

Behavioural responses of juvenile cuttlefish (*Sepia officinalis*) to local water movements

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Abstract

Physiological studies have shown that the epidermal head and arm lines in cephalopods are a mechano-receptive system that is similar to the fish and amphibian lateral lines (Budelmann BU, Bleckmann H. 1988. A lateral line analogue in cephalopods: Water waves generate microphonic potentials in the epidermal head lines of *Sepia officinalis* and *Lolliguncula brevis*. J. Comp. Physiol. A 164:1–5.); however, the biological significance of the epidermal lines remains unclear. To test whether cuttlefish show behavioural responses to local water movements, juvenile *Sepia officinalis* were exposed to local sinusoidal water movements of different frequencies (0.01–1000 Hz) produced by a vibrating sphere. Five behavioural responses were recorded: body pattern changing, moving, burrowing, orienting, and swimming. Cuttlefish responded to a wide range of frequencies (20–600 Hz), but not to all of the frequencies tested within that range. No habituation to repeated stimuli was seen. Results indicate that cuttlefish can detect local water movements (most likely with the epidermal head and arm lines) and are able to integrate that information into behavioural responses.

Keywords: *Cephalopods, mechanoreception, lateral line, hearing*

Introduction

Cephalopods (octopods, cuttlefishes, and squids) have a receptor system that is equivalent to the lateral line system of fishes and aquatic amphibians (Sundermann-Meister 1978; Sundermann 1983; Budelmann & Bleckmann 1988). The system is comprised of lines of polarized epidermal hair cells on the head and arms. The cells are sensitive to local water movements as small as 0.06 μm (Budelmann & Bleckmann 1988); this sensitivity is close to

that of the hair cells in the fish lateral lines (Bleckmann et al. 1991). Octopods have eight, and cuttlefishes and squids ten, epidermal lines that run in anterior/posterior direction over the dorsal, lateral, and ventral sides of the head and continue onto the arms (in cuttlefishes and squids, two of the lines, one below each eye, are restricted to the head). One additional line is located on the ventral side of the funnel (Sundermann-Meister 1978; Sundermann 1983; Lenz et al. 1995; Lenz 1997).

The behavioural significance of the cephalopod epidermal lines remains unclear. Because of their similarity to the vertebrate lateral lines, it is reasonable to assume that they are involved in functions similar to those known for fishes: prey detection, predator avoidance, the localization of stationary objects, and schooling behaviour (Montgomery & MacDonald 1987). Preliminary behavioural experiments have shown that the cuttlefish's ability to catch shrimp in complete darkness most likely depends on the functioning of the epidermal lines (Budelmann et al. 1991).

The present experiments were performed to test whether cuttlefish perceive local water movements (sinusoidal water oscillations of various frequencies but constant amplitudes) and are able to integrate that information into behavioural responses.

Methods

Thirty-four juvenile cuttlefish *Sepia officinalis* were used for the experiments. Twenty-four cuttlefish were one-month old with a dorsal mantle length of 30 ± 1 mm; the remaining 10 cuttlefish were three-month old with a dorsal mantle length of 57 ± 1 mm. All cuttlefish were hatched and reared under standard laboratory conditions at the National Resource Center for Cephalopods in Galveston, Texas (Forsythe et al. 1991).

The cuttlefish were exposed to the water movements and the incidences (occurrences) of the following five behaviours were observed: *pattern changing* (change of coloration, texture, or pattern on the head, arms or mantle), *moving* (skin, mantle, head, or arm movements), *burrowing* (small back and forth movements in place similar to those used when burrowing), *orienting* (change in orientation relative to the stimulus but no change of location), and *swimming* (movements resulting in a change of location).

Experimental apparatus and stimulation

All experiments were performed in a small glass tank ($75 \times 35 \times 35$ cm) with the four sides and the bottom covered with black plastic to minimize visual stimulation from the outside. The tank was placed on an air tire to eliminate potential vibrations of the building (e.g. caused by the air conditioning). Sinusoidal water movements of various frequencies were produced by a vibrating sphere (diameter 14.6 mm) attached to a rod and moved in the direction of the rod by a vibrator (model 102, Ling Dynamic Systems, Royston, England). The vibrator was driven by a function generator to produce sinusoidal water movements with constant amplitudes and oscillation frequencies between 0.01 and 1000 Hz (Budelmann & Williamson 1994). Each stimulus frequency was applied for 30 s, with an inter-stimulus interval of 5 min.

