Thermal response behaviors in larval zebrafish: startle escape, thermotaxis and thermal arousal

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Detection of the changing thermal environment is critical for animal survival. Sensory detection drives motor behaviors which allow animals to rapidly escape from noxious temperatures and initiate search patterns to move back into an optimal thermal environment.
Larval zebrafish have multiple advantages for examining motor behavior and understanding neural circuits. These include: small size for simultaneously recording large numbers of fish, stereotypic swim patterns which can be computationally categorized [1], and the availability of transgenic fish for ablation, monitoring, tracing and activation of targeted neurons. However, very few studies have examined the response of zebrafish to thermal cues. Here we report three types of behavioral response to changes of the thermal environment in larval zebrafish.

Freely swimming zebrafish responses were monitored under three conditions: to a sudden high intensity thermal pulse, to a slow increase in ambient water temperature and to a temperature gradient across the testing arena. To probe the response of larvae to a sudden thermal stimulus, we aimed an infra-red laser at a small region within the testing arena which was triggered when a larva swam into the target zone. Larvae responded robustly to the acute high intensity thermal pulse, and kinematic analysis demonstrated that they performed a swim pattern similar to a Mauthner cell mediated escape response. In contrast, larvae in a flow chamber which were exposed to temperature gradient, showed thermotaxis, turning and swimming away from the region of high temperature. The temperature gradient rose from ambient temperature on one side to 5 C higher on the other side. Under uniform temperature conditions, larvae were evenly distributed across the testing arena. However after establishment of the temperature gradient, larvae aggregated on the room temperature side of the chamber. Kinematic analysis of swimming movements revealed that avoidance of the high temperature zone was due to three factors. First, larvae which were oriented toward the ambient zone showed a high frequency of forward swim movements driving them into this region. Second, fish within the high temperature zone showed a high frequency of turn initiations allowing a local search of water temperatures. Third, turns executed in the high temperature zone showed a strong directional bias, causing larvae to orient away from the high temperature area allowing them to then initiate forward swims toward the ambient zone. The third response to changes in environmental temperatures, thermal arousal, was manifest as a short term change in internal state. When ambient water temperature was gradually increased from baseline 26 C to a noxious 36 C, larvae became hyperactive. Importantly, hyperactivity persisted for several minutes after the water temperature returned to 26 C. We previously reported that water flow induces an arousal state [2], but under the conditions used in these experiments, we used slow flow rates to avoid flow-induced arousal. During thermal arousal, fish showed normal movement initiation frequency but increased velocity during swim bouts. We speculate that thermal arousal prepares fish to anticipate and quickly respond to subsequent changes in environmental temperature.

To investigate the neuronal basis for these different responses, we generated transgenic fish for optogenetic activation of individual trigeminal sensory neurons and tracing of axonal projections. For this, we took advantage of an enhancer trap line which strongly expresses Gal4 in a subset of trigeminal neurons. Ablation of trigeminal neurons using UAS:nitroreductase resulted in a reduction of thermal responses confirming the involvement of the neurons labeled in this line in these behaviors. By injecting a UAS:channelrhodopsin2 plasmid into this fish, we could activate trigeminal neurons in freely swimming fish using a pulse of intense blue light. We calibrated the plasmid dose so that individual fish stochastically expressed ChR2 in only a small subset of trigeminal neurons. ChR2 expressing fish initiated either escape responses or thermal arousal after trigeminal activation, demonstrating that distinct trigeminal neurons mediate these behaviors. Thus individual trigeminal neurons selectively drive different thermal behaviors. We then traced axons from trigeminal neurons with defined behavioral roles revealing distinct projection patterns. We are now using GCaMP imaging to identify neurons throughout the brain which respond to thermal stimuli. These studies will reveal key brain areas which mediate distinct modes of behavioral response to thermal cues.

Reference

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Ethical statement

All in vivo experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the Division of Intramural Research of the National Institute of Child Health and Human Development. The NICHD Animal Care and Use program is administered by the Research Animal Management Branch (RAMB) which is staffed by board-certified laboratory animal veterinarians and pathologists, veterinary and animal care technicians.