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Call it worm sleep

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Abstract

The nematode *Caenorhabditis elegans* stops feeding and moving during a larval transition stage called lethargus and following exposure to cellular stressors. These behaviors have been termed "sleep-like states". We argue that these behaviors should instead be called "sleep". Sleep during lethargus is similar to sleep regulated by circadian timers in insects and mammals, and sleep in response to cellular stress is similar to sleep induced by sickness in other animals. Sleep in mammals and *Drosophila* shows molecular and functional conservation with *C. elegans* sleep. The simple neuroanatomy and powerful genetic tools of *C. elegans* have yielded insights into sleep regulation and hold great promise for future research into sleep regulation and function.

Keywords

sleep; quiescence; *Caenorhabditis elegans*; evolution; development; cellular stress

Behavioral and electrophysiological properties of sleep

The core function of sleep is a long-standing mystery. Theories for sleep function include roles in brain energetics [1], brain recovery [2], somatic functions and thermoregulation [3], biosynthesis [4], neural plasticity [5,6], and allocation of energetic resources [7]. A key challenge for sleep researchers is to determine whether identified sleep functions are particular to the organism of study, or if they represent core functions that led to the evolutionary maintenance of sleep states. Comparative physiology across phylogeny is a powerful approach to distill conserved sleep functions.

Until the mid-1930s, sleep in all animals was identified by three behavioral characteristics: decreased responsiveness to environmental stimuli, rapid reversibility to strong stimuli, and an increased threshold to arousal [8]. The development and use of the electroencephalogram (EEG) revealed that brain activity patterns during sleep are variable [9] and that sleep can be divided into physiologically distinct states based on electrical activity patterns [10]. The subsequent discovery of Rapid Eye Movement (REM) and non-REM sleep [11]

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demonstrated that these different sleep states are generated by distinct physiological processes [12]. Experiments using the EEG have also revealed that slow wave activity can serve as a biomarker for sleep need in certain settings [13,14].

In the 1960s, the ontogenetic hypothesis of sleep, which suggested sleep played an important role in the development of the nervous system specifically in young animals, came to prominence [15]. More recently, it was found that sleep can be triggered by both circadian [16] and non-circadian factors [17], such as bacterial infection [18], through at least partly distinct neural pathways [19,20]. Together, this indicates that there is additional heterogeneity in sleep. While the physiological differences between mechanisms generating REM and non-REM sleep are widely appreciated, the function and regulation of these states are incompletely understood [21]. Likewise, little is known about the physiological and functional differences between infection-induced sleep and circadian sleep, or between sleep at different developmental stages [22].

The past 15 years have witnessed the introduction of non-mammalian model organisms, such as *Danio rerio*, a zebrafish [23], and *Drosophila melanogaster*, a fruit fly [24,25], to sleep research, providing insights into the function and regulation of sleep. Because EEGs are not performed in non-mammals, sleep in such species must be defined by behavioral criteria so as to differentiate this state from quiet wakefulness or pathological quiescence. In addition to locomotion and feeding quiescence, rapid reversibility, and reduced sensory responsiveness, Tobler [26] and others [27,28] have proposed that a stereotypical body posture and a homeostatic response to sleep deprivation should be included in these criteria.

States that fulfill the behavioral criteria for sleep have been also identified in *Caenorhabditis elegans*, a nematode (Table 1). However, unlike in zebrafish and *Drosophila*, in *C. elegans* these behavioral states have been referred to as “sleep-like” states rather than “sleep” states [29,30]. We argue here that these nematode behavioral states should be referred to as sleep.

C. elegans sleeps

C. elegans has two states that fulfill all the behavioral criteria for sleep: developmentally timed sleep (DTS), or lethargus, and stress-induced sleep (SIS).