In each experiment, the cuttlefish were placed individually into a small circular basket (diameter 9 cm, formed from a wide-meshed plastic net) suspended in the middle of the tank. During stimulation, the sphere was positioned 5 mm above the head of the cuttlefish, directly above the dorsal epidermal lines. Sphere movement (in the range of a few micrometers; cp. Budelmann & Williamson 1994) was towards and away from the cuttlefish. Before the stimuli were applied, the cuttlefish were acclimated to the experimental

apparatus for 60–90 min until they were resting calmly on the bottom of the basket and the body patterns had stabilized for a period of at least 10 min. During the experiments, the water circulation of the tank was turned off to eliminate any stimulation side effects caused by the movement of the circulating water.

Preliminary trials using groups of cuttlefish and frequencies ranging from 0.01–1000 Hz indicated that the cuttlefish responded to frequencies ranging from 10–600 Hz. Frequencies in this range were selected for further testing.

For the statistical analyses, behavioural activity was computed as the sum of the incidences of all five behaviours. In Experiment 3, the different behavioural responses were also analyzed separately. All statistics were two-tailed unless otherwise stated.

Experiment 1

Three cuttlefish (one-month old, already used in preliminary trials) were placed individually into the basket in the experimental tank. The following 19 frequencies were applied: 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 180, 200, 550, 600, and 650 Hz.

Experiment 2

Six cuttlefish (one-month old, already used in preliminary trials and in Experiment 1) were individually placed into the basket and, based on the responses seen in Experiment 1, the following five frequencies were applied: 40, 45, 70, 180, and 600 Hz. In addition, the frequencies 0.01 and 1000 Hz were applied as controls because they elicited no response in preliminary trials. Each frequency was tested five consecutive times to test for possible habituation.

Experiment 3

Twenty-eight experimentally naïve cuttlefish (one-month old [$N=18$], three-month old [$N=10$]) were individually placed into the basket and the following six frequencies applied: 20, 45, 70, 180, 300, and 600 Hz. Behaviour immediately before ($T1$) and after ($T3$) the stimulation was recorded as well as during stimulation ($T2$) (cp. Figure 1), for further clarification of the response. First, the number of cuttlefish showing any behavioural activity (sum of all behaviours) during $T1$, $T2$, and $T3$ was determined. Second, the number of cuttlefish that responded to stimulation with burrowing, moving, and pattern changing was determined. Behavioural responses were recorded with a Sony® video camera placed vertically 30 cm above the experimental tank.

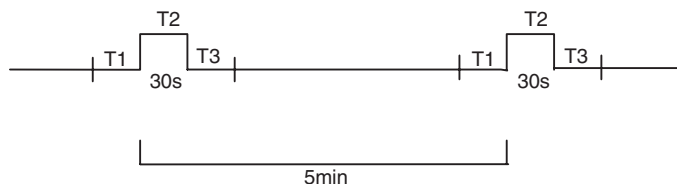


Figure 1. Stimulation diagram. All frequencies were applied at 5 min intervals. $T1=30$ s period before stimulation, $T2=30$ s period during stimulation, and $T3=30$ s period after stimulation.

Results

In all the experiments, each specific frequency was applied for a duration of 30 s; however, almost all responses occurred within the first five to eight seconds of stimulus application.

Experiment 1

The following frequencies caused significantly higher activities than during the control period: 40, 45, 50, 65, 70, 75, 80, 85, 180, and 600 Hz (repeated-measures ANOVA; $N = 3$, $df = 19$, $P < 0.05$ all) (Figure 2); five of these frequencies were again tested in Experiment 2. Frequencies of 55, 60, 90, 105, and 200 Hz did not cause any significant response.

Experiment 2

When stimulated five consecutive times with the same frequency (0.01, 40, 45, 70, 180, 600, or 1000 Hz), between one and five of the six cuttlefish responded to each of the five presentations of the 40, 45, 70, 180, and 600 Hz stimulus (Table I). No habituation to repeated stimulation was seen.

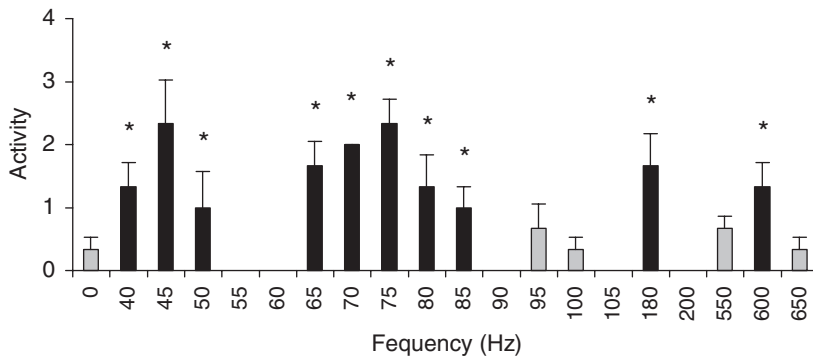


Figure 2. Experiment 1 ($N = 3$, mean + SE). Response activity (mean number of behavioural incidences per cuttlefish; all behaviours combined) to a subset of frequencies; no responses were seen at stimulation frequencies of 55, 60, 90, 105, and 200 Hz. Black columns with asterisks (*) show the frequencies that caused significantly more responses than during the immediately preceding control interval ($P < 0.05$ all).

