogeny. Flies, fish, and worms all exhibit developmentally regulated changes to sleep amount/timing in a manner akin to that of humans. Fruit flies and zebrafish have increased sleep amount in early life compared to mature adults. Nematodes similarly exhibit developmental timing to sleep patterns.

the genetic tractability of this vertebrate model organism has uncovered a role for melatonin in conveying the circadian control of sleep timing (Zhdanova *et al.* 2001; Gandhi *et al.* 2015). Moreover, zebrafish utilize the hypocretin/orexin signaling system (Zhdanova 2006; Appelbaum *et al.* 2010; Panula *et al.* 2010; Yelin-Bekerman *et al.* 2015), abnormalities in which are known to cause the human sleep disorder narcolepsy. Similar to its mammalian analog, the zebrafish hypocretin system is located in the hypothalamus and regulates sleep–wake transitions (Prober *et al.* 2006; Yokogawa *et al.* 2007; Appelbaum *et al.* 2009; Elbaz *et al.* 2013). It comprises merely 20–60 hypocretin neurons, several orders of magnitude fewer than those found in mice (Kaslin *et al.* 2004). Zebrafish genetics were exploited to identify a minimal promoter region capable of mimicking the native expression pattern of fluorescent protein in hypocretin neurons, implicate an AMPA receptor clustering protein in regulation of structural synaptic plasticity, tag and sequence hypocretin neurons, and use a CRISPR-mediated gene inactivation system to implicate a voltage-gated potassium channel in regulating sleep (Faraco *et al.* 2006; Appelbaum *et al.* 2010; Yelin-Bekerman *et al.* 2015). Therefore, the zebrafish hypocretin

system can serve as a simplified model, allowing sleep–wake regulation to be studied at nearly single-neuron resolution (Kaslin *et al.* 2004; Faraco *et al.* 2006).

Zebrafish share with *C. elegans* the optical advantage of transparency, allowing for the ability to monitor neuronal function/structure in real time in an organism that is anatomically similar to mammals. Capitalizing on this advantage, Appelbaum *et al.* (2009, 2010) showed that synaptic outputs of the hypocretin system are under circadian control, perhaps offering a mechanism linking the sleep and circadian systems. Although the neural circuits that regulate sleep in fish are not well understood at this time, the innervation pattern of the zebrafish hypocretin system resembles mammalian circuits for regulating sleep and wakefulness. For instance, serotonergic nuclei are densely innervated by hypocretin immunoreactive neurons (Kaslin *et al.* 2004; Panula *et al.* 2010). Detailed behavioral studies revealed conservation between fish and mammalian responses to pharmacological agents that promote or antagonize sleep (Rihel *et al.* 2010; Sigurgeirsson *et al.* 2011).

Do zebrafish exhibit ontogenetic sleep changes? Research in fish sleep has been undertaken both at the larval stage and

in mature adults; however, until recently, a direct comparison of sleep features throughout development has not been addressed. By examining four different developmental time points within the same monitoring system, it is now clear that zebrafish undergo dramatic sleep changes throughout development, with more sleep early in life. The excess of early sleep derived primarily from an increase in the number of sleep bouts (defined as periods of immobility >6 sec in duration) (Sorribes 2013). Thus, in addition to conserved anatomical and molecular sleep substrates between zebrafish and humans, sleep ontogenetic patterns are also conserved. These similarities support the notion of conservation of neural mechanisms for regulating sleep and wakefulness.

The Dolphin Dilemma

Increased sleep in early life is a widespread phenomenon, but is it universal, and, if not, how does that inform a hypothesized essential role for sleep during development? While controversial, some data suggest cetaceans (bottlenose dolphins and killer whales) actually show *less* typical sleep (rest on the surface) during the first month following birth; mothers likewise suppress sleep during this time, perhaps to remain vigilant in the postpartum period (Lyamin *et al.* 2005). The observed activity was not accompanied by stress as assayed by levels of the hormone cortisol. The authors hypothesize that constant activity could be advantageous to newborns both to avoid predation and to maintain body temperature while development ensues (Lyamin *et al.* 2005). This research was soon followed by two additional studies reaching the opposite conclusion (Gnone *et al.* 2006; Sekiguchi *et al.* 2006). Dolphins exhibit unihemispheric sleep, where slow-wave electroencephalogram (EEG) patterns restricted to a single hemisphere are linked to closure of the contralateral eye (Mukhametov 1987; Lyamin *et al.* 2002, 2004). Swimming and unihemispheric sleep are compatible: the underwater sleeping behavior termed “swim rest” is associated with continuous activity. The initial work may have underestimated swim rest (and perhaps alternative sleep strategies), as both follow-up studies concluded that bottlenose dolphin mothers and calves experience significant cumulative periods of swim rest during the postpartum period.

