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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/344/6188/1150/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S5
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References (*20*–*39*)

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SENSORY BIOLOGY

Marine teleost locates live prey through pH sensing

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We report that the Japanese sea catfish *Plotosus japonicus* senses local pH-associated increases in H^+/CO_2 equating to a decrease of ≤0.1 pH unit in ambient seawater. We demonstrated that these sensors, located on the external body of the fish, detect undamaged cryptic respiring prey, such as polychaete worms. Sensitivity is maximal at the natural pH of seawater (pH 8.1 to 8.2) and decreases dramatically in seawater with a pH <8.0.

Locating food is essential for the survival of any heterotrophic organism. Sensory systems such as vision, hearing, and chemoreception aid in this critical endeavor, because each can provide key information for identifying and locating prey at a distance. Vision is of limited use for species that are nocturnal and/or live in murky environments (such as fish). Chemoreception, however, is especially important for many aquatic organisms in acquiring food (*1*).

While investigating how chemical stimulus information is encoded by the taste system in fish, we discovered a remarkable sensitivity of the Japanese sea catfish (*Plotosus japonicus*) to small transient increases in ambient H^+/CO_2. Extracellular electrophysiological recordings from specific fibers of the facial [cranial nerve VII)/cranial nerve V (trigeminal)] nerve complex that innervates the maxillary barbel (the "whisker") of the catfish (*2*) (Fig. 1A) were excited by slight transient declines in the pH of the ambient seawater (SW) that contacted its barbel (Fig. 1B). These fibers characteristically elicited large-amplitude (hundreds of microvolts to 1 mV) action potentials that were often about double the amplitude of those evoked by other fibers of the nerve complex recorded extracellularly. The recordings revealed that the fibers responded to a decline of ≤0.1 pH unit in SW that washed over the maxillary barbel (Fig. 1B and table S1) (*3*), a similar sensitivity to pH as that observed for respiratory chemosensitive neurons (*4*) and associated astrocytes (*5*) in the mammalian medulla. Not only did small-volume transients (fig. S1) (*3*) and repetitive (fig. S2) (*3*) declines in pH in the SW bathing the barbel receptive field (RF) activate the "pH fibers," the fibers also responded to larger transient drops, even those into the slightly acidic range (Fig. 1C). If the pH of the SW bathing the RF was lowered to <pH 8.0 and maintained for several minutes, either a greater drop in pH was required to activate the same fibers or the fibers became inactivated (table S1 and Figs. 1D, 2, and 3) (*3*). When the flow of control SW (pH ~8.2) over the RF was resumed, however, sensitivity to falling pH was restored (Figs. 1D and 4).

Because sea catfish are benthic nocturnal feeders (*6*) whose stomach contents contain polychaete worms (*7*), we hypothesized that a function of the highly sensitive H^+/CO_2 system of the catfish is to detect polychaete worms, which live in semipermanent U- or Y-shaped burrows in coastal marine sediments (*8*) and release punctate

