CHAPTER 5:

What Physiology Tells Us About the Regulation of Sleep

INTRODUCTION

In the previous chapter, we have made efforts to understand circuit mechanisms that promote sleep-like behavior in *C. elegans*. We found that calcium influx at the sensory neuron is dampened. However, there are many ways to dampen arousal[1], and although neuromodulators such as dopamine[2] and serotonin play key roles in the regulation in the starved and satiated states, their differing effect on select neurons are not always consistent with the sleep-like state. Therefore, we further test for physiological changes within other amphid sensory neurons to understand if sleep is global as well as to find better candidates for neuromodulation of the sensory neurons. The abundance of data concerning the expression of genes in individual neurons and large mutant and RNAi library makes it possible to test potential candidates.

In addition to sensory dampening, we found that the downstream effectors of the ASH sensory neuron were desynchronized during lethargus and that the primary interneuron, that drives the reverse motor neurons, AVA, was less responsive to channelrhopsin activation. In our preliminary results, we find that modulation of responsivity in the command neurons mediates the reversibility of sleep behavior. Careful stimulation with multiple inputs at the same or different sensory neurons allows visualization of activity in the circuit during waking and offer insights into what happens in the awake state.

5.1 SEARCHING FOR SLEEP REGULATORS

The first step for suppression of lethargus phenotypes was to understand the lethargus phenotypes to measure. The first studies of *C. elegans* sleep relied on measurement of locomotion, feeding behavior, and arousal.[3,4] It was through these studies that the conservation of regulators such as EGF[3], PKG[4], and cAMP[4] were confirmed. Because many of the neurotransmitters and signaling pathways are conserved, we began with an initial candidate screen to look for variability in the features that can be detected. Although more time-consuming to measure, the observation of locomotor activity is able to offer more subtle and numerous features in characterizing the mutant lethargus phenotype.

We began by perturbing protein kinase G, PKG, a conserved molecule shown to affect sleep. In Drosophila, increased PKG activity is associated with more sleep.[4] Additionally, preliminary reports suggest that PKG inhibition in the basal forebrain reduces subsequent sleep.[5] In *C. elegans*, PKG is shown to decrease sensory arousal and increase activity in lethargus.[4] We

tested the locomotor phenotype of these mutants in our system to compare our methods and assess interaction with the ALA, a sleep promoting interneuron (Figure 1-2). We confirm that PKG plays a role in promoting lethargus independent or downstream of ALA activity and that duration of lethargus remains unchanged. We also assay a set of neurotransmitter and signaling candidates (Figure 3), and found mutant phenotypes where lethargus duration was shortened.

5.2 MECHANISMS FOR SENSORY DEPRESSION

Calcium influx at the ASH and ALM[6] sensory neurons are dampened during lethargus. These are two disparate neurons: one is a polymodal nocioceptor that senses volatiles, aqueous chemicals, and mechanical stimuli, and the other a simple mechanosensor. It is possible that sensory regulation may occur only in these two neurons or in neurons with mechanosensory functions in general, but confirming modulation of subsets of neurons will narrow the candidates to those components present in these select cells.

Single cell studies of individual neurons have identified gene transcripts present in a handful of sensory neurons (Schwarz & Sternberg et al., unpublished data), and testing them for functional changes in lethargus was logical. We found that sensory dampening as measured by calcium imaging occurred in both the AWC and ASE sensory neurons (Figure 4). These are chemosensory neurons and do not share the same receptors with the ALM, indicating that modulation of these cells cannot be occurring at individual ligand receptors but rather at conserved signaling elements or components that induce changes in membrane potential.

We used channelrhodopsin to induce current in the ASH sensory neuron and assessed calcium changes in the absence of ligand-activated G-protein signaling. To do this experiment, we imaged the cell body using low light that did not induce changes in cellular calcium[7], and additionally illuminated a small area of the sensory cilia to activate channelrhodopsin (Figure 5). This decreased the amount of light contamination that occurs with whole field and allowed better control of the area of activation. Using these methods, we found that there was no exponential increase in calcium in response to channelrhodopsin during lethargus (Figure 6).

