EDITORIAL

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Do *C. elegans* Sleep? A Closer Look

Commentary on Iwanir et al. The microarchitecture of *C. elegans* behavior during lethargus: homeostatic bout dynamics, a typical body posture, and regulation by a central neuron. SLEEP 2013;36:385-395.

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Conserved mechanisms integral to sleep have been identified based on behavioral analyses in animals, using molecular, cellular, and/or genetic tools. In the nematode *C. elegans*, quiescence shares fundamental characteristics with sleep in other species, including decreased activity, rapid reversibility, altered arousal, and homeostatic regulation.[1,2] Behavioral quiescence in *C. elegans* occurs during satiety, after exhaustion, and at specific times during development called lethargus.[1,3,4] In this issue of *SLEEP*, Iwanir and colleagues at the University of Chicago used detailed quantitative analysis to delineate *C. elegans* lethargus quiescence at high resolution, revealing a complex temporal architecture of quiescence/activity bouts, unexpected posture changes, and homeostatic behavior.[5] This precise description of *C. elegans* quiescence is essential to create accurate models and to guide future studies that will identify the mechanisms underlying sleep-like behavior across species.

C. elegans entry into lethargus is defined by a specific behavioral change: initiation of quiescence bouts (QBs). During these short, sleep-like bouts, animals spontaneously and transiently cease feeding and moving. QBs are interspersed with motion bouts (MBs) with overtly normal activity levels. *C. elegans* have increased arousal thresholds during QBs and persistent stimulation during QBs induces subsequent heightened arousal thresholds, which is consistent with homeostatic compensation.[1] *C. elegans* lethargus lasts roughly 2.6 hours, is regulated by developmental expression of the *C. elegans* Period ortholog, and is coordinated with, but does not require, cuticle molting.[1,6,7] Iwanir and colleagues focus on lethargus occurring during the last larval molt and, for clarity, they call L4 intermolt larvae "L4i" and use the term "L4m" for animals in lethargus that are molting from L4 larvae to adults.[5] Hundreds of QBs and MBs occur during the L4m, but the relationship between these bouts and how bouts change as lethargus progresses was not examined carefully until now.

Previous studies of *C. elegans* quiescence primarily addressed the total quantity of sleep-like behavior during lethargus.[1,8,9] However, Iwanir and colleagues[5] focused on the duration of and correlation between QBs and MBs across L4m, thereby revealing new features of *C. elegans* sleep architecture. In early lethargus, they found new evidence for sleep homeostasis. The duration of a QB is directly related to duration of the preceding MB, that is, a long MB is more frequently followed by a long QB, and a short MB by a short QB, which suggests local homeostatic responses. In mice, bout duration is dependent on the previous bout type (NREM, REM, and wake), albeit in a more complicated manner, suggesting that sleep states are interdependent in diverse species.[10] It remains unclear what mechanisms regulate transitions between various sleep states, but useful models have been generated in mammals based on quantitative analysis of behavior and circuit.[11-13] Iwanir et al. also examined the impact of increased cAMP levels on *C. elegans* L4m bout interdependence. Increased adenylyl cyclase function (*acy-1(gf)*) increased locomotion activity at all stages tested[5,14] and decreased L4m quiescence, but the loss of local homeostatic compensation in *acy-1 (gf)* animals suggests cAMP may be required for coupling between states. This extends previous work showing that increased adenylyl cyclase activity decreases *C. elegans* arousal threshold and showing that arousal threshold is highest at the start of lethargus.[1,9] Collectively these results suggest a mechanistic or molecular link between arousal and QB duration that will need to be examined in future studies.

Iwanir and colleagues[5] also present convincing evidence that *C. elegans* adopt a specific posture with reduced body curvature during quiescence bouts, reminiscent of postural changes observed during sleep in other animals, and in *C. elegans* resting after exertion.[4] While posture changes were most profound during quiescence bouts, L4m animals had decreased curvature compared to L4i animals suggesting that the lethargus is a distinct state from a behavioral perspective. Interestingly, GABAergic signaling at the *C. elegans* neuromuscular junction is likely decreased during lethargus as well, based on sensitivity to acetyl cholinesterase inhibitors.[15] Together, these results suggest that lethargus is a distinct state in the *C. elegans* life cycle.[1,15] It is likely that the changes characteristic of lethargus described in this report and elsewhere are necessary for *C. elegans* quiescence.

Previous studies have also suggested, explicitly or implicitly, that there are distinct stages of *C. elegans* lethargus.[8,9] Response to sensory stimuli is lowest in early lethargus (high arousal thresholds), but animals become easier to rouse as lethargus progresses.[9] Also, *C. elegans* feed sporadically during early lethargus, but this activity ceases as lethargus progresses.[8] Additionally, spontaneous activity in *C. elegans* ALM mechanosensory neurons is low in early lethargus and increases during late stage lethargus.[16] The results of Iwanir et al.[5] reveal another facet of quiescence architecture during lethargus. Average QB duration was longer in the early stage of lethargus, but decreased in the middle and late stages of lethargus. By

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contrast, MBs were of short duration in the middle of lethargus, but of long duration in the beginning and end of lethargus. This suggests that while a relationship exists between QBs and MBs in early lethargus, this relationship is not simple and the relationship changes as lethargus progresses. Are there distinct behavioral stages in *C. elegans* lethargus? Further studies addressing this question will likely be forthcoming.

The high-resolution temporal analysis of *C. elegans* quiescence and lethargus microarchitecture presented by Iwanir and colleagues reveals unexpected complexity and relationships. Based on their results and previous studies, it seems likely that *C. elegans* lethargus entry and exit is regulated by mechanisms that are distinct from the mechanisms that regulate transition from QBs and MBs, and that QBs and MBs may be differentially regulated. Also, it seems likely that *C. elegans* lethargus has distinct stages that are evocative of the behavioral stages of sleep observed in vertebrates. Defining the critical mechanisms, circuits, and molecules that regulate *C. elegans* quiescence is likely to shed light on common mechanisms that regulate sleep/sleep-like behavior across species.

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