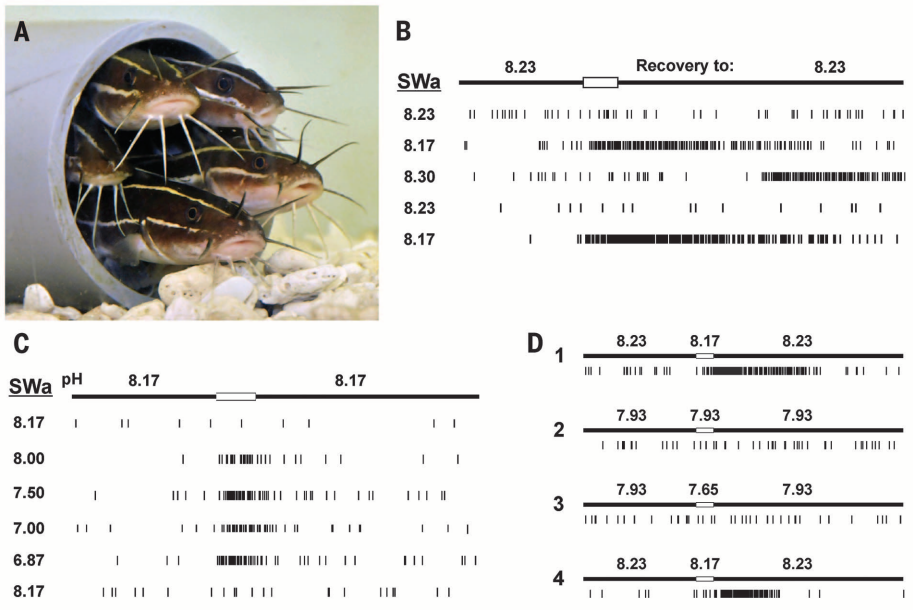

Fig. 1. Representative single nerve fibers that innervate the maxillary barbel respond to the falling pH of ambient SW. (**A**) Sea catfish, *Plotosus japonicus*. (**B**) Only falling pH activates the fiber. Artificial SW (SWa) of pH 8.23 flows over the barbel into which SW of either pH 8.23 (control), 8.17, or 8.3 is added (marked by the clear portion of the stimulus bar). Only falling pH activates the pH fiber (at stimulus onset for SW of pH 8.17 or with a delay after the onset of pH 8.30 SW) as the pH 8.23 SW background replaces the brief application of pH 8.30 SW. (**C**) A typical pH fiber is excited by brief applications of SW of pH < ambient, even to pH values into the acidic range. (**D**) SW of pH <8.0 that continuously bathes the barbel reduces the sensitivity of the sensor and sometimes inactivates the sensor (traces 2 and 3); however, sensitivity recovers to approximately control level (trace 4) after the barbel is bathed in pH 8.23 SW. The clear portion of the stimulus bar represents a 0.5-s stimulus presentation. Fibers shown in (B) to (D) are from different preparations.

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amounts of H^+/CO_2 into the surrounding water during respiration. To test this hypothesis, we first examined whether the respiratory transients of H^+/ CO_2 of the worm would be of sufficient magnitude to be detectable by sea catfish. Therefore, we recorded the pH of the SW within a 1-liter glass beaker every 5 s over 45 min at distances of 5 and 15 mm from the outflow end of a glass U tube used as an artificial burrow containing a single worm. With an electrode distance of 5 mm from the U-tube, the pH was transiently lowered by 0.15 to 0.25 pH units (Fig. 2A), a change in pH that was sufficiently within the range of sensitivity of the pH sensors of the sea catfish (table S1 and Fig. 1B) (*3*); at 15 mm from the U tube containing the worm, pH fluctuations were absent because of the buffering ability of the SW.

We next tested the hypothesis that sea catfish would be attracted to the region of the aquarium that contained a worm in its burrow. All behavioral experiments occurred in the dark (Fig. 2B) and were monitored with an infrared camera. Initial observations showed that sea catfish would approach a U tube containing a worm and suck it out of the tube (Fig. 2C and movie S1) (*3*). For the experimental tests, each of four catfish was placed in a separate aquarium filled with pH 8.0 to 8.1 SW and allowed to acclimate for 1 month. One hour before testing, a glass U tube, covered with netting, either empty or containing a live worm, was placed within the coral substrate of each aquarium at a location distant from the fish's nest; the netting was to prevent ingestion of the worm by the fish during the tests (Fig. 2D). Each 15-min test was conducted over 8 consecutive days. The results indicated that the catfish spent significantly more time within the designated partition of the tank containing the U tube with the worm than in the part of the tank with the U tube that lacked the worm (Fig. 2F).

Although the sensory systems used by the sea catfish to locate the worm in the preceding tests could have been mechanoreception, electroreception, and/or chemoreception, we hypothesized that the respiratory transients of increased H^+/ CO_2 from the worm were a sufficient stimulus to attract the catfish. We therefore tested whether a release from the U tube of SW of pH slightly less than that of the SW within the aquarium (pH 8.0 to 8.1) would result in the catfish spending more time in that region of the aquarium than when only control SW (pH 8.1 to 8.3) flowed from the U tube. A small length of polyethylene tubing was placed in one end of the U tube, and the other end was connected to a peristaltic pump located outside the aquarium that delivered SW at either pH 8.0 to 8.1 (control) or pH 7.8 to 7.9 (test) into the U tube (Fig. 2, B, E, and G). Four fish different than those tested previously were each placed in separate aquaria for at least 1 month before experimentation and were then tested over 8 consecutive days. For each test, 20 ml of pH-adjusted SW was pumped into the aquarium at a rate of 4 ml/min. The results indicated that the catfish spent significantly more time in the partition of the aquarium containing the U tube that emanated the lower-pH SW than when control SW was released (Fig. 2G). Further, the catfish were highly active and in an appetitive search mode when swimming in the vicinity of the U tube emitting the lower-pH SW. The catfish also frequently bit at the end of the U tube, a behavior never observed when control SW at the pH of the aquarium water was released (movie S2)

