RESEARCH ARTICLE

A hydrodynamic sensory antenna used by killifish for nocturnal hunting

Jason S. Schwarz, Tobias Reichenbach and A. J. Hudspeth*

Howard Hughes Medical Institute and Laboratory of Sensory Neuroscience, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA

*Author for correspondence (hudspaj@rockefeller.edu)

SUMMARY

The perception of sensory stimuli by an animal requires several steps, commencing with the capture of stimulus energy by an antenna that, as the interface between the physical world and the nervous system, modifies the stimulus in ways that enhance the animal's perception. The mammalian external ear, for example, collects sound and spectrally alters it to increase sensitivity and improve the detection of directionality. In view of the morphological diversity of the lateral-line system across species and its accessibility to observation and experimental intervention, we sought to investigate the role of antennal structures on the response characteristics of the lateral line. The surface-feeding killifish *Aplocheilus lineatus* is able to hunt in darkness by detecting surface capillary waves with the lateral-line system atop its head. This cephalic lateral line consists of a stereotyped array of 18 mechanosensitive neuromasts bordered by fleshy ridges. By recording microphonic potentials, we found that each neuromast has a unique receptive field defined by its sensitivity to stimulation of the water's surface. The ridges help determine these receptive fields by altering the flow of water over each neuromast. Modification of the hydrodynamic environment by the addition of a supplemental ridge changes the pattern of water movement, perturbs the receptive fields of adjacent neuromasts and impairs the fish's localization ability. On the basis of electrophysiological, hydrodynamic and behavioral evidence, we propose that the ridges constitute a hydrodynamic antenna for the cephalic lateral line.

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Key words: capillary wave, hair cell, neuromast.

INTRODUCTION
Sensory antennae

The initial step of sensory perception is often described as transduction, the process by which a physical stimulus is interpreted as a neural signal. Even before transduction occurs, however, perception depends on the structure of the sensory organ that acts as an interface between the external or internal environment and the receptor cells. Like an antenna for electromagnetic radiation, a sensory antenna captures, concentrates and filters the relevant energy in order to improve transduction and facilitate processing.

Antennal structures occur in organisms at multiple physical scales. At a molecular level, most molecules of chlorophyll and bacteriochlorophyll are termed antennae, for they provide a large surface over which to capture light and transfer the resultant electronic excitation to a photosynthetic reaction center (reviewed in Neilson and Durnford, 2010). On the scale of organelles, the primary cilium is deemed an antenna for the receipt of various morphogenetic signals (reviewed in Marshall and Nonaka, 2006; Singla and Reiter, 2006). At the cellular level, the fibroblasts enwrapping a Pacinian corpuscle constitute an antenna that selectively transmits the high-frequency components of vibratory stimuli through the skin to the axon ending (reviewed in Bell et al., 1994). Finally, on the scale of organs, the cornea and lens of the human eye constitute a sensory antenna that captures light and focuses it onto the retina.

The external ear of a mammal, called a pinna, provides an especially clear example of a sensory antenna. The pinna not only channels sound from a large area into the ear canal, enhancing the animal's sensitivity, but also spectrally modifies the incoming sound (Middlebrooks and Green, 1991; Walsh et al., 2008). The ridges of the pinna generate destructive interference at a frequency that depends on the elevation at which the sound originates relative to the animal. The resultant decrease in sensitivity is called the pinna notch. Because the frequency of the notch depends on the elevation of the source, the brain can use the spectral characteristics of the notch to localize the source of the sound (Hebrank and Wright, 1974). The structure surrounding the ear canal thus acts as an antenna essential for the higher-order processing of sound.

The lateral-line system

The simplest vertebrate acousticolateralis organs are the neuromasts of the lateral-line system found in fishes and aquatic amphibians. Each neuromast includes a cluster of hair cells, the mechanoreceptors also found in vertebrate internal ears, organized in a streak on the surface of the skin or in a subcutaneous canal. Animals utilize neuromasts to detect low-frequency vibrations and water flows (Engelmann et al., 2000). The lateral-line system consists of tens to hundreds of neuromasts distributed across the animal's body. Depending on the species, each neuromast in turn contains tens to hundreds of hair cells covered by a gelatinous cupula that protects the hair cells and transfers water movements to their mechanosensitive hair bundles (McHenry and van Netten, 2007).

The lateral line offers fish an ability that is difficult for us as terrestrial vertebrates to imagine: the sensation of near-field disturbances, also referred to as a sense of distant touch (Dijkgraaf, 1962, Bleckmann, 1993, Ghysen and Dambly-Chaudière, 2007). As

expected from the evolutionary conservation of such a sensory system, the lateral line is involved in a multitude of behaviors. Many species of fish have been shown to use the lateral-line system for rheotaxis, the act of orienting in a water current (Montgomery et al., 1997; Kanter and Coombs, 2003). Blinded fish are able to school or swim in a coordinated manner with conspecifics. However, they lose the ability to do so if the lateral line is destroyed, suggesting that use of the lateral line enables the schooling behavior (Pitcher et al., 1976). Blind cavefish (*Astyanax mexicanus fasciatus*) are able to navigate in their environment (Sharma et al., 2009), discriminate spaces between objects (Hassan, 1986) and detect prey (Yoshizawa et al., 2010), all of which are behaviors presumably driven by the lateral-line system. Numerous other species utilize the lateral line for hunting. The efficiency of prey capture in the muskellunge (*Esox masquinongy*) is inhibited by either blinding or lateral-line inactivation (New et al., 2001). These two manipulations affect distinct components of the behavior. Blinded fish do not stalk their prey, but still strike prey at short distances. Fish whose lateral lines have been inactivated stalk their prey, but are more likely to miss. The nocturnal piscivorous European catfish (*Siluris glanis*) actively tracks prey in three dimensions in darkness (Pohlmann et al., 2004). Inactivation of the lateral line suppresses this behavior, but elimination of the animal's external taste sense does not. Other fish, such as the willow shiner (*Gnathopogon elongatus caerulescens*) and the mottled sculpin (*Cottus bairdi*), are also able to hunt prey in darkness using their lateral lines (Coombs and Janssen, 1990; Mukai et al., 1994; Kanter and Coombs, 2003).

Many species of fish can hunt on the surface of the water when blinded or in darkness using a specialized lateral-line system atop their heads. Groups that have been shown to do so include freshwater hatchetfish (Gasteropelecidae), freshwater butterflyfish (Pantodontidae), halfbeaks (Hemiramphidae) and killifish (Aplocheilidae). Because the striped panchax (*Aplocheilus lineatus*) performs this behavior admirably (Schwartz, 1971), we chose to use it as a model to investigate the role of sensory antennae in the lateral-line system.

Aplocheilus lineatus hunts at the surface of the water (Fig. 1A) and is able to localize and capture insects in darkness by detecting the capillary waves produced by its prey (Schwartz, 1965). The fish detects these waves with neuromasts located on the dorsum of its head. The lateral lines of most fishes, which detect water-borne vibrations and fluid flows, comprise simple rows of neuromasts upon the head and along the trunk (Engelmann et al., 2000). By contrast, the cephalic lateral line of *A. lineatus* is highly specialized, consisting of 18 exceptionally large neuromasts in a stereotyped array (Fig. 1B–D) (reviewed in Bleckmann et al., 1989).