Table I. Experiment 3, individual tests ($N = 6$). Number of cuttlefish responding in each of the five consecutive stimulus presentations.

Frequency (Hz)	Stimulus presentation				
	1	2	3	4	5
0.01	0	0	0	0	0
40	3	5	2	2	3
45	4	2	3	3	3
70	1	2	3	3	1
180	2	2	3	3	1
600	4	3	3	2	3
1000	0	0	0	0	0

Experiment 3

The number of cuttlefish responding (all behaviours combined) was statistically higher with stimulation ($T2$) than without stimulation ($T1$, $T3$) for all frequencies applied except for 600 Hz, which showed no difference between the periods $T2$ and $T3$ (Wilcoxon test for matched-paired data; one-month old cuttlefish: $Z < -2.12$, $N = 18$, $P < 0.05$; three-month-old cuttlefish: $Z < -2.45$, $N = 10$, $P < 0.05$; Figure 3). There was no difference in the level of activity during the no-stimulus periods $T1$ and $T3$ for any frequency applied.

Of the five specific behaviours, only burrowing, moving, and pattern changing (but not swimming and orienting) occurred frequently enough for statistical analysis. Responses of the two age groups were again similar, except for the behaviour of burrowing. One-month-old cuttlefish showed significantly more moving during stimulation for all frequencies tested (20, 45, 70, 180, 300, and 600 Hz; $Z < 2.5$, $N = 18$, $P < 0.05$); three-month old cuttlefish showed significantly more moving during stimulation with frequencies of 45 Hz ($Z = 2.2$, $N = 10$, $P < 0.05$) and 180 Hz ($Z = 2.0$, $N = 10$, $P < 0.05$), although the difference between the periods $T2$ and $T3$ was not significant for 180 Hz. One-month-old cuttlefish showed significantly more pattern changing during stimulation with all frequencies tested ($Z < 2.5$, $N = 18$, $P < 0.05$); three-month-old cuttlefish showed significantly more pattern changing with all frequencies other than 600 Hz ($Z = 2.2$, $N = 10$, $P < 0.05$). One-month-old cuttlefish showed significantly more burrowing with ($T2$) than without ($T1$, $T3$) stimulation for the frequencies 20 Hz ($Z = 2.0$, $N = 18$, $P < 0.05$), 45 Hz ($Z = 2.0$, $N = 18$, $P < 0.05$), 70 Hz ($Z = 2.0$, $N = 18$, $P < 0.05$), and 180 Hz ($Z = 2.2$, $N = 18$, $P < 0.05$); three-month-old cuttlefish showed no significant burrowing response to any frequency. There was no difference between the periods $T1$ and $T3$ for any age group, frequency, or behaviour tested.

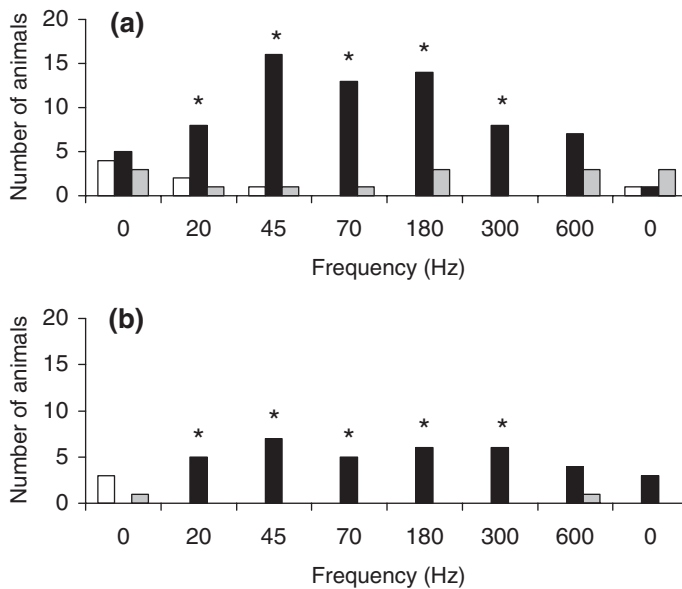


Figure 3. Experiment 3. Number of (a) one-month-old ($N = 18$) and (b) three-month-old ($N = 10$) cuttlefish showing behavioural activity (all behaviours combined) during the 30 s periods before stimulation ($T1$, open columns), during stimulation ($T2$, black columns) and after stimulation ($T3$, gray columns). The * shows the frequencies that caused significantly more responses during stimulation ($T2$), as compared with no stimulation ($T1$ or $T3$) ($P \leq 0.05$ all).