DTS occurs for two to three hours following each of the four larval stages [29,31], and is characterized by behavioral quiescence, stereotypical posture, decreased response to sensory stimuli, and homeostatic response to sleep deprivation [29,32]. Feeding and locomotion, which persist throughout the development of the worm (Video S1), cease during DTS (Video S2), and worms tend to assume a hockey stick-shaped posture [32–34]. The worms are more difficult to arouse during this time [29], partially due to decreases in evoked Ca^{2+} response in sensory neurons [35,36]. Deprivation of DTS, which can be lethal [37], is followed by a homeostatic rebound [29]. These bouts of immobility are interspersed with activity bouts, during which animals move but do not feed [32].

SIS occurs following exposure to environmental stimuli that cause cellular stress, and its duration depends on the severity of the stressor [30,38]. Like DTS, SIS is characterized by a similar cessation of feeding and locomotion and increased arousal threshold [30] (Video S3).

Although a homeostatic response to deprivation of SIS has not been documented, impaired SIS results in increased mortality [30], demonstrating that this sleep state has an adaptive physiological function. SIS can occur during any developmental state, but the possibility that prolonged waking is stressful, and therefore causes SIS, has not been tested.

Satiety behavior is induced by feeding on high quality food or by refeeding after starvation and shares behavioral quiescence with SIS and DIS, but its other sleep properties have not yet been assessed [39,40].

The importance of nomenclature

Why is the correct nomenclature important? One could argue that the term “sleep-like” has sufficed to advance the field. However, “sleep-like” carries the connotation that *C. elegans* sleep is “like” sleep but somehow not quite the same, as if there is a universally accepted definition of sleep that is met by other organisms but not *C. elegans*. What is this universally accepted definition of sleep that *C. elegans* “sleep-like” states fail to meet? Should a qualifying “like” be added to all invertebrate analogs of mammalian behaviors, such as learning, feeding, and reproduction?

This importance of a proper definition is not new to science. In the middle of the 20th century, researchers debated the definition and existence of a circadian clock [41]. However, by the early 1960s, the circadian field came to consensus criteria expected of a circadian clock [42], which were subsequently used to define and then study circadian rhythms in diverse organisms ranging from single cell algae to humans. Such comparative studies have led to great insight into molecular mechanisms governing circadian rhythms, as both similarities and differences between mechanisms of circadian clocks across phylogeny have been informative [43].

Likewise, the *Drosophila* sleep field demonstrates the practical importance of using the term “sleep”. From 2000 to 2005, nearly all publications dealing with *Drosophila* sleep used the term “rest” or “sleep-like” to refer to fly sleep. In 2005, the journal Nature published a paper titled “Reduced sleep in *Drosophila* Shaker mutants” [44]. Following this publication, there was an explosion in the number of labs studying *Drosophila* sleep. It was arguably not new data revealing additional properties of this state, but rather the decision of a prominent journal to use the term “sleep” that led to wide acceptance of this term and expansion of the field of *Drosophila* sleep research.

Molecular conservation of *C. elegans* sleep

Unlike circadian sleep in mammals and *Drosophila*, DTS occurs with an ultradian periodicity (it occurs four times during development, with a period of 7–9 hours) and is linked to the molting cycle rather than the 24-hour light-dark cycle [31]. Despite this difference, many molecular pathways regulating sleep in other species also regulate *C. elegans* sleep. The molecular connection between DTS and circadian sleep was first made via the gene *lin-42*. LIN-42 is the *C. elegans* homolog of PERIOD [45], a protein that cycles its expression in a circadian fashion in *Drosophila* [46] and mammals [47,48]. The *lin-42* gene product does not cycle its expression in phase with circadian time [49], but rather

cycles in phase with molting time and DTS [50,51]. Moreover, *lin-42* mutants show aberrant timing of molting and DTS behavior [52]. Thus, even though the *C. elegans* larval timer does not synchronize to circadian time, it has molecular homology with the circadian timer found in diurnal species.