Approximately 150 ms after the arrival of capillary waves, the prey-localization response of *A. lineatus* commences with a rapid rotation of the body toward the target followed by translation to the source of the stimulus (supplementary material Movie 1) (Bleckmann and Schwartz, 1981). The two determinants of this behavior, estimation of the target direction and of the distance to the source, are largely independent; experimental manipulations can have a disproportionate impact on one component or the other. The removal of an individual neuromast, for example, leads to an error in the estimation of the target direction but has little effect on the determination of distance (Schwartz, 1965). If all the neuromasts except one are ablated, the fish responds identically to targets in any position, turning by an angle that depends on which neuromast remains (Müller and Schwartz, 1982). However, the fish is still able to make accurate estimates of distance. The capillary surface waves that the fish detects exhibit anomalous dispersion: higher-frequency waves travel faster than lower-frequency waves (Lighthill, 1996). Artificial manipulation of wave dispersion can corrupt a fish's estimate of the source distance without affecting the judgment of angle (Bleckmann and Schwartz, 1982). These results imply that the fish analyzes the waveform to determine the distance that the waves have traveled but compares signals between neuromasts to estimate the waves' direction of origin. In view of the distributed pattern of neuromasts (Fig. 1B–D), a fish could compare arrival times of stimuli between neuromasts to calculate the origin of the waves. The head anatomy and stereotypy of the neuromast array – as well as the animal's behavior when all neuromasts but one are removed – suggest that individual neuromasts have unique receptive fields that a fish uses along with timing information to precisely localize the source of a stimulus.

Because of its great morphological diversity in fish species (Coombs et al., 1988), the lateral-line system offers an excellent opportunity to investigate the role of sensory antennae in perception. Fish of most species have both superficial neuromasts, which are located on the animal's surface, and canal neuromasts, which are embedded in a canal beneath the surface and connected to the environment through pores. The neuromasts act as detectors of pressure differentials between these pores, desensitizing the neuromasts to constant flows and allowing an animal to detect accelerations (Coombs et al., 1988). Some superficial neuromasts are embedded in a pit or groove that modifies their sensitivity. The cephalic superficial neuromasts of *A. lineatus* that mediate its mechanosensory prey-localization behavior are structurally unusual in that they are flanked by fleshy ridges, making them superficial receptors that may function like canal organs. When the fish assumes its hunting posture immediately below the water's surface (Fig. 1A),
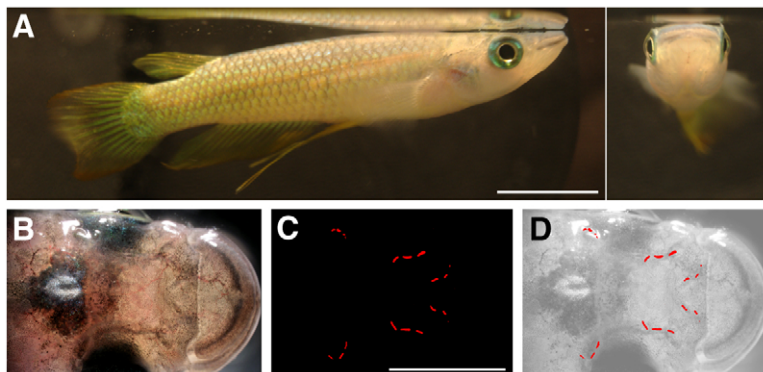

Fig. 1. Head anatomy of *Aplocheilus lineatus*. (A) The killifish hunts with its head immediately below and parallel with the surface of the water, maximizing its sensitivity to capillary waves. Scale bar, 10 mm. (B) The array of specialized neuromasts occurs on the fish's flattened head between the eyes and behind the prominent semicircular lip. (C) In a fluorescence micrograph, labeling with a red fluorophore concentrated by hair cells reveals the positions of the largest 16 of the 18 neuromasts. Scale bar, 5 mm. (D) Superposition of the previous two images reveals the position of the neuromasts on the dorsal surface of the head.

the ridges form a channel with the water's surface acting as its roof. In this work we sought to test the hypothesis that these ridges function in the perception of surface waves by *A. lineatus*. After determining whether modifications of the ridge system affected the receptive fields and the hydrodynamic environment of the neuromasts, we investigated the behavioral effects of perturbations of the system of ridges.

MATERIALS AND METHODS
Animal care

Aplocheilus lineatus (Valenciennes 1846) of the Golden Wonder strain were procured from a fish distributor (Aquatics and Pets, Inc., Riverview, FL, USA) and maintained in filtered water at room temperature. Fish to be used for electrophysiology were kept in water at pH7 adjusted to a conductivity of $2\,mS\,m^{-1}$ with $7\,g\,l^{-1}$ of aquarium salt (Instant Ocean, Aquatic Ecosystems, Apopka, FL, USA). All other fish were maintained at pH7 and a conductivity of $300–400\,\mu S\,m^{-1}$. Each fish used for behavioral testing was isolated in the same solution in a 2 or 4l tank for the 3 week duration of the experiment. Water quality was monitored and the water was regularly refreshed. The experiments were approved by the Institutional Animal Care and Use Committee of The Rockefeller University.

Anatomy

Hair cells were visualized by incubating fish for 5 min in $200\,\mu mol\,l^{-1}$ 4-(4-diethylaminostyryl)-*N*-methylpyridinium iodide (4-Di-2-ASP; Sigma-Aldrich, St Louis, MO, USA), a cationic fluorophore that traverses transduction channels and accumululates in hair cells, and observing them under a fluorescence dissecting microscope. Scanning electron microscopy was performed as described previously (Starr et al., 2004) with one significant modification. Because the cupulae of *A. lineatus* are extraordinarily resistant to enzymatic digestion, we found it necessary in acquiring scanning electron micrographs of hair bundles to reduce the pH of the solution to 3.5 during a 20 min digestion at room temperature with $100\,\mu g\,ml^{-1}$ porcine gastric pepsin, an acid protease. Prior to fixation, the fish were treated for 20 min at room temperature with $100\,\mu g\,ml^{-1}$ subtilopeptidase (protease XXIV, Sigma). Three to four fish were used for each anatomical preparation.

Electrophysiological measurements

Each fish was anesthetized in either $66\,mmol\,l^{-1}$ ethyl carbamate (urethane, Sigma) or $670\,\mu mol\,l^{-1}$ ethyl 3-aminobenzoate methanesulfonate (tricaine or MS-222, Sigma) in fish saline solution consisting of $116\,mmol\,l^{-1}$ NaCl, $2.9\,mmol\,l^{-1}$ KCl, $1.8\,mmol\,l^{-1}$ $CaCl_2$ and $5\,mmol\,l^{-1}$ HEPES at pH7. The fish was secured in a flexible plastic tube with its head protruding and adjusted to a hunting position relative to the surface of the water. In order for the fish to maintain a high sensitivity to capillary waves, it was important that the dorsum of its head lie within $200\,\mu m$ of the surface.

A glass micropipette of resistance $0.5–7\,M\Omega$ was placed approximately $80\,\mu m$ lateral to a neuromast and a ground electrode was situated in the bath. The microphonic signal was detected (Axoclamp 2A, Axon Instruments, Molecular Devices, Sunnyvale, CA, USA), amplified (AM502, Tektronix, Inc., Beaverton, OR, USA) and bandpass filtered between 10 Hz and 3 kHz. To block the transduction channels of the hair cells and abolish the microphonic response in control experiments, we added $100\,\mu mol\,l^{-1}$ 3,5-diamino-6-chloro-*N*-(diaminomethylene)-pyrazine-2-carboxamide (amiloride, Sigma) to the bathing solution (Jørgensen and Ohmori, 1988).