What are the potential implications of these findings for theories of the role of sleep in development? *In utero* sleep is prominently exhibited by all mammalian prenatals that have been carefully observed (Ruckebusch 1972; Drucker-Colin *et al.* 1979; Awoust and Levi 1984; Szeto and Hinman 1985). Human fetal sleep begins early in the third trimester of pregnancy. Synchronous waves of neuronal activity occur during REM sleep and are widely thought to be essential for synaptic formation and thus for the proper development of neurosensory systems, motor systems, and memory circuits (Marks *et al.* 1995; Mirmiran 1995; Segawa 1999). In contrast, there have been no reports of REM sleep in cetaceans. Disambiguating sleep states based on cetacean behavior is challenging, but even twitches (observed in adults) that

may correspond to REM sleep are extremely sporadic (Siegel 2005). Thus, the minimal amounts or absence of REM sleep may reflect peculiarities of the cetacean brain (Siegel 2005; Lyamin *et al.* 2008; Dell *et al.* 2015; Patzke *et al.* 2015). A detailed understanding of prenatal cetacean sleep patterns would provide context to the observations of postpartum behavior. A better mechanistic understanding of the roles of sleep in developing fish, flies, and nematodes would likewise inform our perspective.

Conservation of Sleep-Related Functions with “Developmental” Genes

Using unbiased and candidate-based genetic screens in model organisms, a cluster of genes with essential roles in basic developmental processes have been found to influence sleep in adulthood. Although a dual function in these processes is not unassailable evidence for a core role of sleep in development, it does suggest the two may be intimately linked at a genetic level. For example, several lines of evidence implicate Notch signaling in regulating nematode and fly sleep. In worms, perturbing the functions of either Notch receptors (LIN-12 or GLP-1) or ligands (OSM-7 and OSM-11) altered the total quiescence observed during L4 lethargus, albeit in complex fashion (Singh *et al.* 2011). Moreover, decreasing Notch signaling impaired sensory gating, *i.e.*, reduced the arousal threshold of quiescent L4 lethargus larvae. Overexpression of the Notch ligand OSM-11 induced anachronistic sleep-like quiescence in adult nematodes. Interestingly, Notch signaling regulates quiescence through pathways that were previously implicated in regulating sleep in invertebrates and vertebrates alike, such as epidermal growth factor (EGF) (see below) and cGMP-dependent kinase (PKG) signaling (Singh *et al.* 2011). In *Drosophila*, a specific role for Notch in sleep rebound, *i.e.*, in compensating for lost sleep following a delay in sleep onset or a disruption to sleep quality, has been proposed (Seugnet *et al.* 2011b). Following sleep deprivation, a negative regulator of Notch (*bunched*) is upregulated, while overexpression of Notch or its ligand Delta reduces sleep rebound. This works suggest that sleep deprivation normally suppresses Notch via upregulation of *bunch*, which permits the homeostatic sleep response.

Notably, spatial restriction of enhanced Notch signaling or overexpression of its ligand in a known sleep-promoting region (the mushroom body) is sufficient to reduce rebound sleep, arguing for anatomical specificity in Notch’s role. Despite apparent opposing effects on sleep for Notch in flies and worms, these works emphasize how a canonical developmental gene can adopt a critical role for sleep behavior (Wu and Raizen 2011).