An increase in calcium in response to calcium influx suggests recruitment through voltage-gated or calcium-gated calcium channels. We have selected an available set to measure for arousal. The control wild-type animals showed immediate response to ASH:ChR2, whereas the negative controls, *unc-13* and *unc-31*, showed no significant response to ChR2 activation (data

not shown). *cca-1* and *ccb-1,* voltage-gated calcium channels, show delayed reversal in response to channelrhodopsin (Figure 7).

These long-term changes in the sensory neurons do not change much in preliminary experiments of perturbed animals (Figure 8). Sensory response remains relatively the same, but the response to the sensory input varies dramatically. This observation suggests that long-term regulation occurs at the sensory level, but there are additional dynamic changes that occur in the sensory-interneuron synapses.

FIGURES

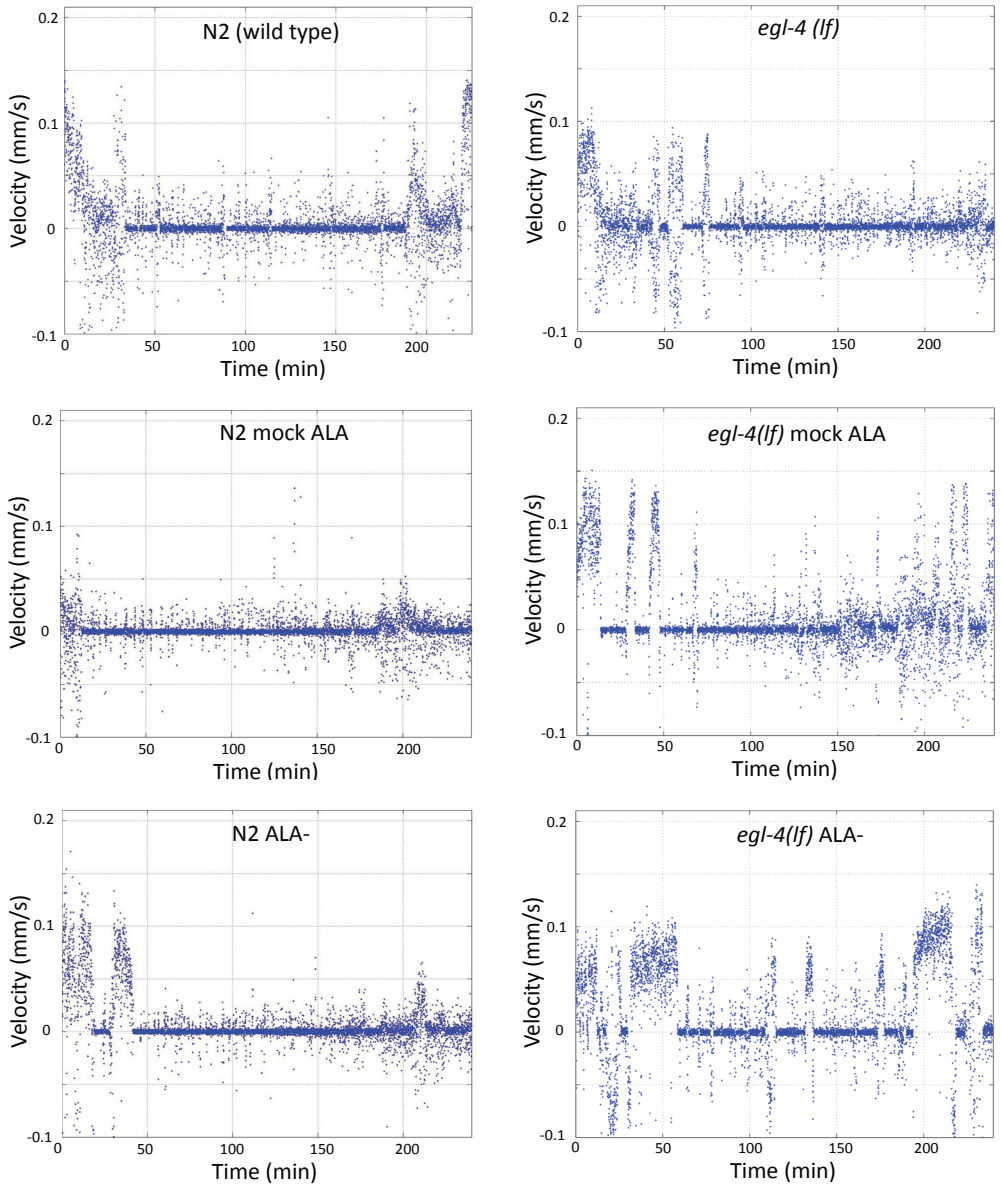

Figure 1. Representative velocity tracks of the N2 and *egl-4(lf)* lethargus are shown. *egl-4(lf)* mutants exhibited increases in locomotion in response to mock and ALA-ablation.

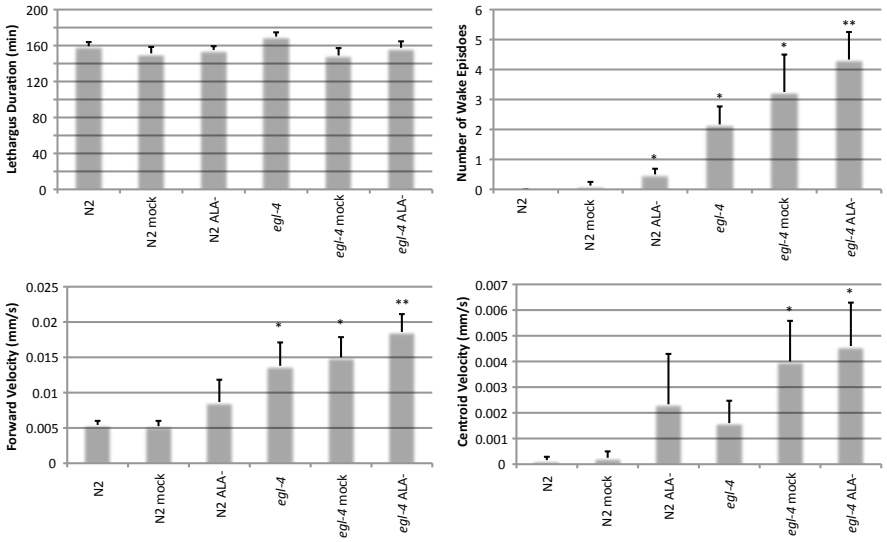

Figure 2. Quantitation of velocity data of the N2 and *egl-4(lf)* lethargus are shown. *egl-4(lf)* mutants exhibited increases in locomotion in response to mock and ALA-ablation.