Neuromasts were stimulated either directly with a piezoelectric actuator attached by a fine glass rod to the cupula and driven by a high-voltage amplifier (Burleigh Instruments, Fishers, NY, USA), or through surface waves generated with air puffs produced by a shielded speaker driven by a stereo amplifier (SA1, Tucker-Davis Technologies, Alachua, FL, USA) that produced sinusoidal wave stimuli on the water's surface. The latter procedure resembled that described previously (Bleckmann and Schwartz, 1982). The speaker's diaphragm was covered with a plastic Petri dish to create a sealed chamber. A hole drilled through the center of the dish held a glass capillary tube 1.2 mm in internal diameter and 76 mm in length. Stimulation of the speaker sent pressure pulses down the capillary, the orifice of which was positioned 1–2 mm above the water's surface. The speaker was driven with 50 cycles of a 150 Hz voltage stimulus of ±3 V. The signal was sinusoidally modulated in amplitude to eliminate contaminants at other frequencies in the surface waves. This stimulus produced a wave amplitude of a few micrometers at the head of the fish, which sufficed to evoke a robust microphonic response (supplementary material Fig. S1). The speaker assembly was secured to a computer-controlled two-dimensional positioning system (Arrick Robotics, Tyler, TX) that was used to stimulate different points on the water's surface. Waveforms were monitored with a heterodyne interferometer (OFV501 and OFV3001, Polytec GmbH, Waldbronn, Germany) whose laser beam was aimed 1–3 mm lateral to the fish's left eye. We used Fourier analysis to confirm that the system produced surface waves with a spectrum strongly dominated by the stimulus frequency. The amplitude of the displacements used for the direct stimulation experiments was calibrated with the interferometer. Experiments were controlled by a computer running LabView 8.5 (National Instruments, Austin, TX, USA). The system measured how microphonic responses changed as a function of the angle at which the waves originated. To do so, we systematically generated waves at different points along an arc 26 mm from the fish's head and compared the ensuing microphonic responses (supplementary material Fig. S1). Microphonic and interferometric data were analyzed in Fourier space with programs written in MATLAB (MathWorks, Natick, MA, USA).

Ridge modifications

The hydrodynamic environment of a fish's head was modified by attaching supplemental ridges made from either cyanoacrylate glue or epoxy cement to the head using the former adhesive. The ridges were removed after experimentation with forceps. Successful experiments were performed on a total of 45 fish. Although it was also possible to remove a fish's normal ridges surgically, we found that damage to the nearby neuromasts precluded interpretation of the electrophysiological results. Neuromasts or ridges were ablated by application of a cauterizing probe (Aaron Medical, St Petersburg, FL, USA) under anesthesia.

Hydrodynamic experiments

With a fish anesthetized and mounted as above, we scattered hollow phenolic beads 3–45 μm in diameter and $130\,g\,l^{-1}$ in density (Polysciences, Inc., Warrington, PA, USA) over the water's surface. The beam of a helium–neon laser was expanded and aimed at the surface of the water with a glancing angle of incidence to illuminate the beads. Stimuli were generated by the speaker system described above, with its orifice positioned 70 mm from the center of the fish's head. The stimulus was delivered at an angle of 45 deg to the left of the fish's longitudinal axis. The origin of our coordinate system lay at the center of the fish's head, which was defined by the

intersection of the fish's midline and a line running across the head between neuromasts LII2 and RII2. A video camera (Motionscope PCI2000S, Redlake Instruments, IDT, Tallahassee, FL, USA) recording at 500 frames s^{-1} was used to visualize bead movements over the head of the fish during continuous sinusoidal stimulation of the water's surface at 30 Hz. Bead trajectories were detected using an ImageJ program for particle tracking (Sbalzarini and Koumoutsakos, 2005). The 30 Hz Fourier components in the longitudinal and transverse directions of the neuromasts were determined with Mathematica 7 (Wolfram Research, Champaign, IL, USA). The corresponding means ± s.d. were computed from approximately 10 trajectories for each specific area.

Behavioral experiments

Fish were trained to localize floating food pellets 1 mm in diameter dropped onto the water's surface. Behavioral experiments were then conducted under infrared light by observers wearing light-converting goggles (Night Optics D-2MV, Huntington Beach, CA, USA). Because the fish lacked their ordinary avoidance response to looming visual stimuli, they were evidently unable to see under these conditions. Video images recorded at 20 frames s^{-1} were processed by subtracting the background, thresholding and tracking the fish's position and orientation with software written in MATLAB. The positions of pellet targets were entered manually. The fish's behavior was tracked for 4 s following presentation of the target. The target angle was defined as the angle between the fish's midline and a line running from the center of the fish's head to the target. The response angle was defined as the angle the fish turned initially, as defined by the first local minimum in the fish's angular velocity following the initiation of its response (supplementary material Fig. S2). For each fish, we found the proportion of successful responses, defined as trials in which the response angle fell within 40 deg of the target angle, and proportion of failures, in which the response angle was less than 20% of the target angle. These thresholds are based on the data from individual fish. The addition of 500 μmol l^{-1} amiloride to the water blocked any prey-localization response under infrared light.

Statistical analysis

The s.e.m. was calculated by dividing the standard deviation by the square root of the number of observations. Statistical significance was determined by a one-tailed paired Student's t-test. We tested for a non-normal distribution of proportions using the Lilliefors composite goodness-of-fit test and the Jarque–Bera hypothesis test of composite normality.

RESULTS

Anatomy of *A. lineatus*

Whereas the superficial neuromasts of most fish are circular and contain a few dozen hair cells (Ghysen and Dambly-Chaudière, 2007), the cephalic neuromasts of *A. lineatus* are elliptical and contain from approximately 70 to over 200 hair cells (Bleckmann et al., 1989). On each side of a fish, the neuromasts form a stereotyped array of three groups (groups I–III, from rostral to caudal) of three neuromasts apiece. Each neuromast is identified from rostral to caudal by group number, followed by a subgroup number provided in Arabic numerals from 1 to 3 (Fig. 1B–D). Each neuromast is bordered by a set of fleshy ridges (Fig. 2). The channel between the ridges is largely filled by the cupula.

An individual hair cell has a single axis of mechanosensitivity: displacement of the hair bundle toward its tall edge depolarizes the cell, whereas movement in the opposite direction hyperpolarizes it (Hudspeth and Corey, 1977). Although the axes of sensitivity of hair cells within a neuromast are parallel to one another, there are two groups of hair cells with opposing polarities (Fig. 3). This arrangement gives each cephalic neuromast of *A. lineatus* the greatest sensitivity to displacements in both directions along the neuromast's long axis. The differing orientations of the neuromasts might be expected to provide them with distinct sensitivities to water-surface waves.

Neuromast receptive fields

The organization of the cephalic lateral line of *A. lineatus* suggests that the individual neuromasts sample different parts of the sensory field on the water's surface. The behavioral effect observed when all neuromasts but one have been ablated also suggests that individual neuromasts have unique receptive fields. Rather than orienting toward the target, the fish consistently turns to a particular angle that depends on which neuromast remains and is independent of the target angle (Müller and Schwartz, 1982). Because both the anatomy and the behavior suggest that neuromasts have unique angular sensitivities, we sought to determine the receptive fields of each neuromast.

