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Larval zebrafish rapidly sense the water flow of a predator's strike

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Larval fishes have a remarkable ability to sense and evade the feeding strike of a predator fish with a rapid escape manoeuvre. Although the neuromuscular control of this behaviour is well studied, it is not clear what stimulus allows a larva to sense a predator. Here we show that this escape response is triggered by the water flow created during a predator's strike. Using a novel device, the impulse chamber, zebrafish (*Danio rerio*) larvae were exposed to this accelerating flow with high repeatability. Larvae responded to this stimulus with an escape response having a latency (mode = 13–15 ms) that was fast enough to respond to predators. This flow was detected by the lateral line system, which includes mechanosensory hair cells within the skin. Pharmacologically ablating these cells caused the escape response to diminish, but then recover as the hair cells regenerated. These findings demonstrate that the lateral line system plays a role in predator evasion at this vulnerable stage of growth in fishes.

Keywords: lateral line; flow; hair cells; fish; predator; prey

1. INTRODUCTION

Larval fishes can evade the strike of a fish predator with an escape response (Blaxter & Fuiman 1990). Upon sensing a threat, the body curls into a 'C' shape and then accelerates away from the predator as the body straightens. Although much is understood about the neuromuscular control of this escape response (Fetcho 2007), it is not clear how larval prey are capable of rapidly sensing a predator's strike.

Fishes sense water flow with a mechanosensory organ within the skin. This organ, the lateral line system, includes receptors with hair cells that produce nervous potentials when deflected by water motion (Dijkgraaf 1963). The signals detected by these receptors, called neuromasts, influence adult fish behaviour such as schooling, prey localization and obstacle detection (Coombs & Van Netten 2006). Although a rapid jet (Liu & Fetcho 1999) or an approaching probe (Blaxter & Fuiman 1989) triggers an escape response in larval fishes, it is unclear whether the lateral line system is capable of detecting a predator at this stage of growth.

The goal of the present study was to test the hypothesis that the lateral line system of larval zebrafish (*Danio rerio*) detects the flow generated by a predator's strike. We developed a device, the impulse chamber (figure 1*a*), that exposes larvae to the flow of a feeding fish predator in a repeatable manner. These experiments focused on zebrafish because they are a model system for the physiology of the lateral line system (Nicolson 2005) and the escape response (Fetcho 2007).

2. MATERIAL AND METHODS

The impulse chamber creates flow with a computer-controlled hydraulic piston that draws water through a rectangular working section from a reservoir (figure 1*a*). The acceleration of flow within the working section matches published values for suction feeding fishes (Wainwright *et al.* 2001; Day *et al.* 2005). Two 130 μm mesh filters at opposite ends of the working section serve to contain larvae in the chamber and reduce non-uniformity of the flow. We confirmed flow velocities to be nearly uniform across the working section by using digital particle image velocimetry with a continuous laser (532 nm, 2.5 W, Laser Quantum) and a high-speed video camera (as in Drucker & Lauder 1999). The responses of zebrafish larvae (*D. rerio* Hamilton 1922, 20–30 individuals of AB line at 5 days post fertilization, dpf) to this stimulus were recorded with high-speed video (250 frames s^{-1} at 256×512 pixels, Photron USA, San Diego, CA, USA).

To test whether escape responses are mediated by the lateral line system, we performed experiments on larvae in which we ablated the lateral line system. Larvae were exposed to a 250 μM solution of neomycin sulphate (Harris *et al.* 2003) 1 hour prior to the first experiment. We confirmed that this treatment did not visibly damage inner-ear hair cells with Nomarski optics. Furthermore, treated larvae could escape in response to sound and exhibited no loss of equilibrium, which suggests that the treatment did not affect inner ear or motor function. The behaviour of treated larvae was compared with a control group that was handled to an equal degree. We repeated this experiment with both groups at 6 hour intervals over the course of 3 days to test whether response probability increases as the hair cells regenerate. This sequence of experiments was replicated three times for both treatment and control groups. Response probability was calculated as the number of larvae that initiated an escape response divided by the total number of larvae. Any larva that was not separated from the other individuals or the walls of the tank by at least 0.5 body lengths was excluded. In order to describe variation among individuals, the 95% confidence interval for the probability of response was calculated from the sampled probability for a binomial distribution (Johnson *et al.* 2005). All analyses were scripted in MATLAB (v. 2007a, Mathworks).

