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Short-term desensitization of fast escape behavior associated with suppression of Mauthner cell activity in larval zebrafish

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ABSTRACT

Escape is among the simplest animal behaviors employed to study the neural mechanisms underlying learning. Teleost fishes exhibit behavioral learning of fast escape initiated with a C-shaped body bend (C-start). C-starts are subdivided into short-latency (SLC) and long-latency (LLC) types in larval zebrafish. Whether these two can be separately modified, and the neural correlates of this modification, however, remains undetermined. We thus performed Ca^{2+} imaging of Mauthner (M-) cells, a pair of giant hindbrain neurons constituting a core element of SLC circuit, during behavioral learning in larval zebrafish. The Ca^{2+} response corresponding to a single spiking of the M-cells was coupled with SLCs but not LLCs. Conditioning with a repeated weak sound at subthreshold intensity to elicit C-starts selectively suppressed SLC occurrence for 10 min without affecting LLC responsiveness. The short-term desensitization of SLC was associated with the suppression of M-cell activity, suggesting that changes in single neuron responsiveness mediate behavioral learning. The conditioning did not affect the acoustically evoked mechanotransduction of inner ear hair cells, further suggesting plastic change in transmission efficacy within the auditory input circuit between the hair cells and the M-cell.

1. Introduction

To elucidate the cellular mechanisms underlying behavioral learning, it is necessary to identify the neuronal circuits involved in a behavior and to evaluate the changes in the activities of key neurons in the circuits. The participation of a large number of neurons in controlling even simple behaviors, however, tends to make this a formidable challenge. Mauthner (M-) cells, which are a pair of giant reticulospinal neurons in the teleost hindbrain (Kimmel et al., 1982; Zottoli and Faber, 2000), provide a unique opportunity to circumvent this issue because the activation of a single M-cell is tightly linked to the initiation of fast escape with a C-shaped body bend, also called a C-start, in response to abrupt stimuli (Eaton et al., 1977; Eaton et al., 1981; Kohashi and Oda, 2008; Liu and Fetcho, 1999; Weiss et al., 2006; Zottoli, 1977).

In larval zebrafish, C-starts are separated into two types: one starting with a fast body bend with a latency less than 16 ms from the onset of sound/vibratory/tactile stimulus (short-latency C-start or SLC), and the other occurring with slow turning with tens of milliseconds latency (long-latency C-start or LLC) (Burgess and Granato, 2007; Issa et al., 2011). Among these, SLCs evoked by auditory stimuli are particularly well studied, and the principal circuit that includes the paired M-cells has been identified from auditory inputs to motor outputs of the M-cell: inner ear hair cells, VIIIth nerve afferent fibers that directly project to the M-cell, and spinal motor neurons and interneurons that are directly innervated by the M-cell (Fig. 1) (Faber et al., 1989; Korn and Faber, 2005; Tanimoto et al., 2009). Although the LLC circuit has not yet been identified, M-cells are not believed to play a role since they are not active during LLCs (Marsden and Granato, 2015).

It has been shown that the occurrence of C-starts can be modified by conditioning with sound/vibration stimuli. Zebrafish larvae exhibit a short-term reduction in C-start probability after repeated exposure to sound/vibration at supra-threshold intensity (Best et al., 2008; Roberts et al., 2011; Wolman et al., 2011). C-start depression was also demonstrated in adult goldfish, using weak stimuli at subthreshold intensity to elicit C-starts (Oda et al., 1998); acoustically induced C-start is suppressed for more than 30 min following repeated weak sound stimulation. The probability of C-start induction in response to a test sound stimulus was reduced without significant change in behavioral kinetics. Further, the conditioning stimulus induced long-term potentiation at inhibitory synapses that mediate feedforward inhibition from the auditory afferent nerves onto the M-cells. These observations strongly sug-

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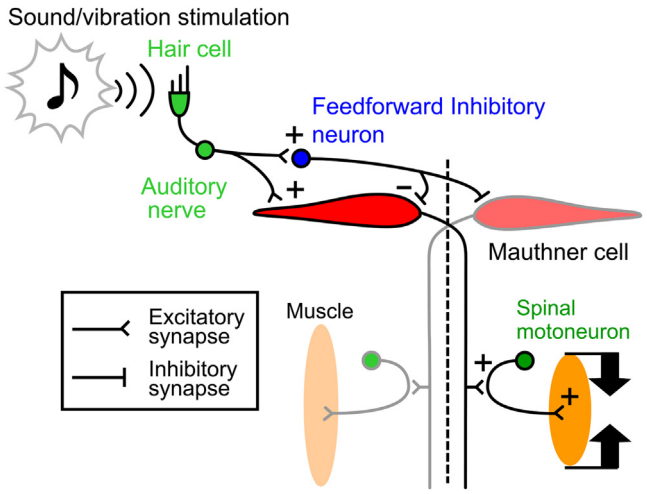

Fig. 1. Principal circuit for acoustically evoked SLC. Sound/vibration stimulation is detected by inner ear hair cells, and activates the VIIIth nerve afferent fibers that directly project to the M-cell. The M-cell directly innervates spinal motor neurons to elicit contraction of the contralateral muscle. The auditory nerve also activates hindbrain interneurons that provide feedforward inhibition onto the M-cells.

gest a tight coupling between behavioral desensitization and the long-term enhancement of the inhibitory input to the M-cell. Conditioning with subthreshold sensory stimulus in this study was useful to narrow down the possible loci of activity-dependent plasticity in the circuit because the postsynaptic circuit of the M-cells was not activated during the conditioning, allowing us to focus on the presynaptic circuit of the M-cells. The M-cell activity during learning has not yet, however, been directly investigated with weak stimulation.