To optimize our stimulation protocol, we first determined the response characteristics of individual neuromasts. While recording the microphonic potential from a given neuromast, we mechanically stimulated the associated cupula with sinusoidal deflections at a particular frequency and amplitude. As previously reported, the neuromasts are sensitive to stimuli as small as tens of nanometers (Bleckmann and Topp, 1981). We found that the microphonic response to a 100 Hz sinusoidal stimulation grew linearly from tens
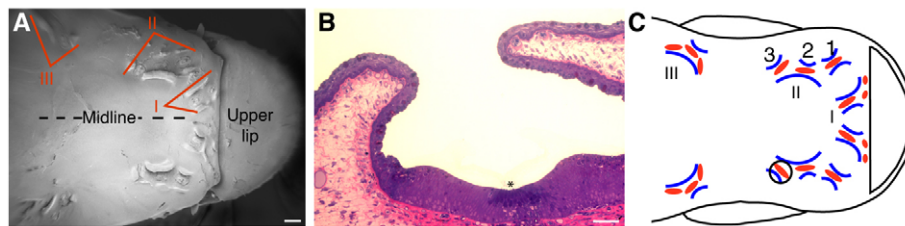

Fig. 2. Ridges bordering the neuromasts in *A. lineatus*. (A) A low-power scanning electron micrograph (SEM) shows that the neuromasts are bordered by fleshy ridges. Each neuromast is an elliptical structure with an outer ellipse indicating the area covered by the cupula and a narrow inner streak representing the hair cells. The three groups of neuromasts on the fish's left side are denoted by Roman numerals. Scale bar, 200 μm. (B) A semithin section across neuromast LII2 portrays the fleshy ridges bordering the neuromast. The hair cells with their hair bundles are marked by an asterisk; the cupula contracts during fixation and is not apparent. Scale bar, 50 μm. (C) A schematic of the cephalic lateral line of *A. lineatus* depicts neuromasts in red and ridges in blue. Each neuromast is identified from rostral to caudal by a group number expressed in Roman numerals from I to III, followed by a subgroup number provided in Arabic numerals from 1 to 3. As an example of the numbering scheme used, the circled neuromast is the third in group II on the right side, or RII3.
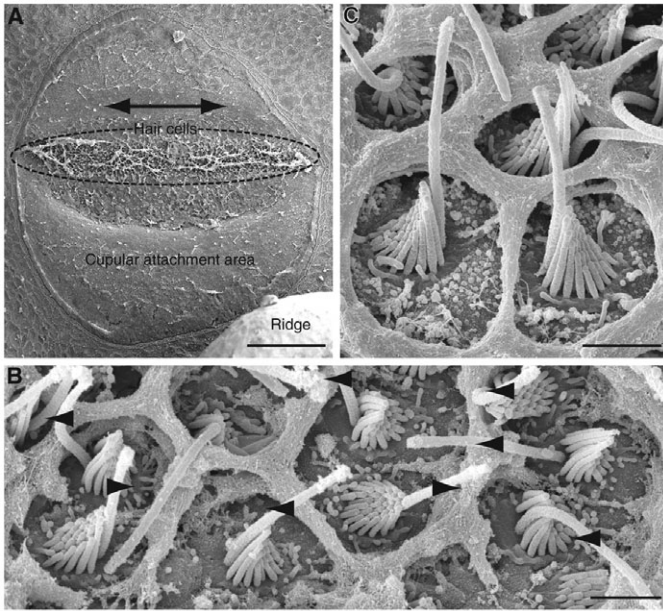
Fig. 3. Hair-bundle orientation. (A) An SEM illustrates cephalic neuromast LII3 of *A. lineatus*. The cupula has been removed enzymatically to expose the hair bundles, whose axes of sensitivity correspond to the long axis of the neuromast, indicated by the two-headed arrow. Scale bar, 50 μm. (B) An intermediate-magnification view of a neuromast resolves the orientations of individual hair bundles. Movement of a bundle toward its kinocilium, the single long process that ordinarily extends into the cupula, depolarizes the hair cell. This direction is indicated for each hair bundle by an arrowhead on its kinocilium. Scale bar, 2 μm. (C) A higher-magnification view shows a pair of hair bundles with opposite orientations. Remnants of the cupula form a porous web over the supporting cells that separate hair cells. Scale bar, 2 μm.
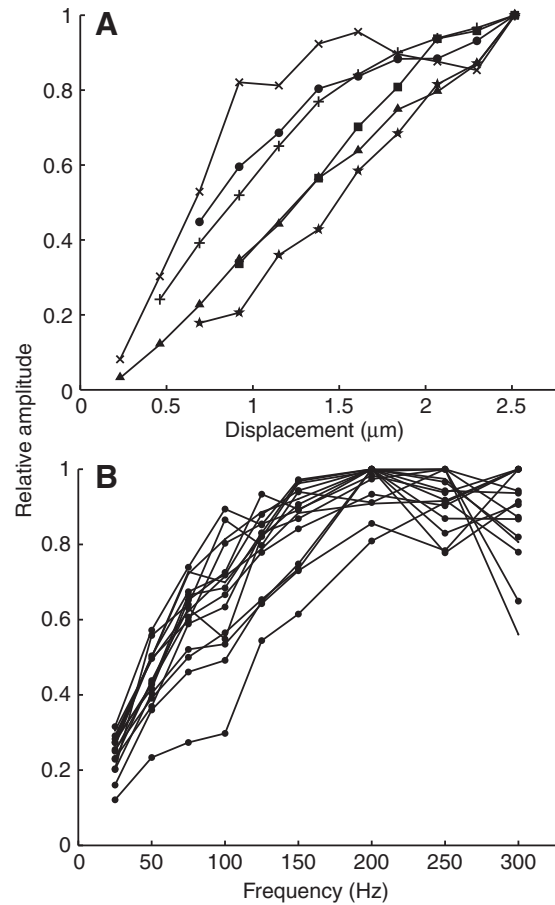

Fig. 4. Microphonic responses of *A. lineatus* neuromasts to direct cupular stimulation. (A) The neuromasts are sensitive to displacements of less than 100 nm up to a few micrometers. The responses are relatively linear over that range, although they begin to saturate at the larger stimulations. Each line represents a different neuromast. The different neuromasts display only minor differences in sensitivity, which lie within the range reported for behavioral responses. (B) Neuromasts act as high-pass filters. Their sensitivity increases briskly as the frequency rises from 25 to 100 Hz, then increases gradually up to 200 Hz before beginning to fall gradually. Each line represents a different neuromast. There are no significant differences in the frequency sensitivities of different neuromasts.

of nanometers up to a few micrometers (Fig. 4A). We observed a spectral sensitivity of individual neuromasts similar to that reported previously, but with greater responsiveness to high-frequency stimuli. When stimulated with sinusoidal deflections of ±1 μm, a neuromast acted as a high-pass filter, with responses increasing rapidly from 25 Hz to 100 Hz (Fig. 4B). We did not observe any differences between neuromasts.

While stimulating the water's surface at controlled locations, we recorded the extracellular microphonic responses of individual neuromasts to determine their sensitivity as a function of source location. We found that each neuromast has a unique receptive field, which may be relatively simple or may include multiple lobes of enhanced and diminished sensitivity (Fig. 5). The receptive field is characterized by the azimuthal angle and shows only modest sensitivity to the source's distance. Although the response of an isolated hair cell varies with the cosine of the angle between the direction of stimulation and the cell's axis of sensitivity (Shotwell et al., 1981), and the response of a neuromast might be expected to vary with the absolute value of the cosine in view of the two oppositely polarized populations of hair cells (Flock, 1965), the receptive fields generally displayed sharper angular sensitivity than expected from a simple cosine relation (Fig. 5).

As anticipated if the receptive fields are essential for an animal's detection of targets, the receptive fields of individual fish were stable over time (Fig. 6A,B). Although differing in some specific characteristics between different fish, the receptive fields of particular neuromasts maintained the same general features (Fig. 6C).

For example, neuromast LII2 consistently displayed a sector of reduced sensitivity directly to the left of a fish, but the size of this notch varied between fish. This variability accords with the observation that although the anatomy of the head is stereotyped across fish, there is individual variability in the exact structure. We noted in particular that neuromasts of group III lie systematically more caudal with respect to the others in larger *versus* smaller fish. Neural plasticity may permit a juvenile fish to learn the particular receptive fields of its own neuromasts.

