



Brain-wide Functional Imaging Reveals Neuronal Dynamics of Working Memory in Larval Zebrafish

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Working memory is a central component of cognition, where sensory information is temporally retained in a form that can be processed and used to assist future decision-making. However, the dynamic nature of working memory, with activity patterns that evolve across multiple areas, presents a challenge for studying its neural circuit mechanisms. We developed a working memory-like assay in larval zebrafish, in which larvae use past visual cues to decide where to escape when an acoustic stimulus is presented after a time delay. We show that this memory consists not only of an overt component, observable during spontaneous swimming, but also a longer-lasting covert component revealed only when the fish performs a long-latency C-start, a delayed escape that facilitates information integration. Furthermore, we found that this memory is used selectively and that it resets after being enacted, sharing characteristics of mammalian working memory. Leveraging the optical transparency of the zebrafish larva, we conducted brain-wide calcium imaging using a volume-scanning light-sheet microscope during this memory paradigm. We found brain areas involved in the creation, maintenance, and usage of this memory, sharing key features with the behavioral observations. In particular, we found regions in the anterior hindbrain showing persistent activity during the delay period with a timescale matching that of working memory, and a region exhibiting reset dynamics, which might be part of a memory gating mechanism. We propose and constrain models of the circuits underlying this memory and predict escape behavior from high-dimensional neuronal data. Our results show that even simple behaviors in teleost larvae can combine to produce an unexpected level of cognitive function, presenting the larval zebrafish as an excellent model to study subcortical brain regions involved in working memory.

working memory, larval zebrafish, brain-wide imaging, subcortical cognition, decision-making