Noxious Stimuli Induce Spitting by *C. elegans* via Spatially-Restricted Calcium Increases

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How neuromuscular systems encode flexible, context-appropriate behaviors is an area of active inquiry in neuroscience. One simple system for the study of this problem is the feeding organ of *C. elegans*, the pharynx, which contracts rhythmically (i.e., pumps) to ingest bacterial food. Recent work in our laboratory showed that this feeding rhythm is interrupted by noxious stimuli, such as hydrogen peroxide (H_2O_2) and short-wavelength light (Bhatla and Horvitz, Neuron 85, 804, 2015). This interruption depends on the gustatory receptor ortholog *gur-3* and the two pharyngeal I2 neurons.

We have discovered that after briefly inhibiting pumping, ultraviolet (UV) light and H_2O_2 subsequently stimulate pumping and these pumps induce robust spitting of food. While ordinary feeding pumps draw bacteria progressively further into the pharyngeal lumen, UV light or H_2O_2 reverse particle flow in the anterior pharynx, ejecting food into the environment.

Laser ablations indicate that the M1 pharyngeal neuron is required for spitting behavior and stimulates pumping in response to UV light. M1 calcium levels increase during UV exposure. The gustatory receptor orthologs *lite-1* and *gur-3* are required both for spitting and M1 calcium influx. *lite-1* is expressed in M1 and is required for 99% of M1 calcium influx. The remaining *lite-1*-independent calcium response in M1 depends on *gur-3* and, surprisingly, is sufficient to drive spitting. Cell-specific rescue indicates that *gur-3* acts in the I2 neurons, previously shown to inhibit feeding, to activate M1 and induce spitting.

We identified a mechanism for spitting: whereas during feeding pumps the anterior-most region of pharyngeal muscle 3 (pm3) relaxes before the rest of the procorpus, closing the anterior end of the pharynx and trapping food (Fang-Yen et al., PNAS 106, 20093, 2009), during spitting pumps the anterior-most region of pm3 remains contracted. This contraction holds the anterior end of the pharynx open, preventing food-catching and permitting spitting when the corpus relaxes. We identified a likely physiological mechanism underlying this local pharyngeal contraction: during UV exposure, calcium increases in a restricted sub-compartment of pharyngeal muscle that corresponds spatially with the site of altered muscle contraction.

Our work identifies a novel *C. elegans* behavior, spitting, a neuron, M1, that controls this behavior, and indicates that spatially-restricted increases in muscle calcium convert feeding motions into spitting motions. We suggest that spatially-restricted calcium influx

might be a general mechanism for encoding multiple, contextual behaviors in a muscle or muscle group.