We hypothesized that the fleshy ridges that border the neuromasts structure the hydrodynamic environment around the neuromasts and thus shape their receptive fields. The ridges might focus wave energy from particular directions onto specific neuromasts and block energy from other directions, for example, thereby sharpening the spatial sensitivity of individual neuromasts. To test this hypothesis, we modified the surface of the head by affixing in a particular position a supplemental ridge similar in size and shape to the natural ridges (supplementary
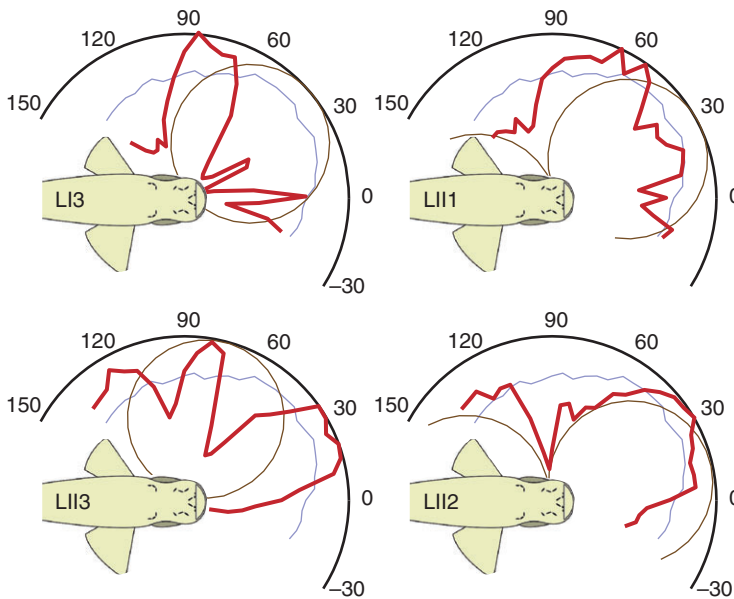
Fig. 5. Receptive fields of cephalic *A. lineatus* neuromasts. The spatial sensitivity of individual neuromasts (LI3, LII1, LII3 and LII2) was determined by recording microphonic potentials while 150 Hz waves were generated at different positions along an arc around the head. Each neuromast has a unique receptive field, here portrayed as a red antenna function centered on the fish's head. For comparison, the blue arc depicts the magnitude of the capillary waves reaching the head as a function of their angle of origin and the brown circle represents a cosine function aligned with the typical orientation of the neuromast represented. 0 deg is straight ahead, positive angles lie to the left of the fish and negative angles occur to its right. See Fig. 2C for locations and orientations of individual neuromasts.

material Fig. S3A,B). Adding such a ridge reversibly altered the receptive fields of the adjacent neuromasts (Fig. 7). Not only was the magnitude of the response affected, but in many instances the pattern of angular responsiveness also changed dramatically. The large alteration of a receptive field in response to a structure similar to the natural ridges suggested that the ridges themselves have a significant effect on water movement.

Hydrodynamics

By tracking the movement of beads on the water's surface, we were able to investigate the effect of the ridges on the movement of liquid over a fish's head. We hypothesized that the ridges structure the hydrodynamic environment of the neuromasts. To test this idea, we first compared water movement lateral to, medial to and directly over the neuromasts of group II in response to sinusoidal stimulation. The particle trajectories over neuromasts LII2 and LII3 differed dramatically owing to the presence of the ridges (Fig. 8A). Lateral to the neuromasts, the water movements were large and resembled those in the free field. In contrast, we observed a substantial reduction in water motion medial to the neuromasts (Fig. 8B). As expected if the ridges serve to channel waves onto the neuromasts, we measured an increase in the longitudinal water movement along a neuromast's axis of sensitivity relative to the transverse movement orthogonal to that axis. Ablating the ridges around group II eliminated this trend (Fig. 8C). Removal of the ridges also rendered the direction of fluid flow uniform: we no longer observed the systematic angular difference in particle trajectories over neuromasts LII2 and LII3. The differences in water movement at distinct positions on the head and the abolition of those differences when the ridges were removed supported the hypothesis that the ridges modulate wave movements over the neuromasts.

We also observed a hydrodynamic effect from a supplemental ridge cemented to the head (Fig. 8D). The addition of a ridge consistently changed the water movement; its subsequent removal always produced at least a partial recovery. The effect on receptive fields of adding a supplemental ridge (Fig. 7) likely reflected the change in the hydrodynamic environment surrounding the neuromast.

Behavior

To test the relationship between the cephalic ridges and the fish's perception of objects on the water's surface, we manipulated the ridges as before and measured the effect on the fish's prey-localization performance. A freely swimming fish was stimulated in darkness with food pellets dropped individually onto the water's surface at a variety of angles with respect to the fish's initial orientation. Using a high-speed video camera and infrared illumination to which the fish was insensitive, we recorded each response and determined the angle to which the fish initially turned.

We observed no behavioral changes upon the addition of a ridge to an intact fish. A fish could also orient effectively following bilateral removal of all of the neuromasts in groups I and III (supplementary material Fig. S3C). After that lesion, however, we found that adding a ridge adjacent to group II impaired localization (Fig. 9A). More specifically, the presence of a supplemental ridge significantly reduced the probability that a fish would accurately estimate the target angle (Fig. 9B) and significantly increased the probability that the fish would fail to respond to a target pellet (Fig. 9C). The mean proportion of successful trials conducted with 16 fish fell from 0.68 to 0.60 and the mean proportion of failed trials rose from a control level of 0.18 to 0.27 when a supplemental ridge was present. For the same 16 fish, we also measured a significant increase in the reaction time to a stimulus, from a mean ($\pm$s.e.m.) value of 444 ± 21 ms in control trials to 528 ± 43 ms when a ridge was added ($P<0.05$). The behavioral impact of the addition of a supplemental ridge varied considerably between fish. Some fish were severely impaired, whereas others were minimally affected. A few even improved their performance in the presence of an artificial ridge: six of the 16 fish increased their proportion of successful trials and five reduced the proportion of failed trials when a ridge was present (Fig. 9B,C). Four of these fish showed improvements by both measures. This variability could reflect differences in localization strategies between individuals or a problem with our experimental manipulation of certain fish. Some fish might depend more heavily on the relative intensities of activity between neuromasts, for example, whereas others might rely more on the relative timing of activity. We would expect the former group
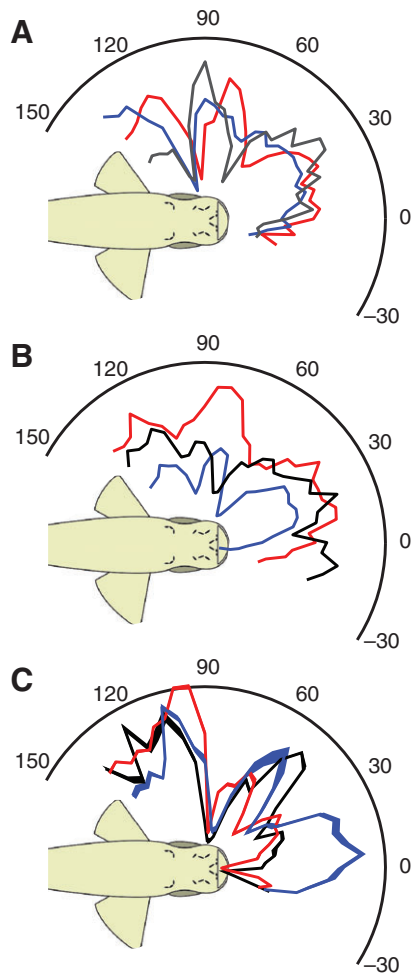
Fig. 6. Consistency of receptive fields in *A. lineatus*. (A) The microphonic receptive field of neuromast LII2 from a single fish reveals some variation on three different days (red, blue and black lines), probably owing to slight changes in the mounting angle and depth of the fish. Nevertheless, the shape of the field is stereotyped. (B) Similar records represent the receptive field of neuromast LII3 from a single fish on three different days. (C) The receptive fields for neuromast LII2 from three different fish display some differences, but the general features – most conspicuously the reduced sensitivity between 60 and 90 deg – are maintained between individuals. Each record, the mean of five determinations, is bracketed by the s.e.m., which is comparable to the line thickness.
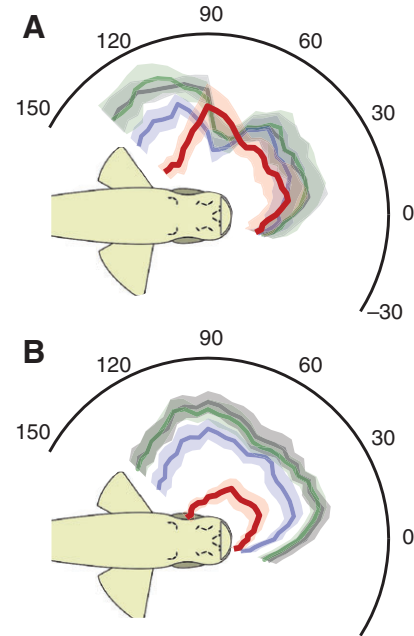