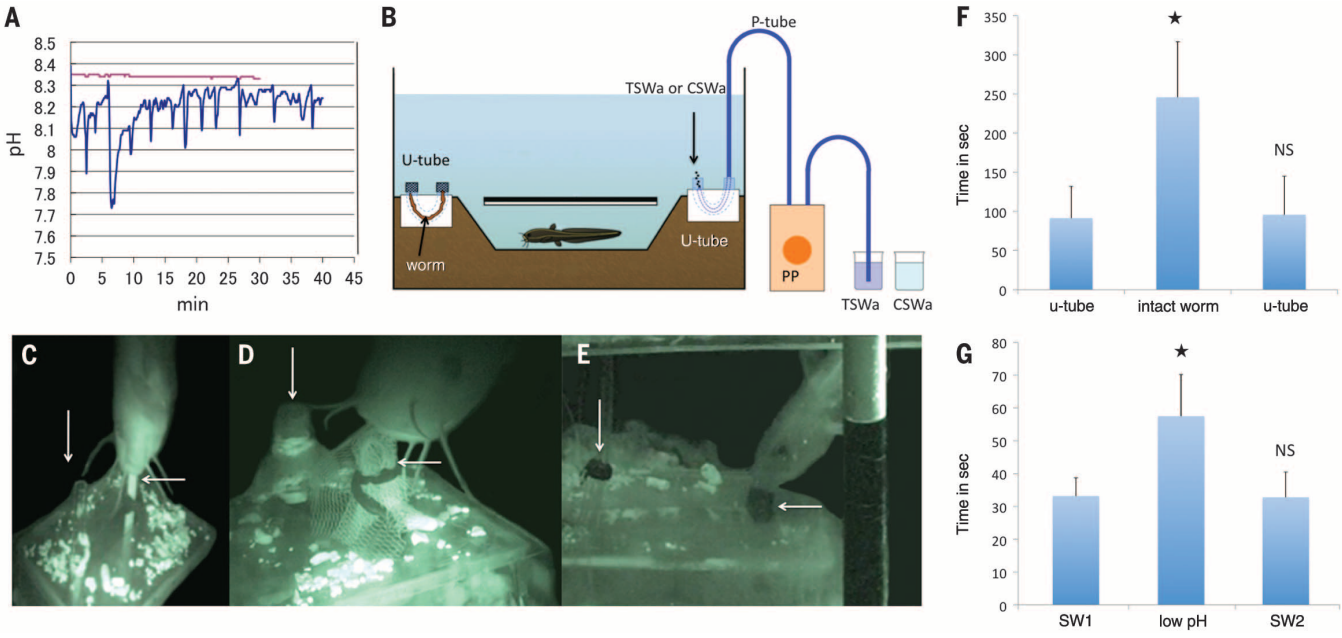

Fig. 2. Chemosensory response of the Japanese sea catfish to polychaetes and falling pH. (**A**) Recorded fluctuations in pH (by 0.15 to 0.25 pH unit) due to polychaete respiration recorded at 5 mm from the outflow end of a U tube containing the worm (blue) immersed in a 1-liter glass beaker containing pH ~ 8.35 SW; no change in pH was detected at 15 mm from the U tube (red). (**B**) Experimental setup: A single catfish constructed a nest within the coral substrate. A glass U tube was inserted into the substrate, containing either a live worm (left) or an empty U tube (right) emitting SW of slightly lower pH than that of the aquarium water. PP, peristaltic pump; TSWa, test SW; CSWa, control SW. (**C**) A catfish biting one end of the U tube and beginning to suck out the worm (movie S1). (**D**) A catfish biting the netting covering one end of the U tube that housed a worm. (**E**) A catfish attracted to and biting at the outflow end of a U tube lacking a worm, but emitting SW of lower pH (7.9) than ambient (pH 8.3) (movie S2). Arrows in (C) to (E) indicate the positions of the two openings of the U tube. (**F**) Catfish spent significantly more time in the quadrant of the aquarium containing a U tube with a worm (asterisk) than in the portion without a worm. Each of four catfish received three treatments (control-test-control) and eight replicates of each treatment; the mean of the eight replicates for each of the four animals was used in the statistical analyses to avoid pseudoreplication. The data were analyzed with a one-factor block design analysis of variance (ANOVA) ($F[2,6] = 27.51$, $P = 0.00095$), followed by a priori one-tailed t tests, $P < 0.05$; NS, not significantly different from the first bar. (**G**) Catfish spent significantly more time in the quadrant of the aquarium containing a U tube that emitted pH 7.9 SW (asterisk) than that emitting pH 8.3 SW, the same pH as the aquarium SW. Each of four catfish different from those tested in (F) received three treatments (control-test-control) with 8 to 10 replicates of each treatment, and the mean of the 8 to 10 replicates for each of the four animals was used in the statistical analyses to avoid pseudoreplication. These data were analyzed with a one-factor block design ANOVA ($F[2,6] = 8.21$, $P = 0.019$), followed by a priori one-tailed t tests, $P < 0.05$; NS, not significantly different from the first bar. Error bars in (F) and (G) are SEM.