In addition to Notch signaling, EGF (Foltenyi *et al.* 2007), Cyclin A (Rogulja and Young 2012), and ecdysone (Ishimoto and Kitamoto 2010) all appear to regulate sleep as well as function in their more conventional developmental roles. Overexpression of EGFR ligands in worms and flies induces sleep-like behaviors (Foltenyi *et al.* 2007; Van Buskirk and

Sternberg 2007; Raizen *et al.* 2008), and this behavioral state change acts through EGFR in both animals. The initial description of nematode sleep onset as molt specific was influential in the examination of a role for ecdysone—the major *Drosophila* steroid hormone—in fly sleep (Ishimoto and Kitamoto 2010). Ecdysone has a critical role in developmental molting transitions, including emergence of the adult fly during eclosion, after which sleep amounts/depth are highest. In mature adults, exogenous administration of ecdysone's active metabolite 20-hydroxyecdysone results in a dose-dependent increase in sleep amount, while sleep is reduced in ecdysone steroid or ecdysone receptor mutants (Ishimoto and Kitamoto 2010). Ecdysone thus represents an attractive candidate in *Drosophila* for coupling developmentally regulated processes to sleep need.

Ramification of Sleep Loss During Development

Ontogenetic sleep changes are conserved across phylogeny, and even in humans it is clear that sleep disturbances during critical developmental windows can have outsized neurobehavioral effects later in life. So, what happens to structural maturation of the brain when sleep in early life is perturbed, and are there global rules governing why certain brain regions might be affected more than others? In adolescent mouse development, most studies have focused on the role for sleep in structural plasticity *after* the phase of robust synaptogenesis (which occurs in first ~3 postnatal weeks), during which time synaptic pruning is, on balance, predominant. *In vivo* studies suggest that changes to dendritic spine number (and likely synapses) depend on sleep–wake state (Maret *et al.* 2011; Yang and Gan 2012), whereas in adults, sleep influences synaptic strength without changing the number of synaptic contacts (Maret *et al.* 2011). More recent work has closely examined the idea that sleep ontogeny in mice, which like in humans is characterized by a notable decline in slow wave activity beginning in adolescence, reflects pruning of synaptic contacts. Interestingly, the decrease in slow wave activity is uncoupled from any measureable changes to synapse number or size, unlike in more mature stages in which slow wave activity is hypothesized to be a marker for and perhaps driver of synaptic homeostasis (de Vivo *et al.* 2014). The data overall support that idea that during highly plastic developmental periods, sleep may sculpt synapses in a manner distinct from adulthood and thereby play a prominent role patterning neural circuits in early life. Indeed, during developmentally timed, robust stages of synaptogenesis, sleep may function specifically to drive addition of new synapses (Kayser *et al.* 2014). Studying sleep longitudinally during this time frame in mice is technically challenging, but work in fruit flies has provided novel insights.

Behavioral work in *Drosophila* has established that, as in mammals, sleep deprivation during sensitive developmental windows has long-lasting ramifications compared to sleep loss in adulthood. Mechanical sleep deprivation in juvenile flies leads to deficits in both courtship behaviors and performance in learning/memory assays as mature adults (Seugnet

et al. 2011a; Kayser *et al.* 2014). Might these changes simply result from heightened stress susceptibility during development? Leveraging the mechanistic understanding of circuits controlling sleep ontogeny in the fly, a specific wake-promoting circuit that is normally quiescent in young flies can be experimentally hyperactivated at inappropriate developmental times to more specifically probe the question. With this approach, sleep deprivation of juvenile flies has been associated with abnormal social behaviors in adulthood, without similar defects when sleep loss occurs in maturity (Kayser *et al.* 2014). Importantly, this effect does not appear to stem from a general “monoaminergic flood” during development, as restricted manipulation of sleep–wake circuits impairs behaviors arising through unrelated brain regions (Kayser *et al.* 2014). This finding, however, resurrects an older question: How are sleep disturbances in early life linked to later behavioral abnormalities? In the fly, courtship deficits in adult flies that were sleep deprived when young are accompanied by abnormal development of a specific olfactory glomerulus, known as VA1v, that plays a pheromone-related role in social behaviors (Dweck *et al.* 2015; Lone *et al.* 2015). Surprisingly, VA1v grows more rapidly during early posteclosion development compared to its neighboring glomeruli (Kayser *et al.* 2014), likely reflecting a higher rate of synapse addition. Together these findings suggest that sleep in early life is particularly crucial for areas undergoing high levels of synaptogenesis. This hypothesis can be tested across species and developmental time windows and could provide a global rule for why sleep amount/depth is highest (and impact of sleep disruptions most severe) during periods of explosive brain growth.