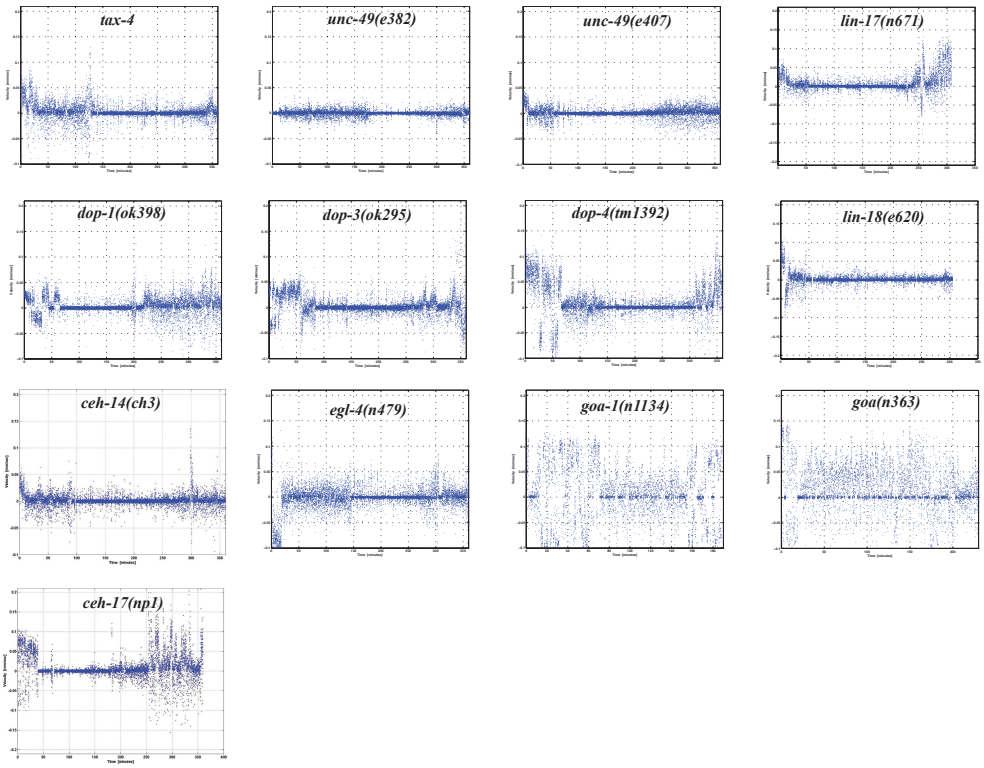

Figure 3. Velocity tracks of mutant animals with genes affecting conserved neurotransmitter and signaling pathways.

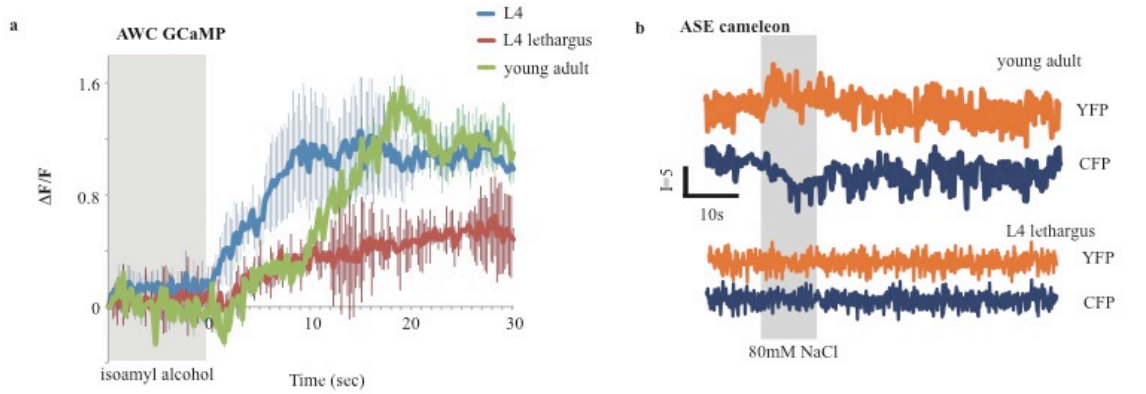

Figure 4. (a) Calcium influx as measured by GCamP in the AWC sensory neurons in response to isoamyl alcohol. (b) Calcium influx as measured by cameleon in the ASE sensory neuron in response to increased NaCl concentration in a single animals during lethargus and adult stages.