Fig. 7. Effect of a supplemental ridge on neuromast receptive fields in *A. lineatus*. (A) Adding a supplemental ridge lateral to neuromast LII3 alters the responsiveness of neuromast LII2. The black record represents the control recording of the receptive field and the nearly identical green record the response after a sham operation. The red record displays the effect of an added ridge, and the blue record documents the recovery after the ridge's removal. Note the effect of ridge addition on the characteristic notch in sensitivity at 60–90 deg. The shaded regions represent ±s.e.m. for 12 fish. (B) As would be expected from the position of the supplemental ridge, the effect of the same added ridge on neuromast LII3 in 14 fish is most significant at the caudal end of the receptive field.

to be more affected by the addition of a supplemental ridge. We did not see a reduced proportion of successes, an increased rate of failure or a greater reaction time in controls subjected to mock ridge additions.

DISCUSSION

Using only its mechanosensory lateral line, *A. lineatus* displays a remarkable capacity to rapidly and accurately localize prey in darkness (Schwartz, 1965; Bleckmann and Schwartz, 1981; Bleckmann and Schwartz, 1982; Müller and Schwartz, 1982), a valuable ability for an insectivorous tropical fish in its native habitat. An untrained *A. lineatus* readily hunts in low light and darkness, suggesting that in the wild the fish continues to hunt as insect activity increases around dusk and into the night (Corbet, 1960; Jones et al., 1967). The structure of the cephalic lateral line underlying the

behavior is stereotyped across individuals, not only in the location and orientation of the neuromasts but also in the presence of the adjacent ridges. Grosser elements of the structure, such as the large elliptical neuromasts flanked by ridges, are conserved throughout the phylogenetic branch to which *A. lineatus* belongs. We observed similar structures not only in a sister species, *Aplocheilus panchax*, but also in another cyprinodontiform fish, the platy (*Xiphophorus* sp.), and even in a beloniform fish, the medaka (*Oryzias latipes*) (J.S.S., personal observations). The order Beloniformes is a sister group to the Cyprinodontiformes that includes halfbeaks, ricefish and flying fish (Steinke et al., 2006). Halfbeaks are capable of mechanosensory prey localization (Schwartz, 1971); the shared cephalic lateral-line structure is presumably an ancestral trait in this group that has been retained for surface-wave detection.

The angular sensitivity of a single hair cell follows a cosine function (Shotwell et al., 1981). Because of the two populations of oppositely polarized hair cells within a neuromast, the angular response of *A. lineatus* neuromasts might in the simplest case be defined by the absolute value of a cosine function. Although we observed some neuromasts with receptive fields that resembled such a function, most neuromasts had receptive fields with multiple lobes of sensitivity over the 160 deg tested. We hypothesized that this complex structure is generated by the ridges adjacent to each neuromast, and specifically that these ridges direct the flow of waves over the head in such a way as to sharpen the angular sensitivity of the neuromasts. To test this hypothesis, we added supplemental
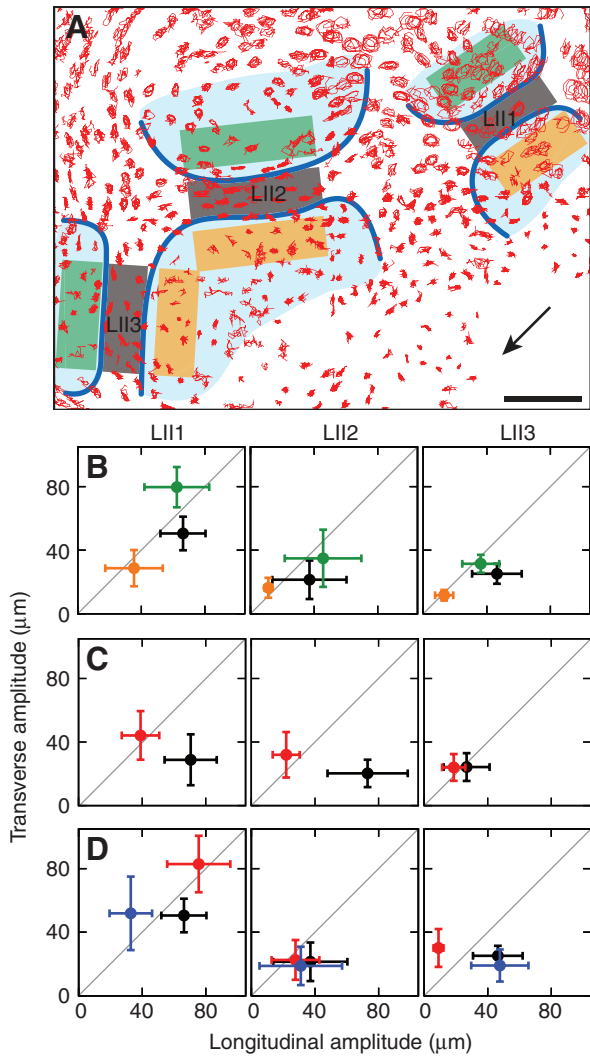
Fig. 8. Hydrodynamic effects of natural and supplemental ridges in *A. lineatus*. (A) In these experiments on group II neuromasts, the regions of interest lie lateral to (green), directly above (black) or medial to the neuromasts (orange). The longitudinal component of a measurement corresponds to the long axis of a rectangle and the transverse component to its short axis. The arrow represents the direction of movement of the incident stimulus waves. The difference in trajectories (red) over LII2 and LII3 apparently stems from the ridges. (B) The natural ridges cause a difference in water movement at different positions over a fish's head. Water movements are largest lateral to the neuromast (green) and smallest medial to the neuromast (orange). Directly above the neuromast (black), the longitudinal amplitude exceeds the transverse amplitude: the ridges direct water movement along the neuromast's axis of sensitivity. (C) After the ridges have been removed we no longer observe a high ratio of longitudinal to transverse amplitudes, suggesting that the ridges are responsible for that effect. Control experiments are shown in black; observations after removing the ridges are shown in red. (D) Adding a supplemental ridge changes the water movements measured directly above the identical neuromasts. The control experiments are shown in black, the trials with the supplemental ridges in red and the recovery data following removal of the ridges in blue. The experiments shown in B and D are from the same fish whereas that in C is from a different fish. Error bars (all panels) are ±s.d. of the trajectories.
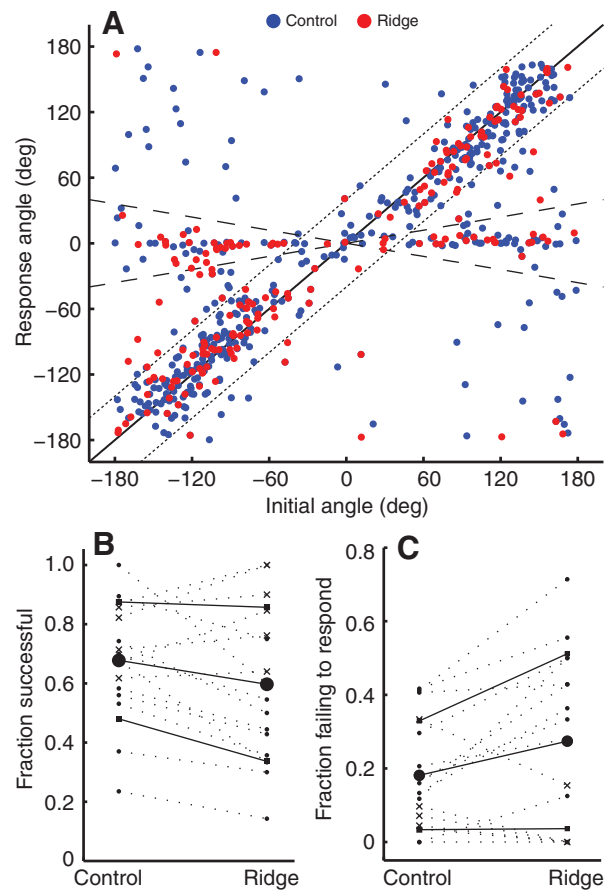