Sleep in Neurodevelopmental Mutants

Further highlighting the important link between sleep and development, sleep disturbances are pervasive across neurodevelopmental disorders in humans (Angriman *et al.* 2015). While difficult in young children to disentangle whether sleep abnormalities precede other neurocognitive symptoms, it is clear that sleep disturbances exacerbate an already challenging constellation of symptoms. Can model systems elaborate our understanding of sleep in neurodevelopmental disorders? Work in *Drosophila* indicates abnormalities in sleep or circadian rhythms are highly conserved across species. Fly models of fragile X syndrome (FXS) display aberrant circadian output (Dockendorff *et al.* 2002) and sleep. In particular, flies with a loss-of-function mutation in *dFmr1* display high sleep need (Bushey *et al.* 2009), perhaps with a specific abnormality of sleep ontogenetic change. Recent work indicates abnormal sleep in a fly model of neurofibromatosis 1 (NF1) (Bai and Sehgal 2015), and NF1 has previously been implicated in circadian output circuitry (Williams *et al.* 2001). Likewise, rest–activity rhythms are weakened in a *Drosophila* Angelman syndrome model (Wu *et al.* 2008) although sleep itself has yet to be examined. These fly models of neurodevelopmental disorders provide an opportunity to dissect sleep by development interactions with the goal of a

Figure 3 The omnipresence of sleep may be a signature of its contribution to satisfying a constraint inherent to development. Should the time pressure of development be thought of as a universal constraint that can be mitigated by a sleep–wake cycle? All nervous systems originate from a single cell. In a restricted period of time, they must undergo structural plasticity (e.g., synaptogenesis) on a scale that is comparable to their full size. If sleep provides vital aspects of the solution to this challenge, its ubiquity in the animal kingdom may be related to the common feat of development (or neurodevelopment in particular). Much like the contributions of simple organisms to understanding developmental processes, facile genetics and tractable anatomy are key to understanding ontogenetic changes, core functions, and evolutionary origins of sleep.

deeper genetic/molecular understanding of sleep in neurodevelopmental disorders. Of note, this line of work will not be limited to the fly going forward: a zebrafish model of Allan–Herndon–Dudley syndrome (ADHS) exhibits both abnormal (high) sleep levels and responses to light–dark transitions (Zada *et al.* 2014), and it is likely that additional models will continue to emerge. There are multiple questions uniquely suited to examination in simple genetic systems: Are specific sleep/circadian neural loci perturbed in neurodevelopmental disorders? Are common sleep-relevant genetic targets affected across these models? And can restoration of normal sleep improve performance in other affected behaviors?

Complex Questions, Simple Models

Simple models were foundational to the development of molecular biology (see, e.g., Cairns *et al.* 2007), and newly

available molecular approaches enabled the launch of neurogenetics, using invertebrate models to investigate the role of genetic pathways in regulating behavior and neurophysiology (Brenner 1974; Harris 2008). A prominent example invoked fly genetics to resolve the question of whether circadian rest–activity rhythms are endogenous or exogenously controlled. The molecular mechanisms underlying circadian clocks, first identified in a *D. melanogaster* screen (Konopka and Benzer 1971), were later found to be largely conserved in mammals (Takahashi *et al.* 2008); moreover, a human circadian rhythm disorder can be caused by mutations in the homolog of *per*, the first identified clock gene (Toh *et al.* 2001).

Perhaps the study of sleep—in particular during development—can similarly benefit from tractable models (Table 1). For example, beyond questions of development alone, an integrated understanding of the neural circuitry

regulating sleep and arousal remains unsolved in any organism. The nervous systems of *D. melanogaster* and *C. elegans* are amenable to systematic analyses toward providing a basic neural logic of sleep–wake balance (Choi *et al.* 2015; Sitaraman *et al.* 2015). Another key set of questions concerns the role played by sleep in reorganization of neural circuits during development. In contrast to numerous studies in vertebrates, data on lasting deficits incurred by invertebrate sleep loss during development are sporadic. Sleep loss in young adult flies leads to lasting deficits in social courtship behaviors traced to impaired development of a specific brain region (Kayser *et al.* 2014). Similarly, behavioral defects can be observed after partial sleep deprivation of *C. elegans*: non-lethal yet severe sleep deprivation during the fourth larval sleep period results in deficits in feeding behavior that persist in the adult animals (D. Biron, unpublished results). The degree of sleep-dependent reorganization in invertebrate neural circuits, activity in these circuits during sleep, and how such activity might promote appropriate neurodevelopment invite careful examination, which we suggest is best accomplished initially in species amenable to sophisticated genetics.

