Underwater sound detection by cephalopod statocyst

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ABSTRACT: The cephalopod receptor of particle motion was identified. In a previous study, it was suggested that statocysts served this function, but there was no direct supporting evidence, and epidermal hair cells had not been conclusively ruled out. Experiments on *Octopus ocellatus* were conducted using respiratory activity as an indicator of sound perception. Intact animals clearly responded to 141-Hz particle motion at particle accelerations below 1.3×10^{-3} m/s^2, and the mean perception threshold at this frequency was approximately 6.0×10^{-4} m/s^2. Specimens in which the statoliths had been surgically removed did not show any response for accelerations up to 3.9×10^{-3} m/s^2 at 141 Hz, which was approximately 16 dB greater than the mean perception threshold at this frequency. Specimens that had undergone a control operation in which the statoliths remained intact showed positive responses at 2.8×10^{-3} m/s^2 for the same frequency stimulus. This indicates that the statocyst, which is morphologically similar to the inner ear system in fish, is responsible for the observed responses to particle motion in *O. ocellatus*. This is the first direct evidence that cephalopods detect kinetic sound components using statocysts.

KEY WORDS: cephalopod, hearing, octopus, particle motion, statocyst.

INTRODUCTION

Propagating sound waves consist of kinetic components (particle motion) and pressure components (sound pressure). Fish detect the kinetic sound components by the inner ear and lateral lines. Both of these detectors contain mechanosensitive hair cells that act as sensory elements by responding to displacement on a nanometer scale.[1,2] As well, the lateral lines detect particle motion when a displacement of the sensory cells relative to the receptor epithelium occurs. However, the working distance of the lateral lines is restricted to one body length.[3] For example, a fish near a sound source receives different particle motions at each point on its body. When the fish is further away from the sound source, the particle motion encompasses the whole fish and causes it to move with the same phase and amplitude, without stimulating the lateral line system. In contrast, the otolith organs in the inner ear are stimulated by whole-body displacements.[3,4] The otolith organs are inertial detectors in which a calcareous otolith is attached to the sensory hair cells. When a fish accelerates, the dense otolith moves, bending the sensory hair cells. Thus, the fish inner ear is a receptor of kinetic sound components.[4–7]

Cephalopod sensitivity to vibration was not clearly demonstrated until the mid-1980s. The epidermal hair cells and statocysts of cephalopods are remarkably similar to the lateral lines and inner ears of fish.[8,9] Budelmann and Bleckmann[9] performed electrophysiological experiments on *Sepia officinalis* and *Lolliguncula brevis* that showed that the epidermal hair cells of these cephalopods were sensitive to local water movements produced by a vibrating sphere located 6–13 mm away. In cephalopods, the statocyst contains the macular and crista systems. The macular system acts as an analog to the otolith organ and contains a dense calcareous statolith attached to sensory hair cells. The crista consists of sensory hair cells on three separate planes that form right angles to each

other.[8] The cephalopod statocyst appears to be sensitive to kinetic sound components because it detects vibrations.[10–13] Packard *et al.*[14] showed that cephalopods are sensitive to particle motion, but not to sound pressure. Furthermore, they suggested that statocysts would detect particle motion in the same manner as fish otolith organs. However, there is no direct evidence that indicates whether cephalopod statocysts detect particle motion.

Underwater sound is distinguished by its long transmission range. For example, a 100-Hz tone suffers only 1 dB of absorption in 1000 km of propagation through sea water, whereas blue-green light attenuates by 1 dB in less than 3 m.[2] It is biologically important to know if and how the statocyst is stimulated by underwater sounds because the working distance of the organism may be determined by this detector. As in fish lateral lines,[3] the working distance of the epidermal hair cells may be restricted to one body length, whereas the statocyst, as an inertial sound detector, would allow an animal to obtain sound information from a distant source.

We performed surgical procedures on the statocysts of *Octopus ocellatus*, which served as our animal model. Previous studies of cephalopods[14,15] have shown that respiratory activity is an indicator of sound reception and is indicated by muscle movements in the cephalopod mantle. Thus, to evaluate sound reception, we measured the mantle muscle movements of *O. ocellatus* using an electromyograph (EMG).[15] After first determining the perception threshold, we examined the effect of surgically removing the statolith on the sound perception of *O. ocellatus*.

