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Using pharyngeal pumping as a readout of associative learning by C. elegans through the pairing of light and odor stimuli.

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Although some mechanisms of molecular and circuit plasticity that underlie learning and memory have been identified, much about the neural circuits involved in memory formation and recall remains unknown. For example, the temporal dynamics of various learning and memory processes are still under active investigation. C. elegans associates paired stimuli such as odors, temperature and food, and can execute learned preferences through locomotory action. Because the timing of presentation and removal of such stimuli cannot be controlled precisely, temporal dynamics underlying the learning are obscured. Furthermore, the complete neural circuits involved in the learning often span many neurons, making it difficult to pinpoint the exact sites of plasticity when learning occurs. Previous studies in our laboratory described neural circuits in the pharynx that inhibit pharyngeal pumping and initiate spitting in response to noxious stimuli such as short-wavelength light or hydrogen peroxide. The temporal precision of light administration and the short latency of the pharyngeal response makes light-induced pumping inhibition a good system with which to study learning. Furthermore, if the learning occurs within the pharyngeal nervous system, the relatively simple circuitry that is distinct from the rest of the somatic system should allow easier investigation into the learning mechanisms. We have demonstrated classical conditioning of C. elegans by pairing light (as the unconditioned stimulus) with a neutral odor, Isoamyl alcohol (IAA) (as the conditioning stimulus), and scoring inhibition of pharyngeal pumps as a quantitative readout for learning. Worms learned to inhibit pumping when exposed to IAA after they were trained to associate the IAA stimulus with noxious light. The simultaneous presence of both IAA and light was necessary, as training with either stimulus alone did not produce learning. The learning was also dependent upon the relative timing of stimulus presentation: worms failed to learn when the light preceded the odor.

Many neurons can respond endogenously to light, potentially complicating our efforts to specify the circuit components involved in our learning paradigm. Thus, we are also seeking individual cell types that when optogenetically activated will inhibit pumping and might be used as an alternative to our current unconditioned light response. To this end, we have demonstrated that channelrhodopsin-based activation of the ASH neurons is sufficient to cause pumping inhibition. We expect that the temporal precision of the training stimuli and simplicity of the pharyngeal circuit that underlies this learning paradigm will greatly facilitate analyses to uncover the circuitry underlying learning and memory formation at the level of single cells and small networks.