A novel associative learning paradigm for investigating the spatiotemporal control of learning and memory in *C. elegans*

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Associative learning allows animals to adapt to multiple environmental stimuli that occur proximally in space and time. How molecular and cellular interactions control the formation, maintenance and degradation of learned memories in precise spatiotemporal terms is not fully understood. The nematode *C. elegans* can be trained to associate multiple cues and exhibit learned locomotor responses. Short wavelength light is an aversive stimulus that triggers an escape response by *C. elegans* and also causes worms to stop feeding. We have developed a novel trial-by- trial associative conditioning paradigm for *C. elegans* that utilizes the pairing of a noxious light stimulus and a neutral odor stimulus. After training, worms not only learned to reverse to the once-neutral smell but also learned to stop feeding - a new learned response that has not been previously described. Interestingly, locomotor and pharyngeal learning occur independently of each other, suggesting that multiple parallel neural circuits can be trained in response to a single stimulus pairing (light/odor). As neuromodulators are known to be involved in some learning and memory processes, we conducted a screen of neuromodulator mutants. Mutants defective in dopamine, glutamate, and octopamine exhibited defects in learning rates and efficiencies. Intriguingly, mutants defective in serotonin learned more rapidly and more consistently than wild-type worms. Additionally, an animal's physiological state could also influence its learning capabilities. For example, "food-deprived" worms learned more efficiently than "well-fed" worms. Finally, temporal order and contiguity of stimuli presentation is a key feature of associative learning. Worms were even able to learn associations in a trace-conditioning procedure where the light and odor stimuli presentation was separated by a time interval. Although the effects of the temporal order of stimuli presentation, an animal's physiological state and neuromodulation on learning have been studied individually, how the interplay of these elements produce an optimal adapted learning process is incompletely understood. By studying how these factors influence each other at a single-cell level resolution of analysis, we hope to gain a detailed understanding of the molecular, cellular and circuit mechanisms that underlie learning behavior.