Other signaling pathways are conserved between *C. elegans*, *Drosophila* and mammalian sleep, suggesting that the regulation of these states is broadly conserved (Table 2). Here, we discuss five of these conserved pathways in detail: the pigment dispersing factor (PDF), protein kinase A/cyclic adenosine monophosphate (PKA/cAMP), epithelial growth factor receptor (EGFR), dopamine, and protein kinase G (PKG) signaling pathways. We focus on these five pathways because they are the ones for which the most evidence has accumulated for similarities between nematodes and other species, but there are others that are likely conserved (e.g., serotonin [53] and glutamate [54,55]), which we do not discuss here.

PDF

The neuropeptide PDF was first implicated in the circadian regulation of behavior in *Drosophila*, as flies lacking PDF showed defects in the circadian timing of their locomotive activity [56]. Its effects on circadian behavior and sleep in flies have since been extensively documented [57]. PDF is a key output of two populations of circadian clock cells: when PDF is lost, these clock cells lose their synchronization [57]. PDF-expressing cells affect activity and other circadian behaviors most strongly in the morning, and flies mutant for PDF or its receptor PDFR display increased morning sleep [58]. In *C. elegans*, mutants for the PDF homolog *pdf-1* or its receptor *pdfr-1* have increased locomotion quiescence and decreased touch sensitivity during DTS [59], consistent with a wake-promoting effect of PDF. Release of *C. elegans* PDF-1 is greatly reduced during DTS [59] suggesting that activity of PDF-secreting neurons promotes arousal in *C. elegans*, as they do in *Drosophila* [60].

EGFR

Intracerebroventricular (ICV) injection of EGF promotes sleep in rabbits [61]. In hamsters, EGF and transforming growth factor alpha (TGF-α) can activate the EGF receptor (EGFR), and infusion of either ligand into the hamster ICV suppresses locomotion and feeding in an EGFR-dependent fashion [62]. Similarly, activation of the EGFR signaling pathway in *Drosophila* promotes sleep [63]. In *C. elegans*, overexpression of the EGF homolog LIN-3 causes cessation of feeding and locomotion [64]. EGF signaling appears primarily necessary for SIS, rather than DTS [30,64], suggesting that the EGFR signaling pathway may serve a similar role in other species. While reduced EGFR signaling in *Drosophila* has relatively minor effects on circadian sleep (shorter and more frequent sleep bouts) [63], its effects on stress-induced sleep in *Drosophila* and mammals have not yet been tested.

cAMP

In the hamster suprachiasmatic nucleus (SCN), activation of the cAMP-regulated binding protein (CREB) by the cAMP-dependent protein kinase (PKA) is controlled by the circadian clock [65]. CREB mutations also lead to decreased cortical arousal and increased sleep in mice [66], suggesting that the cAMP/PKA/CREB signaling pathway promotes wake. This

signaling pathway plays a similar role in *Drosophila*, where duration of sleep is inversely related to cAMP levels and CREB expression [67]. *C. elegans* mutants have been identified with increased PKA signaling caused by increased cAMP levels (due to increased synthesis or reduced breakdown) or by reduced function of the PKA regulatory subunit. During DTS, these mutants are hyperactive [32,53,68] and have increased responsiveness to sensory stimuli [29,53,68].

Dopamine

Dopamine release is positively correlated with arousal [69] and compounds that promote dopamine release also promote wakefulness in mammals [70]. Clinical drugs that increase dopamine release, such as the amphetamines, strongly promote wake in humans. In *Drosophila*, endogenous dopamine promotes wake [71,72], an effect attributed to two dopaminergic neurons [73,74]. In *C. elegans*, mutations in the dopamine D1 receptor gene *dop-1* cause increased quiescence, and loss of function mutations in the dopamine transporter gene *dat-1* results in reduced quiescence [53], effects that parallel those observed in *Drosophila*.