Lasting deficits from developmentally specific sleep loss naturally lead to asking how such ill effects might be mitigated; *i.e.*, what mechanisms facilitate compensation for disruptions or aid recovery on longer timescales. Stress response pathways, *e.g.*, insulin/insulin-like (IIS) or heat-shock factor (HSF)-dependent signaling, are activated by disrupting sleep in flies and worms (Shaw *et al.* 2000, 2002; Cirelli *et al.* 2006; Naidoo 2009; Brown and Naidoo 2010; Varshavsky 2012; Driver *et al.* 2013; Nagy *et al.* 2014). These findings indicate that, like mammalian sleep, invertebrate sleep can be a period of heightened vulnerability when otherwise benign stimuli act as stressors (Shaw *et al.* 2002; Driver *et al.* 2013; Nagy *et al.* 2014). In addition to the mechanistic links between sleep loss and other stressors, broader parallels can be drawn between research performed in the two fields. Typically, protective responses, *e.g.*, to heat shock, are complex and employ both cell-autonomous and cell-nonautonomous signaling pathways (Carvalho Marques *et al.* 2015; Mardones *et al.* 2015). Likewise, pathways that protect sleep and aid recovery after deprivation can span scales from the cellular to the organismal, involve intertissue communication, and employ complex cellular signaling. Notably, tractable models prominently contribute to understanding classical protective responses (Carvalho Marques *et al.* 2015; Mardones *et al.* 2015). Precisely because susceptibility to sleep loss is elevated in early life, considering the protective responses evoked by sleep disruptions during development is a natural approach for shedding light on generalizable functions of sleep. A deeper understanding of how developing organisms cope with sleep loss at a cellular level would likewise inform why sleep is so tightly protected in immature brains and delineate cellular signatures of sleep states throughout life.

A Link Between Development and the Ubiquity of Sleep?

Sleep remains a mysterious behavior, from its core functions at the cellular and systems level, to how sleep itself is adaptive (or maladaptive), to mechanisms compensating for sleep loss, to even whether sleep is universally essential (Campbell and Tobler 1984; Cirelli and Tononi 2008; Siegel 2009; McNamara *et al.* 2010). There are many arguments in support of the universality of sleep, but conservation of ontogenetic sleep changes with an active role in the developing nervous system is emerging as one of the strongest (Roffwarg *et al.* 1966; Jouvet-Mounier *et al.* 1970; McGinty *et al.* 1977; Shaw *et al.* 2000; Kirov and Moyanova 2002; Raizen *et al.* 2008; Hasan *et al.* 2012; Todd *et al.* 2012; Sorribes 2013; Kayser *et al.* 2014). The observation that sleep amount is highest during developmental periods across species has led to extensive and ongoing explorations of the ontogenetic hypothesis, suggesting that sleep promotes normal brain development by providing necessary endogenous activity (Roffwarg *et al.* 1966; Jouvet-Mounier *et al.* 1970; Oksenberg *et al.* 1996; Shaffery *et al.* 1999; Frank 2011; Blumberg *et al.* 2013; Kayser *et al.* 2014; Tononi and Cirelli 2014). The self-evident fact that all nervous systems originate from a single cell can be viewed as a truly universal constraint imposed by the very nature of development. Within a restricted period, which cannot be trivially prolonged, all nervous systems must undergo structural plasticity on a scale that is macroscopic with respect to their own size (Figure 3). Thus, the feat facing developing nervous systems (and perhaps other tissues) across the animal kingdom shares a common barrier, for which sleep may provide a vital solution. Genetically tractable organisms are particularly useful for understanding such ancient functions of sleep during development and the conserved (or convergent) mechanisms that regulate them.

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