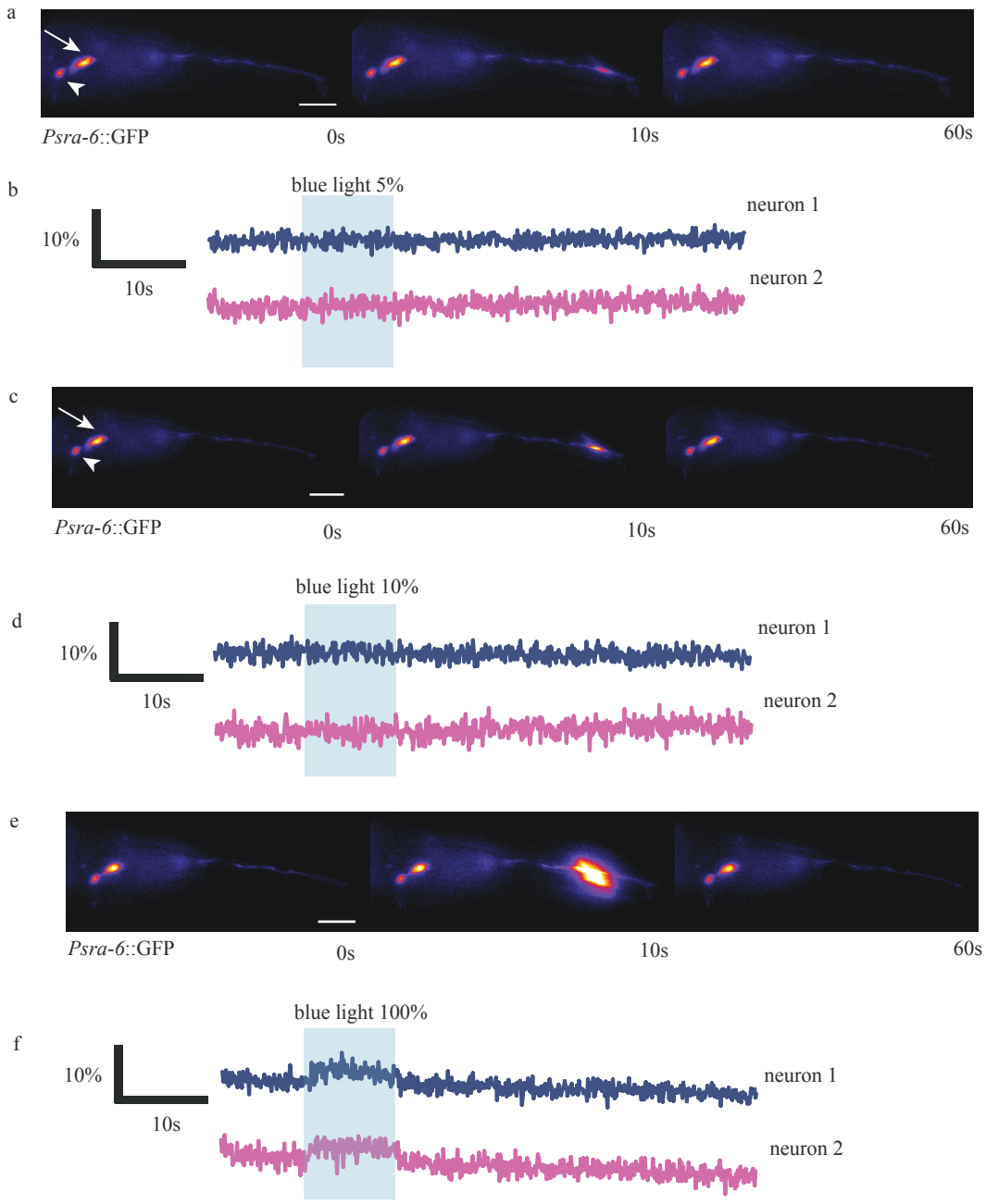

Figure 5. Technical tests for light contamination at the neuronal cell body in response to regional illumination. Chosen images of the GFP expressing animals before, during, and after regional illumination (a)(c)(e). There is no apparent increase in GFP intensity at lower levels of regional stimulation and a small increase when the highest intensity is used. Note arrowhead indicating neuron 1 and arrow indicating neuron 2.