Here, we demonstrate for the first time that acoustic conditioning at subthreshold intensity to elicit C-starts suppresses SLCs but not LLCs in larval zebrafish. The transparency of the larvae enabled the imaging of brain neurons *in vivo*, and partially restrained preparation with the head embedded in agar allowed us to simultaneously monitor the caudal body bend during neural imaging. Ca^{2+} imaging of the M-cell during C-start shows that the M-cell fires in association with SLCs but not LLCs. Repeated weak sound/vibration induces short-term desensitization of SLCs but not LLCs in association with the suppression of M-cell firing without changes in the acoustic responsiveness of the inner ear hair cells. The present study suggests that acoustic conditioning plastically alters the synaptic efficacy from the hair cells to the M-cell, resulting in the reduced probability of M-cell firing and the initiation of SLCs.

2. Materials and methods

2.1. Animals

We used wild-type and *nacre* mutant zebrafish (*Danio rerio*) larvae at 5–6 days postfertilization (dpf) raised at 28.5 °C. Experiments were performed at 25–29 °C. The *nacre* mutant was suitable for Ca^{2+} imaging because homozygous larvae lack melanophores and are thus transparent. There was no significant difference in behavioral kinetics and plasticity between wild-type and *nacre* mutants. The care of the fish and all of the experimental procedures in this study were approved by the Institutional Animal Care Committee of Nagoya University (Approval number #13-26).

2.2. Behavioral analysis of sound-/vibration-evoked escape responses

Behavioral experiments were performed as described previously (Kohashi et al., 2012; Satou et al., 2009). To observe the escape behaviors of unrestrained, freely swimming fish, larvae were kept in a Petri dish (60 mm diameter). Escape responses were elicited by sound/vibration via a custom-made vibration platform (Fig. 2A) onto which the Petri dish was tightly glued with dental wax (GC Corporation). The vibration platform consists of a loudspeaker (FRS8, Visaton) attached to an acrylic plate. Sinusoidal stimulus waveforms (500 Hz, 2 cycles) were generated by a function generator (WAVE Factory 1941, NF Corporation), amplified by an audio amplifier (AX-S313, Victor), and delivered to the loudspeaker. Peak acceleration amplitude was measured by an acceleration meter (PEA320, WACOH-TECH Inc.) attached to the acrylic plate, and was reported as the stimulus intensity. Twenty-five larvae in the Petri dish were tested in every experiment after 20 min of acclimation. Sequential images of the escape responses were captured at 1000 frames/s by a high-speed camera (FASTCAM-ultima1024, Photron) at 512 × 512 pixel resolution.

Behavioral movements that started within 80 ms after stimulus onset were regarded as escape behaviors and analyzed. The responses of larvae that contacted the dish wall at the time of stimulation were excluded from analysis. The initial C-shaped body bend was detected by automated tracking software. The body silhouette was first thinned to a curved line and then divided into eight equal sections by nine dots from head to tail (Wriggle Tracker, Library). A line connecting the second and fourth dots, corresponding to the midpoint of the eyes and the caudal end of the swim bladder, respectively, was defined as head orientation (Move Tr2D, Library). Onset latency, flexion angle, and duration of C-bends were quantified by measuring the head orientation in sequential images. As shown in Fig. 2B and F, escape responses were divided into SLCs and LLCs, respectively, with a criterion latency of 16 ms (dotted line in Fig. 2F). LLC probability (LLC/R) was determined as the percentage of larvae that responded with LLCs out of all larvae that did not exhibit SLCs (Burgess and Granato, 2007).

2.3. Conditioning of escape behavior

To evaluate startle sensitivity, sound-/vibration-evoked responses were measured at least three times before conditioning (pretests). The stimulus at test intensity (range: 7.3–10.5 m/s^2) elicited SLCs in 50–90% of larvae. Five minutes after the last pretest trial, a conditioning stimulus was delivered for 5 min: sound/vibration at subthreshold intensity to elicit SLCs (25 cycles at 500 Hz, with intensity of 1.0 m/s^2 that induced SLCs in 5–20% of larvae) was repeated every 5 s. Escape behaviors were tested 1, 5, 10, 15, 20, and 25 min after the end of conditioning. In control trials, a 5 min period without stimulation was employed instead of the conditioning period.

2.4. Simultaneous monitoring of the M-cell activity and escape responses

Ca^{2+} imaging of the M-cells during escape was performed as described previously (Kohashi et al., 2012; Kohashi and Oda, 2008; Satou et al., 2009). Briefly, M-cells were retrogradely labeled by injecting 20% Oregon Green 488 BAPTA-1 dextran (10,000 molecular weight, Life Technologies) into the caudal spinal cord at approximately the level of the 20th myotome. After incubating in 10% Hank's solution (in millimolars: 13.7 NaCl, 0.54 KCl, 0.025 Na_2HPO_4, 0.044 KH_2PO_4, 0.13 $CaCl_2$, 0.10 $MgSO_4$, 0.42 $NaHCO_3$, pH 7.2) for more than 6 h, each larva was briefly anesthetized with 0.02% tricaine methanesulfonate (MS-222; Sigma-Aldrich)