A trial-by-trial associative-learning paradigm reveals neuromodulatory effects of physiological states and stimulus timing on the efficacies of learning by 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 learnt memories in precise spatiotemporal terms under various contexts remains under active investigation. The nematode *C. elegans* can be trained to associate multiple cues and exhibit learnt locomotor responses. Comprehensive genetic and cellular manipulation tools and a deep understanding of its neural circuits allow a single-cell level resolution of analysis to be applied to *C. elegans* learning and memory. 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 conditioning paradigm for *C. elegans* that utilizes the pairing of a noxious light stimulus and a neutral odour stimulus. After training, worms not only learnt to reverse to the once-neutral smell but also learnt to stop feeding - a new learnt 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/odour). As neuromodulators are crucial factors in learning and memory processes, we conducted a screen of neuromodulator mutants. Mutants defective in dopamine and octopamine exhibited defects in learning efficiencies. Strikingly, mutants defective in serotonin learnt faster 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 in which the light and odor stimuli presentation was separated in time. How the interplay of the temporal order of stimuli presentation, an animal's physiological state and neuromodulation 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 behaviour.