MATERIALS AND METHODS

Experimental animals

We used *Octopus ocellatus*, which is a small benthic cephalopod that occurs in coastal waters. The animals were captured by hand in September 2006 on the tidal sand flats at Futtsu, Tokyo Bay, Japan, and carefully transported to the test site. For at least 3 days before the experiments, the animals were kept in individual closed tanks at approximately 20°C in the laboratory of the Tokyo University of Marine Science and Technology. During this adaptation period, the animals were fed live clams. Five intact specimens [mean body weight (BW) 16.5 g, standard deviation (SD) 5.0 g] were used to determine the perception threshold. Five specimens (mean BW 15.0 g, SD 1.9 g) were selected for the surgical removal of the statolith and control operations.

The experimental tank was a 180 mm × 330 m × 230 mm transparent plastic container placed on a 15-mm thick rubber sheet. Individual specimens were enclosed in a soft nylon net and placed in the experimental tank. The net was slightly bigger than the animal to allow it to breathe and jet, but not to change positions.[15,16] Electrodes from an EMG (T-1202, Fukuda, Tokyo, Japan) were attached to the net to record the muscle movements of the specimen's mantle.[15] We compared the EMG profiles to visual observations to confirm that the profiles indicated muscle movements of the mantle, and thus the respiration of the animal. Because the distance between the animal examined and electrodes would not be equal among tests, amplitudes of EMG profiles were not strictly comparable. We therefore do not discuss the differences of amplitude of EMG profiles among tests.

Sound projection

We produced 10-s audio files of sinusoidal waves with a 1-s rise and decay time. We played these back on a personal computer using audio software (Cool Edit 2000, Syntrillium Software, Scottsdale, AZ, USA) and amplified the sound signals using a power amplifier (KR-V55R, Kenwood, Tokyo, Japan), which drove an air speaker (C250L24s, Foster, Tokyo, Japan) fixed 15 cm above the water surface. Particle acceleration was detected using an acceleration pickup package (Fig. 1), based on a single-axis acceleration pickup (PV87, Rion, Tokyo, Japan). The weight of the package was approximately 5 g when it was in sea water, and its density was approximately 1.7% greater than that of sea water. When measuring particle acceleration, the package was suspended by a string at the position where the experimental animal was to be placed, with the sensitivity axis oriented horizontally to make it free from the tension of the string. Because the package contained air, which is a compressive material, the wall of the package to which the pickup was attached would vibrate because of the pressure components of sound. The axis of vibration was set at a right angle to the sensitivity axis of the acceleration pickup so that it would not detect the vibration caused by pressure sound components. An accelerometer (VM83, Rion) was used to measure the signals detected by the acceleration pickup, and an oscilloscope (Wavesurfer 424 LeCroy, Chestnut Ridge, NY, USA) was used to observe the waveforms visually. When the particle acceleration was lower than the detection limit of the acceleration measurement system (4×10^{-4} m/s^2), we assumed that the sound project system was linear and used the following equation:
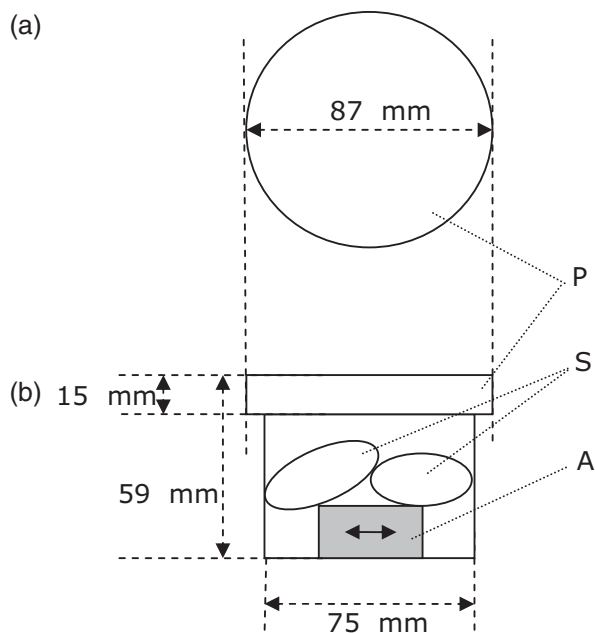
Fig. 1 Schematic diagram of the acceleration pickup package, (a) plane and (b) side views. P, polypropylene container; A, acceleration pickup; S, sandbag (weight). Solid arrow indicates the sensitivity axis of the acceleration pickup.

