Supplementary Materials for

Marine teleost locates live prey through pH sensing

John Caprio,* Mami Shimohara, Takayuki Marui, Shuitsu Harada, Sadao Kiyohara

*Corresponding author. E-mail: jcap@lsu.edu

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Other Supplementary Materials for this manuscript include the following:
(available at www.sciencemag.org/content/344/6188/1154/suppl/DC1)

Movies S1 and S2

Materials and Methods

Experimental animals. Marine catfish, *Plotosus japonicas* (2), were collected with large net traps by local fishermen along the coast near Nagashima, Kagoshima Prefecture, Japan; some were also caught near Kagoshima with small net traps baited with either thawed krill (*Meganyctiphanes norvegica)* or live marine polychaete worms (*Perinereis nuntia*). The catfish were transported to the Division of Chemistry and Bioscience, Graduate School of Science and Engineering, Kagoshima University and maintained in a holding tank (90cm x 45cm x 45cm) filled with artificial sea water (SW) (Sealife, Marinetech Co., Ltd., Japan) at 24-26ºC under a 12:12 light:dark cycle. All SW in this report was made from the commercial sea salts. The SW was re-circulated after filtration by top and bottom sand bed filters. The pH and specific gravity of the SW were maintained at 8.1-8.2 and 1.024-1.026, respectively. The fish either in groups or as individuals were hand fed daily with thawed krill. At least one month prior to behavioral experimentation, individual specimens were transferred to test aquaria (60cm (L) x 30cm (W) x 35 cm (H) and maintained similarly as in the holding tank. All experimental procedures were approved according to the guidelines for the care and use of experimental animals of both Kagoshima University and Louisiana State University.

Electrophysiological Methods:

Animal immobilization, anesthesia, surgical preparation & data collection: Each catfish was initially immobilized with an intramuscular injection of the neuromuscular blocking agent Flaxedil (gallamine triethiodide, 0.05-1.0 mg/50g body wt), wrapped in wet tissue paper and positioned in a Plexiglas^{TM} container. Aerated water containing the anesthetic, ethyl-m-aminobenzoate methane sulfonic acid (MS-222, 0.05%), irrigated the gills via a recirculating system. Supplemental Flaxedil and MS-222 were provided as necessary to maintain animal immobility and a constant level of anesthesia. SW (9 ml/min without MS-222) was maintained over the maxillary barbel or lip. Neural activity was recorded with a tungsten hook electrode, a.c.amplified (Grass Instruments P511 with high impedance probe; band-pass 30-3,000 Hz), monitored aurally, displayed on an oscilloscope, integrated (0.3s) and printed on chart paper and recorded on an audio channel of a VCR along with a verbal description of the experimental procedures. For multifiber preparations, the neural activity was integrated (0.3s) and printed on chart paper; for single/few fiber preparations, action potentials were digitized at 32 kHz and analyzed off-line by Discovery software (Brainwave Systems Discovery package; DataWave Technologies, Longmont, CO) and printed.

Stimulus delivery & pH determination. Stimulus delivery was via a gravity-fed device employing a spring-loaded valve (model No. 5300, Rheodyn, Cotati, CA) driven by a pneumatic actuator. Stimulus pH solutions and the SW that continually bathed the maxillary barbel between stimulations were delivered through separate Teflon tubes at a rate of 9 ml/min. A foot switch connected to an electronic timer triggered the valve to introduce the stimulus (generally a 2.0 or 3.0 sec stimulus duration; 200 msec for S. Fig.1) without a change in either pressure or temperature and with minimal dilution. The stimulus delivery system reliably delivered the SW to the receptive field at the specified pH of the injected SW. In tests, the variation of the stimulus pH emitted from the stimulus delivery system was within 0.01-0.03 pH unit of that injected. The continual flow of SW over the maxillary barbel or lip protected the mucosa from desiccation, avoided mechanical artifacts associated with stimulus presentation and thoroughly

rinsed the RF between stimulations. Test solutions of SW of various pH values adjusted primarily with HCl (or in a few experiments with NaOH) were prepared immediately prior to each experiment, and the pH of these solutions was carefully checked during the experiments. SW of a lower pH than that bathing the maxillary barbel of lip served as a search solution to locate pH responsive nerve bundles/single & few fibers. pH measurements (NBS scale) were made with either a Horiba Navi pH Meter, F-52 equipped with a Horiba Laqqua pH electrode 9615-10D (Kyoto, Japan) or a Thermo Orion Co., Ltd. Benchtop pH/ISE meter, Model 720 Aplus equipped with an 8102 BN, Ross Combination pH electrode. The buffers used to calibrate the electrodes were: 0.34% potassium dihydrogenorthophosphate (pH 7) and 0.2% disodium tetraborate decahydrate (pH 9). Preparation of the V/VII nerve complex that innervated either the maxillary barbel, which was inserted into a sleeve formed from the barrel of a glass pipet, or lip was similar to that described previously (33).