(*3*). These results clearly showed that an elevation in ambient H^+/CO_2 alone was sufficient to attract the catfish to the worm prey.

The sensory origin of this extraordinary sensitivity to H^+/CO_2 is currently unidentified, because recordings were obtained from fibers within the facial/trigeminal complex that innervate the head, including the barbels (*9*, *10*). The sensors could be located in cells within taste buds innervated by VII fibers (*10*) and/or within solitary chemosensory cells (SCCs) that are scattered across the surface epithelium innervated by either V or VII fibers (*11*–*13*). Because both tastebuds (*10*, *14*, *15*) and SCCs (*16*) also occur along the flank of catfishes, the sensors might also be located within flank tastebuds innervated by VII nerves or on SCCs innervated by either VII and/or spinal sensory nerves (*17*, *18*). It is possible that the entire surface epithelium is H^+/CO_2-sensitive, as it is to amino acid stimuli (*19*). The molecular sensors of the sea catfish to H^+/CO_2 are unknown; however, if the sea catfish sensors are activated by H^+, they could possibly be related to acid-sensing (ASIC) (*20*, *21*) or TASK-2 (*22*) channels. If, however, the sensors detect CO_2, they might be related to connexin 26 hemichannels found in medullary respiratory neurons (*23*) or to currently unknown sensors located on neuroepithelial cells in fish gills (*24*). Whether the olfactory system of the sea catfish is also sensitive to H^+/CO_2 is unknown.

The decline in sensitivity of the H^+/CO_2–detecting system in SW <pH 8.0 suggests the possibility of compromised feeding behavior of these fish in their estuarine habitat due to transient drops in ambient pH commonly occurring in that environment (*25*). It is possible, however, that other sensory systems of the sea catfish not so affected during times of pH transients could compensate for locating prey. In addition to transitory alterations in the pH of the marine environment, anthropogenic activities are causing ocean acidification that can adversely affect marine organisms over the long term. The pH of ocean waters has been reduced from 8.2 to 8.1, a 30% rise in acidity since preindustrial times, and is predicted to decline to ~7.8, a 150% rise in acidity, by the end of the 21st century (*26*, *27*). Declining oceanic pH is shown to be detrimental to numerous aquatic species, including fish populations, because it alters various neurosensory systems and natural behaviors that affect species survival (*28*–*32*). If the rapidity of ocean acidification exceeds the plasticity (*24*) of the H^+/CO_2-sensing system of the Japanese sea catfish to adapt and reset its sensitivity to a lower-pH environment, then this detection system along with possibly other sensory systems will be compromised. Whether such a highly sensitive prey-detecting system is common among other benthic teleosts and how ocean acidification might impinge on the sensory capabilities of those organisms remain unknown.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/344/6188/1154/suppl/DC1
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Reference (*33*)
Movies S1 and S2
Databases S1 and S2 (Excel spreadsheets)

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PLURIPOTENCY PROGRAM

Defining an essential transcription factor program for naïve pluripotency

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The gene regulatory circuitry through which pluripotent embryonic stem (ES) cells choose between self-renewal and differentiation appears vast and has yet to be distilled into an executive molecular program. We developed a data-constrained, computational approach to reduce complexity and to derive a set of functionally validated components and interaction combinations sufficient to explain observed ES cell behavior. This minimal set, the simplest version of which comprises only 16 interactions, 12 components, and three inputs, satisfies all prior specifications for self-renewal and furthermore predicts unknown and nonintuitive responses to compound genetic perturbations with an overall accuracy of 70%. We propose that propagation of ES cell identity is not determined by a vast interactome but rather can be explained by a relatively simple process of molecular computation.

Mouse embryonic stem (ES) cells exhibit the capacity to self-renew indefinitely, the plasticity to generate all somatic lineages and germ cells, and the ability to reenter embryogenesis after blastocyst injection. Collectively these properties are described as ground-state pluripotency (*1*). The pluripotent state of mouse ES cells has been considered as controlled by a vast network of genetic interactions (*2*–*6*). However, only a limited number of transcription factors (TFs) have been validated rigorously. Two have been found to be indispensable: Oct4 and Sox2. In contrast, factors such as Nanog, Klf4, and Esrrb are individually dispensable, yet their overexpression can support self-renewal (*7*–*9*).

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