in simulating realistic, complex materials in natural illumination fields has made it possible to manipulate the physics of image generation directly and assess the impact these manipulations have on perception. Once the boundary conditions on perceptual performance are identified, they can be used to formulate hypotheses about the image properties and mechanisms that underlie these perceptual abilities. Although the scientific study of material perception is in its infancy, there is a rapidly growing set of tools that can be used to study this remarkable and fundamental aspect of visual experience.

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Reduced activity of a sensory neuron during a sleep-like state in *Caenorhabditis elegans*

Juliane Schwarz, Ines Lewandrowski, and Henrik Bringmann

Sleep-like states occur in the life of all animals carefully studied and are characterized by reduced behavioral and neural activity as well as reduced responsiveness to stimulation [1]. How is reduced responsiveness to stimulation generated? We used calcium imaging to investigate a sleep-like state in larvae of the nematode *Caenorhabditis elegans*. We found that overall spontaneous neural activity was reduced during the sleep-like state in many neurons, including the mechanosensory neuron ALM. Stimulus-evoked calcium transients and behavior were reduced in ALM during the sleep-like state. Thus, reduced activity of ALM may contribute to reduce responsiveness during a sleep-like state.

At the end of each larval stage, before the molt, *C. elegans* larvae go through a phase of behavioral quiescence called lethargus, during which animals don't feed and move only little [2]. Lethargus has sleep-like properties, such as reduced responsiveness to mechanical stimulation [3]. How does neural activity generate sleep-like behavior? We set up a system that allowed us to observe behavior and neural activity over the sleep-like–wake-like cycle. We generated transgenic *C. elegans* expressing the calcium sensor GCaMP3.35, a shortened version of GCaMP3 [4], to visualize neuronal calcium and mKate2 as an expression control [5]. We cultured and filmed larval *C. elegans* inside microcompartments from the first to the second larval stage [6]. We defined sleep-like and wake-like states based on the behavior of the worms. During the sleep-like state, worms did not feed and their movement was reduced (mean nose speed wake-like state: 7 ± 0.3 μm/sec, sleep-like state: 2.8 ± 0.2 μm/sec, $p < 0.01$, N = 10 worms; Figure 1A). We first looked at worms that expressed GCaMP3.35 in all neurons. The overall GCaMP3.35 signal increased over time with a clear reduction during the sleep-like state. Also, the mKate2 signal increased over time, but did not show a reduction during the sleep-like state. The GCaMP3.35/mKate2 ratio was reduced during the sleep-like state by 6% (GCaMP3.35/mKate2 ratio wake-like state: 1.04 ± 0.03, sleep-like state: 0.98 ± 0.02, $p < 0.001$, N = 16 worms; Figure 1B, and Supplemental Figure S1A). This suggests that overall spontaneous neural activity is reduced during the sleep-like state.

Little is known about which neurons are involved in control of the sleep-like state. Which neurons have decreased activity during the sleep-like state? We tried to identify sleep-regulated neurons in the transgenic worms expressing GCaMP3.35 in all neurons. Based on its morphology and isolated position, we could identify the mechanosensory neuron ALM as one of several neurons with reduced spontaneous activity during the sleep-like state (data not shown). To verify this observation, we expressed GCaMP3.35/mKate2 specifically in mechanosensory neurons and analyzed neural activity. ALM calcium signals were reduced during the sleep-like state by about 20% (GCaMP3.35/mKate2 ratio wake-like state: 0.66 ± 0.02, sleep-like state: 0.53 ± 0.01, $p < 0.001$, N = 15 worms; Figure 1B,C, and Supplemental Figure S1A). This suggests that the spontaneous activity of ALM is reduced during the sleep-like state.

ALM senses gentle mechanical stimulation [7]. How is reduction of spontaneous ALM activity influenced by sensory input? Sensory input could be caused by mechanical stimulation caused by worm locomotion, which is decreased during the sleep-like state. Mechanosensitive calcium currents in ALM depend on the mechanosensory channel subunit MEC-4 [8]. In *mec-4(u253)* mutant worms, spontaneous ALM activity was still reduced during the sleep-like state by about 14% (GCaMP3.35/mKate2 ratio wake-like state: 0.69 ± 0.05, sleep-like state: 0.59 ± 0.06, $p < 0.01$, N = 8 worms). Thus, mechanosensory input plays only a minor role in spontaneous