We tracked the regeneration of hair cells over the recovery period in a separate treatment group. The number of hair cells per neuromast was measured for the second infraorbital, second middle line and fifth posterior lateral line neuromasts using 2-(4-(dimethylamino)styryl)-*N*-ethylpyridinium iodide (DASPEI) staining (Harris *et al.* 2003). The 95% confidence interval for the mean hair cell number was calculated by assuming a normal distribution (Sokal & Rohlf 1995).

3. RESULTS

A majority (68%) of larvae responded to the flow stimulus with an escape response. Among 703 measured responses, the latency from stimulus onset (i.e. the instant the linear motor started to move) to the start of the escape response exhibited a mode between 13 and 15 ms and most (64%) responses occurred within 25 ms (figure 1*b*,*c*). The 4 ms temporal resolution of these recordings do not provide a precise measure of the maximum speed of response. Therefore, the few responses that we report at <6 ms (figure 1*b*) may actually be closer to 10 ms.

Larvae treated with neomycin sulphate exhibited a nearly complete loss of lateral line hair cells within an hour of treatment (figure 2*a*–*c*). One hour after treatment, larvae exhibited a response probability ($R = 0.16$, $R_L = 0.05$ and $R_U = 0.30$, where R_L and R_U are the respective lower and upper 95% confidence intervals,

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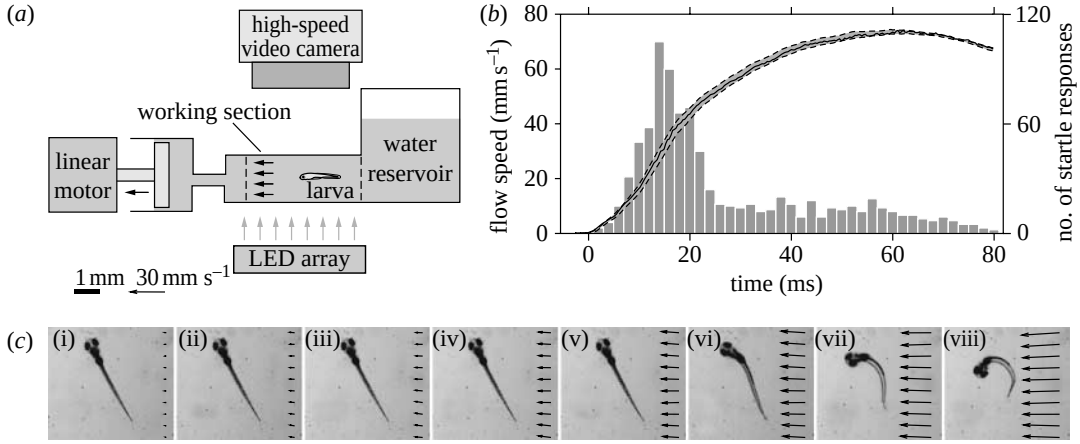

Figure 1. Water flow stimulates an escape response in zebrafish larvae. (*a*) The impulse chamber used to generate flow includes a computer-controlled linear motor that actuates a hydraulic piston. The motion of the hydraulic piston (black arrow) creates flow through the working section. A high-speed video camera (250 frames s^{-1}) recorded the responses of larvae that were backlit with an array of infrared LEDs in a darkened room. (*b*) The speed of the flow stimulus is shown for a representative flow visualization measurement (black curve) and the 95% confidence intervals (dashed area, $n=15$ trials) from repeated measurements. The frequency of larvae that responded to this stimulus (grey bars) is overlaid in 2 ms intervals. (*c*) Video stills of a representative fast start response for a single larva (5.90 dpf) from a dorsal view with velocity vectors from the representative flow stimulus in (*b*) ((i) 1 ms, (ii) 3 ms, (iii) 5 ms, (iv) 7 ms, (v) 9 ms, (vi) 11 ms, (vii) 13 ms and (viii) 15 ms).