$$a_1 = a_2 \times \frac{p_1}{p_2}$$

where a_1 (less than the detection limit) and p_1 are the acceleration and sound pressure to be measured, respectively, and a_2 (greater than the detection limit) and p_2 are the acceleration and the sound pressure of the sound stimulus at a particular intensity, respectively. The sound pressure was measured using a hydrophone (B&K 8103, Bruel and Kjaer, Naerum, Denmark) located at the position where the experimental animal was to be placed. Particle motion is presented as the root-mean-square of particle acceleration.

The particle acceleration measured using our acceleration measurement system is underestimated for two reasons. First, the acceleration pickup package is somewhat denser than sea water. Second, the measurement system detects horizontal particle motion only, whereas the air speaker projects compression waves in the air, and these waves vibrate the water surface and the walls of the experimental tank to cause particle motion in various directions.

Perception threshold determination

We recorded the rhythmic respiratory activity of mantle muscle movements using an EMG. We con-

sidered a response to a sound stimulus as positive if we observed a marked change in the respiratory activity during the stimulus or if any respiratory activity during the stimulus exceeded the longest of the 20 previous respiratory activities by at least 10%.[5,14] The animals sometimes experienced periods of hyperventilation and jetting, despite the absence of stimulation. When this happened, we halted testing until the animals relaxed.[14] The stimuli were administered starting at a low intensity and increasing in steps of approximately 2 dB. We determined the perception threshold as the average of the highest intensity at which there was a negative response and the lowest intensity at which there were two successive positive responses. The specimens were not conditioned because all of them clearly showed initial responses to certain stimuli.
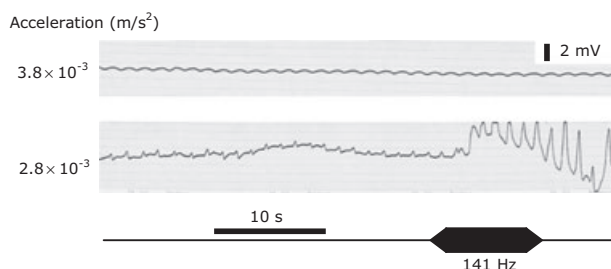
Surgery

Three specimens were selected for surgical removal of the statolith, and two specimens were used as surgical controls. The animals were anesthetized with 1% ethanol in sea water at 20°C prior to surgery. They were allowed a 20-h recovery period after the operation before testing. For the experimental manipulation, we cut the ventral surface above the statocyst and removed the statolith using a pair of tweezers. For the control subjects, we cut the ventral surface above the statocyst in the cartilage layer, but left the statocysts untouched. After surgery and recovery, we tested the 141-Hz perception threshold of the specimens that had had the statoliths removed. We tested the reaction of the control subjects by exposing them to a 141-Hz sound level 10 dB greater than the perception threshold of intact animals.