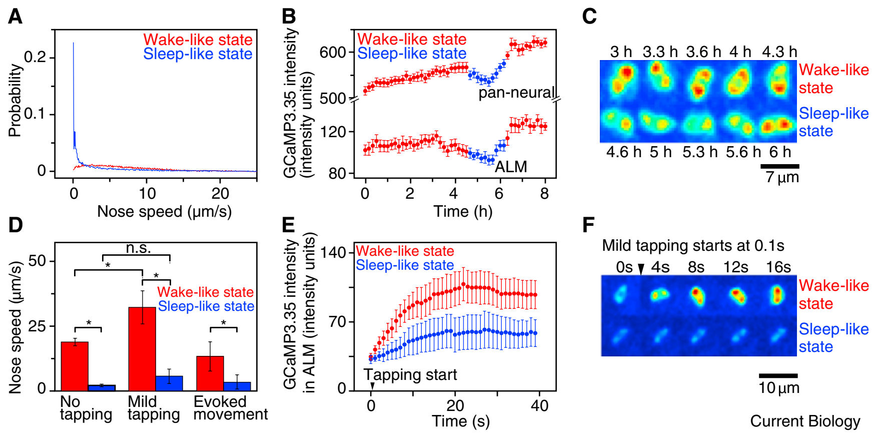

Figure 1. Reduced spontaneous and evoked activity of the mechanosensory ALM neuron during the *C. elegans* sleep-like state lethargus.

(A) Reduced nose movement during a sleep-like state. Shown is a probability distribution after normalizing areas to one. (B) Spontaneous neural activity is reduced in several neurons, including ALM, during the sleep-like state. Shown is GCaMP3.35 intensity versus time for all neurons (top) and ALM (bottom). (C) Spontaneous activity in ALM is reduced during the sleep-like state. Gauss-filtered false-color time-lapse images showing the GCaMP3.35 signal in an individual experiment during the wake-like state (top) and during the sleep-like state (bottom). (D) Behavioral response to dish tapping is decreased during the sleep-like state. Nose speed was measured before and after dish tapping. (E) Mechanosensitive calcium transients in ALM were reduced during the sleep-like state. Quantification of tap-evoked calcium transients in ALM versus time. (F) Mechanosensitive calcium transients in ALM were reduced during the sleep-like state. Gauss-filtered false-color time-lapse images show the GCaMP3.35 signal of an individual tapping experiment during the wake-like state (top) and during the sleep-like state (bottom). All errors are SEM. All statistical tests are Wilcoxon Signed Ranks. Asterisks indicate $p < 0.05$.

ALM activity. This result suggests that an endogenous mechanism reduces spontaneous neural activity of ALM during the sleep-like state.

ALM is required for increased movement after gentle mechanical stimulation and shows mechanosensitive calcium transients [8]. How are stimulus-evoked behavior and calcium transients altered across the sleep-like–wake-like cycle? We measured dish tap-evoked behavior and calcium transients over the sleep-like–wake-like cycle. We cultured and filmed transgenic *C. elegans* expressing GCaMP3.35 like before. Every 30 minutes we applied several taps to the dish containing the microcompartment with the worm inside. We measured nose speed and calcium before and after tapping. Dish tapping caused an increase in nose speed. During the sleep-like state, dish tapping caused 74% less increase in nose speed compared with the wake-like state (evoked movement wake-like state, 13 ± 6 µm/s; sleep-like state, 4 ± 3 µm/s; N = 6 worms, $p < 0.05$; Figure 1D). Dish tapping-induced calcium transients first increased linearly and then saturated. During the sleep-like state, calcium transients were reduced by more than half, with a slower linear phase and reduced amplitude (initial slope wake-like state, 6 ± 1 intensity units s^{-1}; initial slope sleep-like state, 2 ± 1 intensity units s^{-1}; amplitude wake-like state, 72 ± 14 intensity units; amplitude sleep-like state, 32 ± 14 intensity units; $p < 0.05$, N = 6; Figure 1E,F). We confirmed this result with the FRET-based calcium sensor chameleon [8] (Supplemental Figure S1B–D). During the sleep-like state, both behavior and neural activity appeared to be reduced after tapping. The results suggested that reduced excitability of a sensory neuron caused reduced responsiveness during the sleep-like state.

Sleep-like states are defined by behavioral criteria and by changes in the nervous system. In mammals, electroencephalogram patterns are used to define neural activity during sleep [1]. In *C. elegans*, however, direct observations of neural changes during sleep-like states have not yet been described. We found that activity of a sensory neuron was reduced during the sleep-like state. Thus, our results corroborate previous findings that suggested that *C. elegans* lethargus constitutes a sleep-like state.

In vertebrates, thalamocortical relay neurons are thought to control signal transmission from sensory systems to the cortex during sleep and wake [9]. In *C. elegans*, the only neuron previously implicated in the control of sleep is the interneuron ALA [10]. Here, we found that sleep-like regulation occurred at an early step in information processing, namely at the level of a sensory neuron. It may be a unique adaptation to the extremely small size of the *C. elegans* nervous system.

Supplemental Information

Supplemental Information includes one Figure and Experimental Procedures, and can be found with this article online at doi: 10.1016/j.cub.2011.10.046.

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