Behavioral Methods:

Experimental animals. Eight healthy adult sea catfish (length: 15.0 – 19.5cm; weight: 30.8 - 45.6g) were selected from the stock and used for the behavioral experiments.

Behavioral tests. A room containing four aquaria [each 60cm (L) x 30cm (W) x 35cm (H)] for behavior testing was isolated from natural light. The bottom of each aquarium was covered with black coral stones, and a black plastic plate was placed on the stones suspended by four wires. Within a few days of holding, the catfish dug nests under the plates. Each aquarium was partitioned by external markers into four sections that housed the sea catfish in its nest in one of the sections. All behavioral tests were performed daily at the same time (1300-1500) over eight (Fig. 2f) to 10 (Fig. 2g) consecutive days with an inverted day:night (12:12) laboratory schedule under infra-red lighting (overhead fluorescent lamp, 20 W; mean light intensity of 1500 lux at the water surface). All behavioral experiments were recorded with a digital infra-red video camera (DCR-PC300 or DCR-SR220, Sony Co., Ltd. Japan).

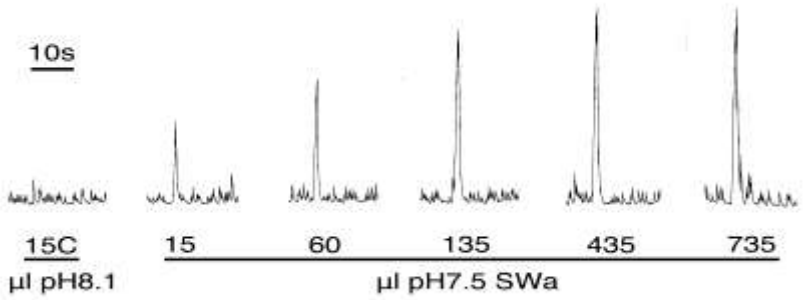

Fig. S1.Responses of maxillary barbel nerve to small volumes of pH 7.5 artificial seawater (SWa). Integrated (0.5 sec) neural responses from the maxillary barbel nerve of the sea catfish to repetitive 200 ms stimulations of various (15-735µL) aliquots of pH 7.5 SW into background pH 8.1 control (C) SW.

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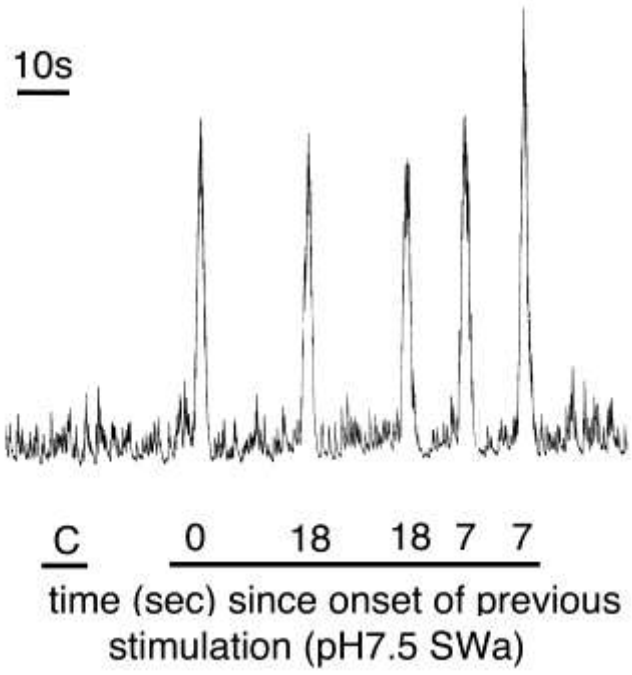

Fig. S2. Repetitive stimulationsof the maxillary barbel to pH 7.5 artificial seawater (SW_a). Integrated (0.3 sec) neural activity from the maxillary barbel nerve of the sea catfish to repetitive ~285 µL stimulations of pH 7.5 SW in a background of pH 8.1 SW (control) occurring without sensory adaptation

Table S1. Sensitivity of the maxillary barbel pH sensors at different pH backgrounds

Fish & Single Fiber #	Background pH[a]	Transient decline in pH units for activation[b]
1	8.23 7.80	0.13 1.00
2	8.30 8.06	0.13 (0.07) 0.41
3	8.23 8.10 7.50	0.05 0.16 NR down to 6.8
4	8.21	0.21
5	8.17 7.50	0.17 0.70
6	8.48 7.40 8.48 7.64 7.78 8.48 9.00 7.0 8.48	0.38 (0.10) 1.3 0.4 0.59 0.6 0.38 0.52 NR 0.38
7	8.26	0.05
8	8.10	0.1
9	8.30	0.13

[a] pH of SW bathing the maxillary barbel listed in tested sequence in each experiment.
[b] The incremental drop in pH from that bathing the maxillary barbel (i.e. background pH) which resulted in activation of the pH-responsive single fiber; # in parenthesis, smallest drop in pH that activated the fiber earlier in that experiment.
NR, no response

Movie S1

A Japanese sea catfish emerges from its nest, locates a polychaete worm and ingests it.