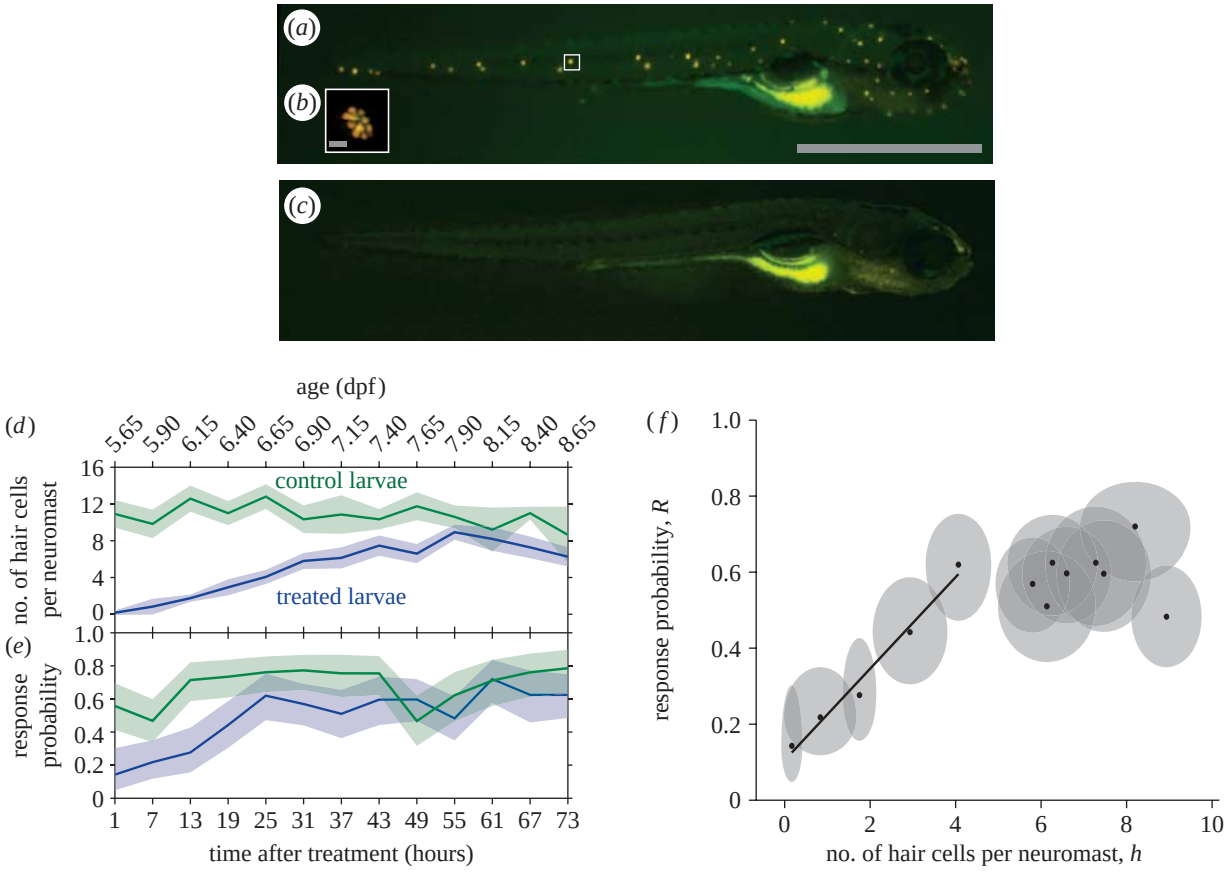

Figure 2. The effects of lateral line hair cells on startle response probability. (*a*) The lateral line hair cells stained with DASPEI are visible as yellow points along the body of a control larva (5 dpf) with a single neuromast highlighted (white box; scale bar, 1 mm). (*b*) The individual soma of hair cells within a neuromast are visible at high magnification (scale bar, 5 μm). (*c*) A larva treated with neomycin stained with DASPEI. (*d*) The mean number of hair cells per neuromast among three loci (see §2 for details) for larvae after treatment with neomycin (blue curve) and an untreated control group (green curve) of the same age. At each age, 95% confidence intervals (shaded areas) were calculated from variation among individuals ($n=3$–5). (*e*) The probability of an escape response after recovery for treated (blue curve, $n=77$–93 at each age) and control (green curve, $n=66$–107 at each age) larvae with 95% confidence intervals (shaded areas). (*f*) Measurements of response probability (from (*e*)) versus the number of hair cells (from (*d*)) for treated larvae with 95% confidence intervals (shaded areas). A linear least-squares curve fit (black line: $R=0.12h+0.11$, $r^2=0.98$, $p=0.001$) to the five sets of measurements made 25 hours after treatment.

$n=35$ larvae) that was substantially less than a control group of the same age ($R=0.56$, $R_L=0.41$ and $R_U=0.70$, $n=52$ larvae). However, larvae recovered the ability to respond as the hair cells regenerated (figure 2*d–e*). On average, larvae recovered one hair cell per lateral line neuromast every 6.1 hours in the day following the neomycin treatment (figure 2*d*) and the response probability increased by 0.12 for each regenerated hair cell, up to four hair cells per neuromast (figure 2*f*).

4. DISCUSSION