PKG

PKG is a protein kinase activated by elevation of cyclic guanosine monophosphate (cGMP), and it promotes sleep or sleep drive across different species. Reduced activity of the *Drosophila* PKG encoded by the *foraging* locus is associated with decreased sleep [29,75]. In mice, brain-specific knockout of the PKG homolog PRKG1 is associated with reduced slow wave power in the cortical EEG, a finding supporting a reduced drive to sleep in these mutants [76]. Gain-of-function mutations in *C. elegans pkg-1* (previously called *egl-4*) cause increased quiescence and arousal threshold, while loss of function mutations in *pkg-1* cause decreased quiescence and decreased arousal threshold [29].

Putative sleep functions are conserved in *C. elegans*

In addition to the molecular conservation, there is also evidence that DTS and SIS serve functions similar to the proposed functions of mammalian sleep, including synaptic plasticity [5], increased anabolic metabolism [4], and stress response [18,77]. During DTS, GABAergic neuromuscular junctions are less active [78], consistent with the notion that sleep is a state that promotes synaptic plasticity. In addition, cuticle synthesis [37,51,79,80] and DNA synthesis [81] both occur during DTS, demonstrating anabolic/synthetic metabolism. As its name implies, SIS is important for recovery following exposure to cellular stressors and pathogens [30], a conserved process in *Drosophila* [82] and mammals [20]. State-dependent neural responses to sensory stimuli, a well-established feature of the mammalian visual system [83], have been demonstrated in mechanosensory and chemosensory neurons in *C. elegans* [35,54,59]. However, other putative sleep functions, such as a role in learning and memory, have not yet been addressed in *C. elegans*. Nevertheless, *C. elegans* sleep serves functions similar to those described in other animals, indicating that the study of sleep function in this animal model will be illuminating.

Prominent developmental sleep is seen across species

Implicit to its name, DTS occurs only during larval development and is not observed in adult animals. Interestingly, across all terrestrial animals sleep is most prominent during development, where it has long been thought to play an important role [15]. In *Drosophila*, sleep in young animals plays a role in the development of neural circuitry required for courtship behavior [84]. Sleep in neonatal rats has been described as distinct from both REM and non-REM sleep in adult animals, suggesting that developmental sleep may serve different functions than sleep in adults [22]. Thus, *C. elegans* DTS may prove to be a useful model for understanding the importance of sleep in growth and development. The apparent absence of natural sleep-wake cycles in adult worms (unless triggered by cellular stress) suggests that across phylogeny, developmental sleep is more fundamental and conserved than adult sleep.

New insights gained from studying *C. elegans* sleep

Though still in its nascent stages, *C. elegans* sleep research has already revealed a surprising degree of neural and molecular complexity.

Sleep regulating circuitry

Two types of peptidergic interneurons have been identified that are required for quiescence during DTS, the single GABAergic RIS interneuron and the paired glutamatergic RIA interneurons [85,86]. The RIS interneuron is active during DTS and is required for locomotion quiescence during this state. Optogenetic activation of RIS inhibits locomotion, an effect that requires neuropeptide processing but not GABA synthesis, suggesting that RIS releases an unidentified somnogenic neuropeptide [85]. The RIA interneurons express the gene encoding NLP-22, a neuropeptide that inhibits both feeding and locomotion during DTS [86]. However, the role of the RIA neurons is complex, since acute optogenetic activation of RIA does not induce quiescence during the active adult stage. Rather, RIA activation inhibits locomotion quiescence during DTS [86]. This complexity might be explained by regulation of NLP-22 release primarily by modulation of expression rather than by neural activity. Consistent with this idea, *nlp-22* mRNA shows prominent cycling during larval development.

A third type of peptidergic interneuron, the single ALA neuron, is required for quiescence during SIS [30,64,87]. The ALA neuron releases neuropeptides encoded by the gene *flp-13* in response to EGF signaling [30,64,87], and *flp-13* mRNA levels are increased following exposure to cellular stress. Optogenetic depolarization of the ALA neuron causes cessation of feeding and locomotion in a *flp-13*-dependent manner [87].