RESULTS

The respiratory activity of *Octopus ocellatus* exposed to a 141-Hz sound at an acceleration of $1.2–2.8 \times 10^{-4}$ m/s² was stable in the pre-stimulus period in all EMG profiles and also during the stimulus period in three profiles that were declared negative (Fig. 2). *Octopus ocellatus* displayed a positive response to sounds in the range of $2.2–2.8 \times 10^{-4}$ m/s² by suppressing its respiration (Fig. 2); the respiration suppression period was longer at greater sound intensity. All of the intact animals tested responded to certain stimuli without conditioning. Most of the responses were suppressed respiration, but there were instances of
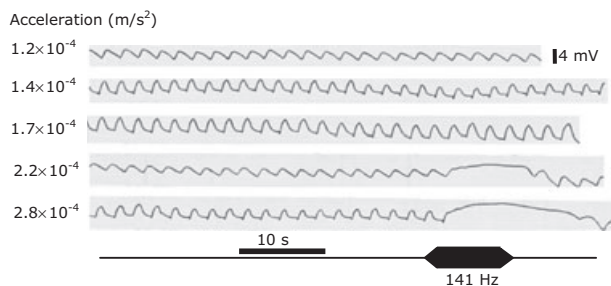
Fig. 2 Example electromyograph profiles of mantle muscle movements of intact *Octopus ocellatus* exposed to sound of 141 Hz. Particle acceleration is indicated to the left. The bottom trace indicates the time course and stimulus period (bold). The scale, indicated by the vertical bar at the upper right, is the same for all five profiles. The three upper profiles indicate no response to the sound stimulus. The perception threshold of this individual for 141-Hz sound was determined as 1.95×10^{-4} m/s^2, which is the average of 1.7×10^{-4} m/s^2 and 2.2×10^{-4} m/s^2.

Fig. 3 Example electromyograph profiles of mantle muscle movements of a specimen from which the statolith had been surgically removed (upper profile) and a specimen that had undergone control surgery with no manipulation of the statolith (lower profile). The specimens were exposed to 141-Hz sound. Particle acceleration is indicated to the left. The bottom trace indicates the time course and stimulus period (bold). The lower profile indicates a response of hyperventilation or jetting to the sound stimulus. The scale, indicated by the vertical bar at the upper right, is the same for both profiles.

hyperventilation or jetting (Fig. 3) for both intact and surgically operated specimens.

In terms of the acceleration threshold of intact animals for the 50–283 Hz frequency range, the acceleration threshold was relatively stable at approximately 5.0×10^{-4} m/s^2 for frequencies below 141 Hz and increased sharply above this frequency (Fig. 4). The lowest acceleration threshold of 3.4×10^{-4} m/s^2 occurred at 50 Hz, which was the lowest frequency that we tested. At 283 Hz, which was the highest frequency tested, the acceleration threshold was 4.3×10^{-2} m/s^2. The upper acceleration threshold was 42 dB greater than the lower acceleration threshold. As explained above, these
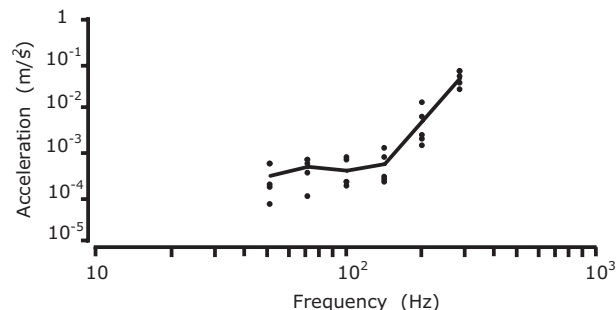
Fig. 4 Particle motion perception threshold of intact *Octopus ocellatus*, determined by respiratory activity. The line indicates averaged thresholds and (●) indicate individual thresholds at 50, 72, 100, 141, 200 and 283 Hz.

thresholds may be underestimated because of the design of the acceleration measurement system.

We next examined the responses of *O. ocellatus* to 141-Hz stimuli following the 20-h recovery period after statolith removal ($n = 3$) or control surgery ($n = 2$) (Fig. 3). For the two of the three specimens that had their statoliths removed, we could not determine the acceleration threshold because the animals did not respond even at a level of 3.0×10^{-2} m/s^2, which was the highest acceleration that our sound system could emit. This was approximately 34 dB greater than the acceleration threshold of the intact animals. For the other specimen that had its statoliths removed, the acceleration threshold at 141 Hz was 3.8×10^{-3} m/s^2, which was 16 dB greater than the acceleration threshold of the intact animals. The two specimens that had undergone the control operation were exposed to 141-Hz sound at a level of 2.8×10^{-3} m/s^2, which was 10 dB greater than the acceleration threshold of the intact animals. Both specimens showed positive responses to the sound stimuli. Based on these results, we concluded that the statocysts of *O. ocellatus* detected particle motion.