Fig. 9. Behavioral effects of a supplemental ridge in *A. lineatus*. (A) When the responses to target pellets dropped onto the water's surface were measured, successful responses clustered along the line of unity slope (solid line). Control fish (blue) consistently estimated the target angle accurately; the addition of a supplemental ridge (red) did not have a noticeable effect on the fishs' angle-estimation ability in this pooled representation. However, when individual fish were analyzed, the addition of a supplemental ridge both increased the proportion of trials in which the fish failed to respond, which we defined as trials in which the fish's response angle was less than 20% of the target angle (dashed lines), and reduced the incidence of successful responses, which we defined as trials in which the response angle was within 40 deg. The dotted lines contain most of the trials in which the responses clustered along the line of unity slope whereas the dashed lines contain of the trials in which the responses clustered along the abscissa. Note that these are not entirely independent sets. (B) The proportion of trials in which the fish successfully estimated the target angle dropped when a ridge was present. The large circles represent the mean proportion for each condition and the squares represent the standard deviations. Individual fish are represented by the smaller symbols (small circles, the 10 fish that followed the trend; ×, those that opposed the trend). The addition of a supplemental ridge significantly reduced the proportion of successful responses (*P*<0.04). (C) The proportion of trials in which the fish failed to respond was significantly increased by the addition of a supplemental ridge (*P*<0.02). Symbols are the same as in B. Of the five fish that opposed the trend, four overlapped with those that countered the trend shown in B, suggesting an idiosyncrasy of those particular fish.

ridges to the fish's head and documented their effect on the receptive fields, which proved to be considerable. We also measured water movements over the head in relation to both the natural and supplemental ridges, finding that the natural ridges block the propagation of surface waves and direct the energy of waves along the axes of sensitivity of the neuromasts. The presence of a supplemental ridge perturbed water flows over the head of the fish. Together these results imply that the ridges structure the

hydrodynamic environment of the head in a manner that affects the fish's perception of surface waves.

When all neuromasts except one are removed from the cephalic lateral line of *A. lineatus*, the fish always responds at a particular angle regardless of the target's direction (Müller and Schwartz, 1982). This response angle correlates with the location on the head of the fish of the remaining neuromast: anterior neuromasts drive responses through small angles whereas posterior neuromasts elicit responses through large angles. This position code is consistent with distance estimation based on the relative timing of activity between neuromasts. However, there are instances in which small distances between neuromasts result in different response angles, such as between neuromasts II2 and II3. In other cases, such as neuromasts III1 and III3, two neuromasts respond at similar angles despite the substantial distance between them. These results suggest that something more complicated is occurring. The response angle also correlates with the angle at which a neuromast is oriented on the head (Bleckmann et al., 1989). Comparison of the response angles with the peaks of angular sensitivity in the neuromast receptive fields that we observed is complicated by the fact that many of the receptive fields display multiple lobes of sensitivity. In every case, however, one of these peaks for each neuromast corresponds to the observed response angle (Müller and Schwartz, 1982; Bleckmann et al., 1989). The single neuromast for which the orientation was a poor predictor of response angle, neuromast II2, has a receptive field that peaks at precisely the reported response angle of 50 deg, suggesting that it is a neuromast's receptive field rather than its orientation that defines that neuromast's role in determining the angle of a target.

Given that the addition of a supplemental ridge affected the receptive fields of the contiguous neuromasts, the absence of behavioral impairment in fish with a normal complement of neuromasts suggests that there is considerable redundancy in this sensory system. For an intact fish with a supplemental ridge, it seems likely that the neuromasts of groups I and III compensated for deficits in those of group II. The observation that a fish with only group II neuromasts was impaired when a supplemental ridge was added nearby suggests that the fish utilize differences in receptive fields between neuromasts to make directional estimates of prey location. By analogy to the use by higher vertebrates of interaural sound-level differences for sound localization (Takahashi, 1989), a fish might compare the magnitude of activity between neuromasts, which depends on their respective receptive fields, to estimate the direction of a target. In a manner analogous to the analysis to interaural time differences, the fish could also localize prey by comparing the timing of activity between neuromasts, which is sensitive to the neuromasts' positions relative to the target and to each other.

Disruption of the receptive fields of at least two neuromasts by an added ridge did not significantly affect the angle-estimation ability of the fish. However, the reduced probability of responding and the delay in the responses indicate that the aberrant directional information that the fish received affected its performance. The fish apparently utilized the corrupted signals but overcame the misinformation through redundancy in its prey-localization system. The fish might have compensated by utilizing the timing differences of 1 ms or more between adjacent neuromasts.

In this study, we attempted to test the hypothesis that the ridges surrounding the cephalic neuromasts function as sensory antennae and that they are crucial components in the prey-localization response of *A. lineatus*. The ridges might alternatively or additionally protect the neuromasts from mechanical damage, allow the fish to determine its depth below the water's surface or merely relate to other adaptations to surface feeding such as the flattened dorsum

of the head. Although we cannot refute these alternative hypotheses, we have provided substantial evidence that the ridges function as sensory antennae. The receptive fields of individual neuromasts differ dramatically from the expected cosine structure because of the ridges. Perturbation of the ridge system through the addition of an artificial ridge alters the receptive fields of individual neuromasts. Hydrodynamic measurement of water trajectories over the neuromasts show that the ridges direct waves along the axes of sensitivity of the neuromasts and that manipulation of the ridges changes water trajectories. Finally, the addition of a supplemental ridge has a significant effect on a fish's prey-localization behavior.

CONCLUSIONS

The head of the surface-feeding killifish *A. lineatus* bears mechanosensitive neuromasts that underlie its ability to hunt in darkness. When stimulated with capillary waves on the water's surface, each neuromast displays a unique angular receptive field. The fleshy ridges that border the cephalic neuromasts modulate these receptive fields. The addition of a supplemental ridge reversibly affects the flow of water over the head, alters the neuromasts' receptive fields and produces a measurable behavioral impairment. The ridges thus act as sensory antennae that shape the stimuli reaching the fish's sensory transducers.

REFERENCES

Bell, J., Bolanowski, S. and Holmes, M. H. (1994). The structure and function of Pacinian corpuscles: a review. *Prog. Neurobiol.* **42**, 79-128.

Bleckmann, H. (1993). Role of the lateral line in fish behavior. In *Behaviour of Teleost Fishes*, 2nd edn (ed. T. J. Pitcher), pp. 201-251. London: Chapman and Hall.