The neuropeptide PDF-1 is expressed in *C. elegans* chemosensory neurons as well as interneurons [88]. Since sensory neuron activity is decreased during DTS [35,36] and restoration of *pdf-1* in sensory neurons rescues the increased quiescence observed in *pdf-1* mutants, PDF-1 is likely released from sensory neurons to promote arousal during wake. The PDF-1 receptor PDFR-1 promotes the touch sensitivity of mechanosensory neurons to stimulate locomotion during wake [59]. This role of PDF-1 offers molecular insight into the

reduced sensitivity of sensory neurons, one mechanism of sensory gating during *C. elegans* sleep [35,36]. Sensory gating, a universal feature of sleep in all species, is understood better in *C. elegans* than any other animal [35,36,59], a testament to the simplicity of the nematode nervous system and the powerful genetic and optogenetic tools available in this system.

Distinct mechanisms of different sleep states

The FLP-13 neuropeptides released by the ALA neuron are not required for quiescence during DTS [87], and NLP-22 and RIS neuron function are not required for quiescence during SIS [89], indicating that these states are governed by at least partly distinct mechanisms. Despite behavioral similarities between DTS and SIS, the mechanisms of quiescence generation downstream of the FLP-13 and NLP-22 neuropeptides during these states are distinct [89]. During SIS, optogenetic stimulation of the cholinergic motor neurons of the pharynx, the *C. elegans* feeding organ, causes an increase in feeding rate similar to that observed following neuron stimulation during wake. In contrast, feeding rate during DTS is not increased by stimulation of pharyngeal cholinergic motor neurons, or even direct stimulation of pharyngeal muscle, demonstrating that pharyngeal muscle excitability is strongly decreased during DTS. Thus, despite being behaviorally indistinguishable, SIS and DTS are generated by distinct mechanisms. A comparison between DTS and SIS is shown in Table 3.

The demonstration of mechanistically distinct sleep states in a non-mammalian animal is relevant to results obtained in the study of other animals in which sleep is identified strictly by behavioral criteria. Recent studies have shown that *Drosophila* daytime sleep and nighttime sleep are regulated by at least partly distinct mechanisms [90,91], and that mechanisms regulating sleep in young flies are partially distinct from mechanisms regulating sleep in older flies [84]. Consistent with lessons learned in *C. elegans*, *Drosophila* mutations that impair stress-induced sleep have no effect on circadian sleep [92]. Together, these results demonstrate that though sleep appears behaviorally homogenous, invertebrates have physiologically distinct sleep states. Given the high degree of molecular and functional conservation of sleep between *C. elegans*, *Drosophila*, and mammals, it would be surprising if sleep in all species were not similarly complex.

Sleep homeostasis

C. elegans sleep research has also provided insights into the regulation of sleep homeostasis. A recent study demonstrated that distinct molecular mechanisms are required for different types of homeostatic responses to sleep deprivation during DTS: deprivation with a gentle stimulus has a short-lasting effect on sleep bout durations immediately following the stimulus and requires neuropeptide signaling through an NPY receptor homolog NPR-1 [93], while deprivation with a harsh stimulus has long-lasting effects on sleep and requires signaling via the FOXO transcription factor DAF-16 [37,93]. The notion that different types of wake-promoting perturbations can have distinct effects on the sleep homeostatic response was recently also shown in *Drosophila* [94]. A role for NPY in sleep regulation in humans and flies has been described [95,96], and a role for FOXO signaling in *Drosophila* sleep has recently been identified [91], suggesting the homeostatic roles for these signaling pathways are conserved in species beyond *C. elegans*.