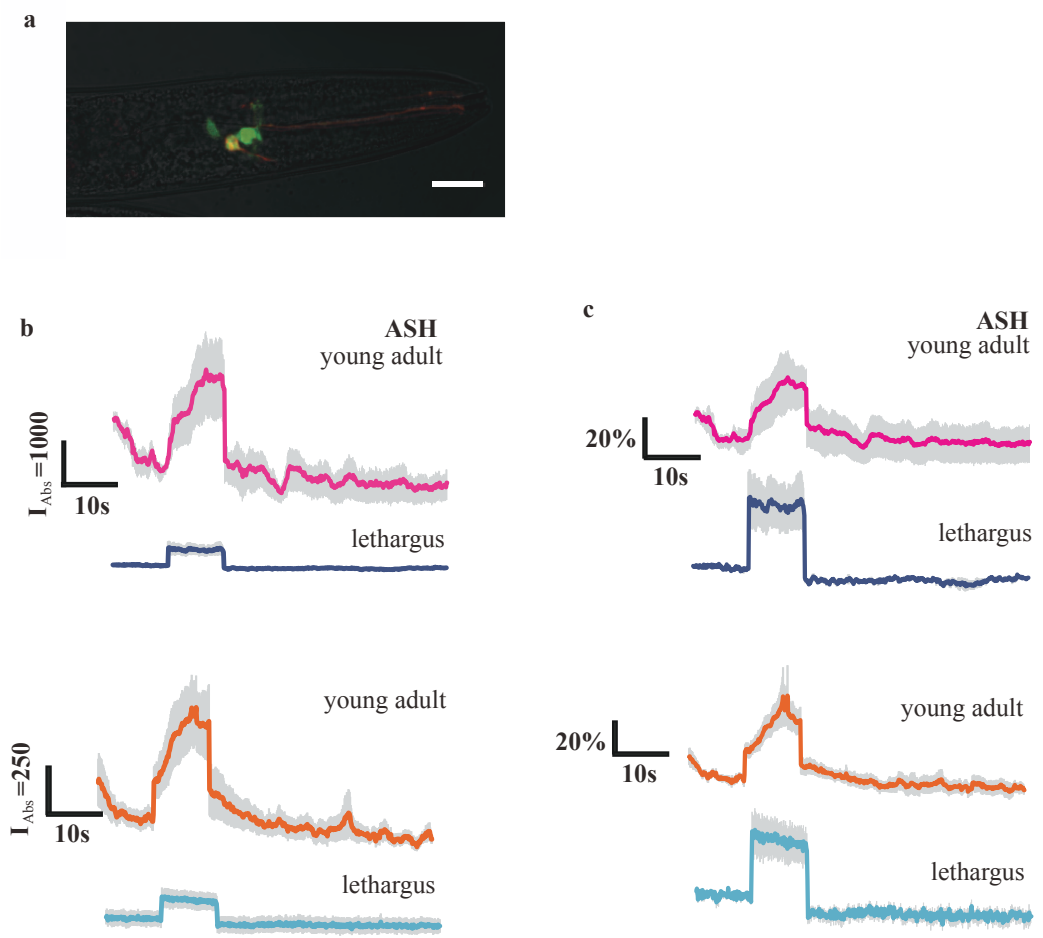

Figure 6. (a) Image of strain in which ASH GCaMP3 was measured. ASH is marked by the presence of both GCaMP3 and mCherry. (b) (c) (d) (e) Additional influx of calcium is only present in young adult animals. Lethargus animals show a flat pulse which does not look physiological.

