A Behavioral Assay for Neurophysiological Studies of Habituation in Larval Zebrafish

Abstract

We seek to understand how the brain mediates an organism's response to its environment analyzing habituation at a behavioral and neuronal level. When an organism is repeatedly presented with a stimulus, it will eventually decrease or eliminate its response to that stimulus; this process is known as habituation. We developed a behavioral assay in larval zebrafish to identify a stimulus that could evoke habituation for neurophysiology studies. Electric shocks and dark flashes are convenient stimuli for neuroimaging because they evoke robust responses with minimal motion artifact. Presentation of an electric shock results in a well-defined behavioral output called the “escape response," in which fish rapidly bend their tails to swim away from the stimulus. In contrast, fish respond to dark flashes with a slower "O-bend," a less violent stimulus that reverse their direction. We monitored the behavior of fish in response to electric shocks and dark flashes. Fish presented with repeated electric shocks showed periods of missed or diminished responses but spontaneously dishabituated. Repeated dark flashes appeared to induce habituation more rapidly and exhibited a sustained decline of response. To identify a neuronal correlate for determining whether or not fish habituate, we labeled reticulospinal neurons with a calcium indicator, and imaged them during presentations of electric shocks. The activity of the Mauthner cell, the largest reticulospinal neuron, was correlated with electric shock presentations. We have established a paradigm for habituation which can be applied to in vivo imaging studies to determine a neural correlate for vertebrate habituation.