Bleckmann, H. and Schwartz, E. (1981). Reaction time of the topminnow *Aplocheilus lineatus* to surface waves determined by video- and electromyogram recordings. *Experientia* **37**, 362-363.

Bleckmann, H. and Schwartz, E. (1982). The functional significance of frequency modulation within a wave train for prey localization in the surface-feeding fish *Aplocheilus lineatus* (Cyprinodontidae). *J. Comp. Physiol. A* **145**, 331-339.

Bleckmann, H. and Topp, G. (1981). Surface wave sensitivity of the lateral line organs of the topminnow *Aplocheilus lineatus. Naturwissenshaften* **68**, 624-625.

Bleckmann, H., Tittel, G. and Blübaum-Gronau, E. (1989). The lateral line system of surface-feeding fish: anatomy, physiology, and behaviour. In *The Mechanosensory Lateral Line System* (ed. S. Coombs, P. Görner and H. Münz), pp. 501-526. New York: Springer-Verlag.

Coombs, S. and Janssen, J. (1990). Behavioral and neurophysiological assessment of lateral line sensitivity in the mottled sculpin, *Cottus bairdi. J. Comp. Physiol. A* **167**, 557-567.

Coombs, S., Janssen, J. and Webb, J. (1988). Diversity of lateral line systems: evolutionary and functional considerations. In *Sensory Biology of Aquatic Animals* (J. Atema, R. R. Fay, A. N. Popper and W. N. Tavolga), pp. 553-594. New York: Springer-Verlag.

Corbet, P. S. (1960). Patterns of circadian rhythms in insects. *Cold Spring Harb. Symp. Quant. Biol.* **25**, 357-360.

Dijkgraaf, S. (1962). The functioning and significance of the lateral-line organs. *Biol. Rev.* **38**, 51-105.

Engelmann, J., Hanke, W., Mogdans, J. and Bleckmann, H. (2000). Hydrodynamic stimuli and the fish lateral line. *Nature* **408**, 51-52.

Flock, Å. (1965). Transducing mechanisms in the lateral line canal organ receptors. *Cold Spring Harb. Symp. Quant. Biol.* **30**, 133-145.

Ghysen, A. and Dambly-Chaudière, C. (2007). The lateral line microcosmos. *Genes Dev.* **21**, 2118-2130.

Hassan, E.-S. (1986). On the discrimination of spatial intervals by the blind cavefish (*Anoptichthyes jordani*). *J. Comp. Physiol. A* **159**, 701-710.

Hebrank, J. and Wright, D. (1974). Spectral cues used in the localization of sound sources on the median plane. *J. Acoust. Soc. Am.* **56**, 1829-1834.

Hudspeth, A. J. and Corey, D. P. (1977). Sensitivity, polarity, and conductance change in the response of vertebrate hair cells to controlled mechanical stimuli. *Proc. Natl. Acad. Sci. USA* **74**, 2407-2411.

Jones, M. D. R., Hill, M. and Hope, A. M. (1967). The circadian flight activity of the mosquito *Anopheles gambiae*: phase setting by the light regime. *J. Exp. Biol.* **47**, 503-511.

Jørgensen, F. and Ohmori, H. (1988). Amiloride blocks the mechano-transduction channel of hair cells of the chick. *J. Physiol.* **403**, 577-588.

Kanter, M. J. and Coombs, S. (2003). Rheotaxis and prey detection in uniform currents by Lake Michigan mottled sculpin (*Cottus bairdi*). *J. Exp. Biol.* **206**, 59-70.

Lighthill, J. (1996). *Waves in Fluids.* Cambridge: Cambridge University Press.

Marshall, W. F. and Nonaka, S. (2006). Cilia: tuning in to the cell's antenna. *Curr. Biol.* **16**, R604-R614.

McHenry, M. J. and van·Netten, S. M. (2007). The flexural stiffness of superficial neuromasts in the zebrafish (*Danio rerio*) lateral line. *J. Exp. Biol.* **210**, 4244-4253.

Middlebrooks, J. C. and Green, D. M. (1991). Sound localization by human listeners. *Annu. Rev. Psychol.* **42**, 135-159.

Montgomery, J. C., Baker, C. F. and Carton, A. G. (1997). The lateral line can mediate rheotaxis in fish. *Nature* **389**, 960-963.

Mukai, Y., Yoshikawa, H. and Kobayashi, H. (1994). The relationship between the length of the cupulae of free neuromasts and feeding ability in larvae of the willow shiner *Gnathopogon elongatus caerulescens* (Teleostie, Cyprinidae). *J. Exp. Biol.* **197**, 399-403.

Müller, U. and Schwartz, E. (1982). Influence of single neuromasts on prey localizing behavior of the surface feeding fish, *Aplocheilus lineatus. J. Comp. Physiol. A* **149**, 399-408.

Neilson, J. A. and Durnford, D. G. (2010). Structural and functional diversification of the light-harvesting complexes in photosynthetic eukaryotes. *Photosynth. Res.* **106**, 57-71.

New, J. G., Fewkes, L. A. and Khan, A. N. (2001). Strike feeding behavior in the muskellunge, *Esox masquinox*: contributions of the lateral line and visual sensory systems. *J. Exp. Biol.* **204**, 1207-1221.

Pitcher, T. J., Partridge, B. L. and Wardie, C. S. (1976). A blind fish can school. *Science* **194**, 963-965.

Pohlmann, K., Atema, J. and Breithaupt, T. (2004). The importance of the lateral line in nocturnal predation of piscivorous catfish. *J. Exp. Biol.* **207**, 2971-2978.

Sbalzarini, F. and Koumoutsakos, P. (2005). Feature point tracking and trajectory analysis for video imaging in cell biology. *J. Struct. Biol.* **151**, 182-195.

Schwartz, E. (1965). Bau und Funktion der Seitenlinie des Streifenhechtlings (*Aplocheilus lineatus*). *Z. Vgl. Physiol.* **50**, 55-87.

Schwartz, E. (1971). Die Ortung von Wasserwellen durch Oberflächenfische. *Z. Vgl. Physiol.* **74**, 64-80.

Sharma, S., Coombs, S., Patton, P. and de·Perera, T. B. (2009). The function of wall-following behaviors in the Mexican blind cavefish and a sighted relative, the Mexican tetra (*Astyanax*). *J. Comp. Physiol. A* **195**, 225-240.

Shotwell, S. L., Jacobs, R. and Hudspeth, A. J. (1981). Directional sensitivity of individual vertebrate hair cells to controlled deflections of their hard bundles. *Ann. N. Y. Acad. Sci.* **374**, 1-10.

Singla, V. and Reiter, J. F. (2006). The primary cilium as the cell's antenna: signaling at a sensory organelle. *Science* **313**, 629-633.

Starr, C. J., Kappler, J. A., Chan, D. K., Kollmar, R. and Hudspeth, A. J. (2004). Mutation of the zebrafish choroideremia gene encoding rab escort protein 1 devastates hair cells. *Proc. Natl. Acad. Sci. USA* **101**, 2572-2577.

Steinke, D., Salzburger, W. and Meyer, A. (2006). Novel relationships among ten fish model species revealed based on a phylogenomic analysis using ESTs. *J. Mol. Evol.* **62**, 772-784.

Takahashi, T. (1989). The neural coding of auditory space. *J. Exp. Biol.* **146**, 307-322.

Walsh, W. E., Dougherty, B., Reisberg, D. J., Applebaum, E. L., Shag, C., O'Donnell, P. and Richter, C.-P. (2008). The importances of auricular prostheses for speech recognition. *Arch. Facial Plast. Surg.* **10**, 321-328.

Yoshizawa, M., Goricki, S., Soares, D. and Jeffery, W. R. (2010). Evolution of a behavioral shift mediated by superficial neuromasts helps cavefish find food in darkness. *Curr. Biol.* **20**, 1-6.