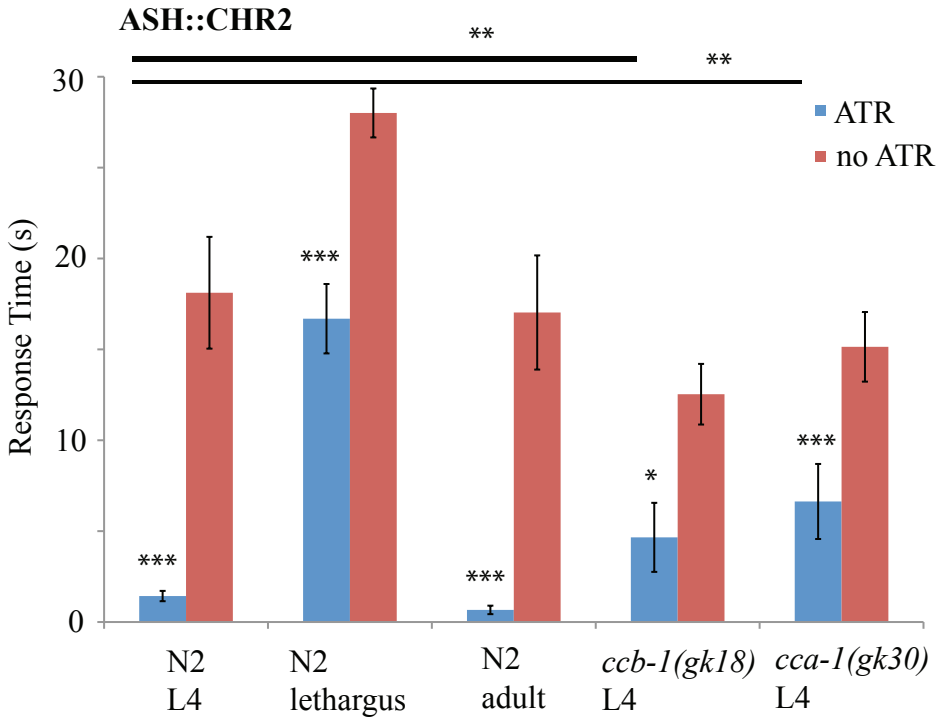

Figure 7. Time of sensory response of L4 N2, *cca-1*, and *ccb-1* animals. N2 lethargus and adult animal responses are also shown. All animals showed a significant response in the presence of the cofactor ATR as compared to conditions without (*p<0.01, ***p<0.0001). In the presence of ATR, *cca-1* and *ccb-1* animals showed a significant (p<0.001) difference from N2 animals in response to light-activated ChR2.

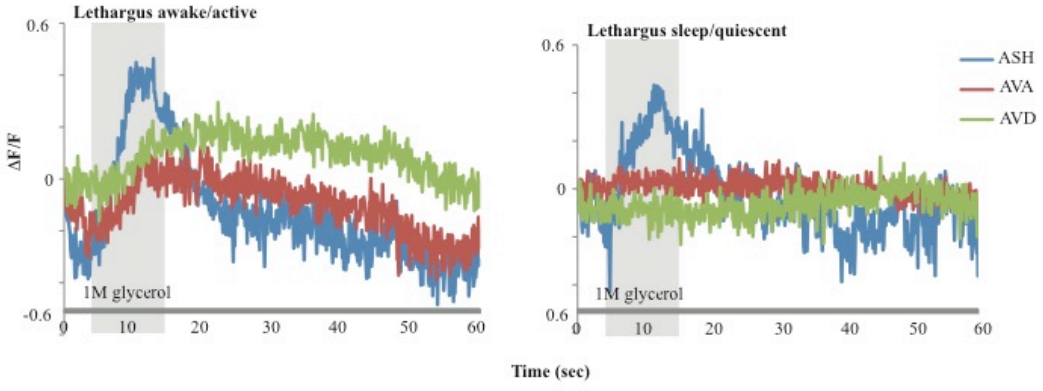

Figure 8. Activity of ASH, AVD, and AVD in response to chemical stimulus after perturbation and the following rest.

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