

Evidence for Biphasic Habituation and Darkness-Shock Associative Learning in *Hydra vulgaris*

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Hydra are aquatic invertebrates with decentralized nervous systems, meaning their neurons are completely dispersed throughout their bodies. These organisms possess the most basal nervous system within the phylum Cnidaria. Since studies on learning in Hydra are scarce and outdated, I aimed to address whether they were capable of habituation, response decrement, and/or classical conditioning, associating two distinct stimuli. The evolutionary context and neural architecture of Hydra provide insight into the widely-debated question of evolutionary biology: how did learning first arise? To test for habituation, I utilized a home-built apparatus, consisting of a ruler, electromagnet, and Arduino board, to deliver constant pulsing stimuli. To test for classical conditioning, I paired darkness and electric shock at various timings to observe if the animals would learn to contract in darkness alone. My findings revealed that habituation in Hydra occurs biphaseically: an initial steep decline in contractions followed by a gradual, oscillating decrease. The original response is restored when introduced with the same stimulus at a stronger magnitude (dishabituation). My results also showed partial evidence for classical conditioning, with increases in contraction frequency and duration when comparing the pre- and post-conditioning tests. However, additional testing is required with larger, more even sample sizes to solidify this finding. There is convincing evidence that *Hydra vulgaris* are capable of both categories of learning. Evolutionarily, these findings indicate that basal neural architectures are sufficient to sustain learning, suggesting that these processes either convergently evolved or were inherited from a common ancestor of Cnidaria and Bilateria.

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