Movie S2

A Japanese sea catfish is attracted to and bites at the end of a tube releasing seawater of slightly lower pH than that of the aquarium water.

Fig2f data	u-tube 1	intact worm	u-tube 1	u-tube 1	intact worm	u-tube 1
fish 5	60	212	20			
	92	428	72			
	64	214	16			
	128	310	76			
	100	290	214			
	200	157	120			
	88	236	84			
	176	352	176	113.5	274.9	97.3
fish 6	6	234	28			
	10	120	11			
	16	128	12			
	32	320	16			
	8	102	12			
	12	74	48			
	48	52	28			
	60	216	72	24.0	155.8	28.4
fish 7	28	76	12			
	29	180	24			
	36	44	4			
	0	106	44			
	92	230	64			
	60	254	10			
	8	24	12			
	4	40	16	32.1	119.3	23.3
fish 8	72	328	142			
	200	478	134			
	324	404	200			
	128	410	292			
	360	530	202			
	108	428	400			
	220	468	264			
	160	422	240	196.5	433.5	234.3
			Average	91.5	245.8	95.8
			N	4	4	4
			SD	80.8	141.7	98.3
			SEM	40.4	70.8	49.1

Summary of all Effects; design: (summary.stat)

1-WORM

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2	30904.943	6	1123.423	27.50961	0.000950

Thus, there is an overall significance difference amongst the 3 groups: U-tbue 1, Intact Worm, and U-tube 2

A priori one-tailed t-tests (paired)

U-tube 1 vs. Intact Worm	Mean	Std.Dv.	N	Diff.	Diff.	t	df	p value (two tailed)	p value (two tailed)
UTUBE1	91.525	80.8158555							
WORM	245.875	141.6337148	4	-154.35	62.97711	-4.9017808	3	0.016251	0.008125

Thus, there is a significant difference between U-tube1 and Intact Worm

Utube 1 vs.Utube 2	Mean	Std.Dv.	N	Diff.	Diff.	t	df	p value (two tailed)	p value (two tailed)
UTUBE1	91.525	80.8158555							
UTUBE2	95.825	98.29124664	4	-4.3	23.903416	-0.35978120	3	0.7428468	0.371423

Thus, there is not a significant difference between U-tube 1 and U-tube 2

Fig2g data

		SW1	low pH	SW2
1	fish 9	56	58	37
2		35	29	21
3		30	30	29
4		48	48	40
5		18	18	12
6		41	47	42
7		23	24	22
8		27	25	13
9		18	24	20
10		15	18	26
1	fish 10	16	46	42
2		26	100	52
3		20	63	35
4		51	77	50
5		59	144	76
6		26	52	24
7		29	73	44
8		95	106	95
1	fish 11	63	16	51
2		50	113	50
3		72	100	29
4		21	19	5
5		58	161	50
6		49	100	44
7		40	73	27
8		25	60	33
9		10	46	25
10		40	70	53
1	fish 12	15	36	21
2		26	110	27
3		21	72	24
4		29	34	6
5		19	25	24
6		16	18	12
7		9	5	5
8		14	17	10

Average of replicate trials for each of 4 fish

	SW1	low pH	SW2
Fish 9	31.1	32.1	26.2
Fish 10	40.3	82.6	52.3
Fish 11	42.8	75.8	36.7
Fish 12	18.6	39.6	16.1
	SW1	Low pH	SW2

Average	33.2	57.5	32.8
N	4.0	4.0	4.0
SD	10.9	25.4	15.4
SEM	5.5	12.7	7.7

ANOVA

Effect	df effect	MS Effect	df error	MS error	F value	P value
1	2	801.5	6.0	97.6	8.2	0.01917

Thus, there is an overall significance difference amongst the 3 groups: SW1, Low pH, and SW2

A priori one-tailed t-tests (paired)

SW1 vs Low pH		Mean	Std.Dv.	N	Diff.	Diff.	t	df	p value (two tailed)	p value (two tailed)
	SW1	33.2	10.9565							
	LOWPH	57.525	25.3670	4	-24.325	17.8275	-2.7289182	3	0.07200625	0.036003125

Thus, there is a significant difference between SW1 and Low pH

SW1 vs SW2		Mean	Std.Dv.	N	Diff.	Diff.	t	df	p value (two tailed)	p value (two tailed)
	SW1	33.2	10.9565							
	SW2	32.8187	15.4394	4	0.38125	8.35796	0.0912303	3	0.933059825	0.466529912

Thus, there is not a significant difference between SW1 and SW2

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