We have learned that larvae respond rapidly to the flow of a predator's strike with an escape response. Our results predict that the greatest proportion of escapes will complete within 30 ms from strike initiation, due to the 16 ms necessary for the manoeuvre (Liu & Fetcho 1999). This is too slow to evade a strike from the fastest of cichlid species, but is rapid enough for many teleost predators (Wainwright *et al.* 2001; Day *et al.* 2005). Although fast, the direction and timing of the escape response relative to a predator's strike will also be critical to evasion success because this manoeuvre only propels a larva by a few body lengths (Hale 1999).

Our results provide, to our knowledge, the first demonstration that prey fish use the lateral line system to detect a predator. This conclusion is based on the finding that the escape response diminishes with lateral line ablation, but then recovers as its hair cells regenerate (figure 2). This recovery demonstrates an extraordinary ability of afferent neurons to re-establish their connectivity and suggests that behavioural sensitivity is limited by the number of hair cells (figure 2*f*). However, more hair cells than four per neuromast do not appear to enhance sensitivity. This finding is consistent with the idea that the enhanced neurobiological signal created by more hair cells is offset by their negative effect on the mechanical sensitivity of a neuromast (McHenry & Van Netten 2007).

The hydrodynamics of flow detection in larval fish contrasts the lateral line function of predators. A prey fish may sense the pressure gradient created by a predator's feeding (Wainwright & Day 2007) if its body possesses a density that is different from the surrounding water. This difference causes the body to accelerate at a differential rate from the water and thereby generate flow with respect to the body, which may be sensed by the neuromasts. By contrast, predator fishes directly detect pressure gradients with neuromasts that are recessed within canals (Coombs *et al.* 1996). Despite this mechanistic difference between predators and prey, it is now clear that the lateral line is capable of playing a role on both sides of their interaction. Therefore, the lateral line system has the potential to play a critical role in the ecology of fishes (Bailey & Houde 1989) at all stages of growth.

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