Prospects for *C. elegans* sleep research

Sleep arises from networks of neurons rather than individual neurons, so the small number of neurons and known anatomical connectivity of the *C. elegans* nervous system provides an advantage both for identifying sleep circuits and for propelling our understanding of the neural basis of sleep. Whole brain calcium imaging can be used to detect activity patterns of neurons across behavioral states [97], and optogenetic techniques for manipulating individual neurons *in vivo* can be used to identify those that modulate sleep-related behaviors, such as feeding [98] and locomotion [99–101]. Further, the ease of manipulation of *C. elegans* allows the independent study of the neural subprograms of sleep, such as decreased sensory response [35,36,54,59], behavioral quiescence [89], and homeostasis [37,93], which will shed light into how these behaviors are coordinated.

In addition to defining and understanding sleep circuits, *C. elegans* research benefits from an advanced set of genetic tools and from rapid genetic analysis. These techniques can be used to determine how conserved genetic pathways, such as those discussed here, affect sleep behavior, and to discover novel pathways regulating sleep. The description and understanding of the genetic landscape of *C. elegans* sleep can then be translated to research in other animals. These genetic data can also be used to uncover functions of sleep in *C. elegans* and their relative importance for organismal fitness, which will shed light onto the core function of sleep. Wider acknowledgement of sleep states in this model organism will facilitate the leveraging of knowledge gained from the study of *C. elegans* into a better understanding of sleep in general.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Trends

C. elegans has two states that meet the behavioral criteria for sleep, developmentally timed sleep (DTS) and stress-induces sleep (SIS).

DTS and SIS are regulated by the same signaling pathways that regulate mammalian sleep, including PDF, cAMP, EGFR, dopamine, and PKG signaling.

C. elegans sleep has proposed functions similar to those of mammalian sleep, including synaptic plasticity, anabolic metabolism, and stress response, and is similarly prominent during development.

Animals in DTS and SIS display behaviorally identical cessation of feeding and locomotion, but this quiescence is produced by different mechanisms in each state.

Homeostatic responses to disruption of DTS by gentle and harsh stimuli occurs through molecularly distinct pathways.

Table 1

C. elegans fulfills all behavioral criteria of sleep

	Mammals	***Drosophila***	***C. elegans***	**References for *C. elegans***
Reversible quiescent behavior	yes	yes	yes	[29,31,89]
Increased arousal threshold	yes	yes	yes	[29,35]
Stereotypical posture	yes	yes	yes	[32–34]
Homeostatic response to deprivation	yes	yes	yes	[29,37,93]
Sleep deprivation can be lethal	yes	yes	yes	[37]

Table 2

Molecular conservation of pathways regulating sleep

	Mammals	*Drosophila*	*C. elegans*	References for *C. elegans*
PERIOD regulates timing	yes	yes	yes	[52]
PDF signaling promotes wake	unknown	yes	yes	[59]
EGF signaling promotes sleep	yes	yes	yes	[30,64]
cAMP signaling promotes wake	yes	yes	yes	[29,32,68,53]
Dopamine signaling promotes wake	yes	yes	yes	[53]
PKG activity is associated with sleep or sleep intensity	yes	yes	yes	[29]

Table 3

Comparison of *C. elegans* DTS and SIS

	DTS	SIS
Occurs during	development	any stage
Trigger	larval timer	cellular stress
Duration	2–3 hours	depends on severity of stressor
Feeding quiescence	yes	yes
Locomotion quiescence	yes	yes
Increased arousal threshold	yes	yes
Homeostatic response to deprivation	yes	unknown
Deprivation can be lethal	yes	yes
Stereotypical posture	yes	unknown
Peptidergic interneuron modulation	RIS, RIA	ALA
Associated neuropeptides	NLP-22, others	FLP-13, others
Mechanism of feeding quiescence	muscular	neuronal
Mechanism of locomotion quiescence	neuronal*	neuronal*
Putative functions	anabolic metabolism synaptic plasticity	recovery of cellular homeostasis

*the neuronal mechanism for locomotion quiescence differs between DTS and SIS