DISCUSSION

Packard *et al.*[14] suggested that the cephalopod statocyst could detect particle motion, based on their experimental design that resulted in the stimulation of the fish inner ear, but not the lateral line.[1] However, there was no direct evidence that indicates whether cephalopod statocysts detect particle motion. Our results show that the statocysts of *Octopus ocellatus* detected particle motion and resulted in a response to the presented sound stimuli. This is the first direct evidence that cephalopods detect kinetic sound components by the statocyst. Lovell *et al.*[17] showed that sound projected

from an air speaker stimulates the prawn statolith, which has a basic structure similar to that of the cephalopod statocyst.[8] Our result that sound projected from an air speaker stimulated the cephalopod statocyst is in agreement with this study.

It was shown that cephalopods are sensitive to particle motion but not to sound pressure,[14] and our study showed that the cephalopod statocyst detects particle motion. The basic structure of the cephalopod statocyst is identical to that of the fish otolith organ in that they are mass-loaded inertial accelerometers that contain dense statoliths or otoliths attached to sensory hair cells.[18,19] Therefore, the cephalopod statocyst could detect particle motion in the same manner as the fish otolith organ; the cephalopod statocyst could be stimulated when particle motion encompasses the whole animal body and causes it to move. Moreover, the otolith organ of fish has been modeled theoretically as a simple harmonic oscillator because of its basic structure.[6,20] Because of the similarity between the fish otolith organ and the cephalopod statocyst, the latter might also be modeled as a simple harmonic oscillator.

In the specimen that had had its statolith removed, but responded to a stimulus 16 dB greater than the threshold of the intact animals, the epidermal hair cell likely acted as a secondary receptor. The epidermal hair cells in cephalopods detect local water movements when the sound source is extremely close.[9] The air speaker projected compression waves in the air, which vibrated the water surface and the walls of the experimental tank as incidental sound sources. The experimental animals were close to these incidental sound sources; the center of the experimental tank was only 90 mm from the nearest wall. The local water movements may have stimulated the epidermal hair cells; thus, an animal lacking a statolith could detect the stimulus by its epidermal hair cells.

The particle motion perception thresholds of *Sepia officinalis*, *Loligo vulgaris* and *Octopus vulgaris* are constant at low frequencies, but increase sharply at high frequencies.[14] We found a similar frequency dependence of the perception threshold in *O. ocellatus*. Despite this similarity, however, the transition frequency was approximately 10 Hz for *S. officinalis*, *L. vulgaris* and *O. vulgaris*, but approximately 150 Hz for *O. ocellatus* (Fig. 4). This indicates that *O. ocellatus* has a wider detection range than the other three species.

The biological significance of particle motion sensitivity in cephalopods is not clear. Nevertheless, underwater acoustic cues may be important in prey–predator interactions. Maniwa[21] reported that the capture of *Todarodes pacificus* increased in the presence of a 600-Hz pure tone combined with the sound produced by a fishing boat. Hanlon and Budelmann[22] reported that *Sepioteuthis sepioidea* in the wild show greater fright behavior 3–4 s before predatory fish of the Carangidae family are observed approaching the squid school and suggested that the squid use a sense other than vision to detect the predatory fish. Moreover, *O. ocellatus* responds to sound stimuli with respiratory suppression and the retraction of the basal parts of the eyes, which seem to be related to defense against predation.[15] It is also possible that underwater sound is biologically important to cephalopods for migratory purposes. Infrasound patterns in the ocean may be used by fish for orientation and navigation during migration.[5,23,24] Infrasound noise propagates over long distances with little attenuation. This noise is reflected off of the continents and causes a directional pattern of infrasound in the oceans. The particle motion sensitivity of cephalopods is comparable to that of fish.[14] Our statolith-removal experiment clearly demonstrates that particle motion is detected by the cephalopod statocysts, which would allow to detection of sound information from a distant sound source. Thus, underwater sound cues may be used extensively by cephalopods.

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