To determine whether certain frequencies caused stronger responses than others, the numbers of cuttlefish responding with each specific behaviour was compared between frequencies. For one-month-old cuttlefish, the 45 Hz stimulus caused more individuals to respond by moving than did any other frequency tested (Friedman two-way ANOVA, $F_{r}=14.70$, $N=18$, $df=5$, $P<0.01$); no significant differences were found between frequencies for either burrowing or pattern changing. For three-month-old cuttlefish, no significant differences between frequencies were found for any behaviour.

Discussion

The juvenile cuttlefish showed behavioural responses to a wide range (10–600 Hz) of frequencies that were specific to the presence of the stimulus; the stimuli did not significantly change the post-stimulus behaviour. All responses to stimulation were shown in the first five to eight seconds of the 30 s stimulation, indicating high sensitivity to these frequencies. Clearly, cuttlefish can perceive local water movements and integrate that information into behavioural responses.

The context in which these vibration frequencies are relevant for cuttlefish behaviour remains to be determined. Results suggest that responses were greatest to water movements of frequencies around 20, 45, 75, 180, and 300 Hz, and there may be frequencies to which the cuttlefish do not respond (Experiment 1). When frequencies were applied five consecutive times, no habituation was detected (Table I). It is possible, therefore, that the frequencies are important in a context in which the exact frequency provides salient information, and habituation may be inappropriate or detrimental, such as in predator avoidance. Results to particular frequencies should be studied in greater detail, however, before such conjectures are pursued.

The present data give some evidence that the readiness to respond to water movements of certain frequencies with a particular behaviour may change with increasing age. In general, the one-month old cuttlefish appeared more responsive, specifically with regard to moving, and only one-month-old cuttlefish responded to any of the frequencies with burrowing. It could be that readiness for burrowing declines with age because burrowing, as protection behaviour, may become less important as the cuttlefish increase in size and become less vulnerable to predation. Additional experiments are needed to more thoroughly investigate such possible age-dependent changes.

Hydrodynamic receptor systems are widespread among aquatic invertebrates and primarily serve in predator avoidance and prey detection (see Budelmann 1989, for a review). Some are narrowly tuned to certain frequencies, e.g. chaetognath arrow-worms show prey capture behaviour best at frequencies of 12, 30, and 150 Hz, depending upon the species; some chaetognaths may even select specific prey by the frequency of vibration the prey produce (Horridge & Boulton 1967; Newbury 1972; Feigenbaum & Reeve 1977). Interestingly, the squid *Todarodes pacificus* is attracted to a pure tone of 600 Hz (Maniwa 1976), and this behavioural response is used to enhance commercial squid catches in Japan (Hanlon & Budelmann 1987).

A number of different receptor systems could have played a role in the cuttlefish's detection of local water movements, including epidermal head and arm lines, statocysts, and eyes. We consider the most likely receptor system to be the epidermal lines. These lines are a hydrodynamic receptor system similar to the fish and amphibian lateral lines (Sundermann-Meister 1978; Budelmann & Bleckmann 1988; Lenz et al. 1995); based on microphonic potential recordings, their highest sensitivity is in the range of 75–100 Hz (Budelmann & Bleckmann 1988; Bleckmann et al. 1991). In cuttlefish, preliminary

experiments have shown that the epidermal lines can be used for prey detection when vision is restricted (Budelmann et al. 1991).

Cephalopod statocysts are another possible receptor system for detecting small water movements. Angular acceleration receptor systems in cephalopod statocysts detect fluid (endolymph) motion within the sense organ and not water movements outside (surrounding) the animal (Young 1960; Stephens & Young 1982; Williamson & Budelmann 1985; Budelmann et al. 1987; Williamson 1991); however, statocysts are also sensitive to linear accelerations, including substrate-borne vibrations (M.J. Wells & J. Wells 1956; Young 1960; Maturana & Sperling 1963; Budelmann 1976; Williamson 1988). When the cuttlefish rest on a substrate, the linear acceleration receptor systems cannot sense a relative movement between the cuttlefish and the surrounding water because the receptor systems are internal and their sensory structures are not exposed to the external water movement. The linear or angular acceleration receptor systems could be stimulated by external water movements if the cuttlefish are not attached to a stationary substrate but move or vibrate in synchrony with the water column (see also Dijgraaf 1963; Packard et al. 1990). Because the cuttlefish were tested in a suspended basket, and the firmness of their contact with the meshed bottom of the basket was not controlled, it is possible that the statocyst's linear acceleration receptor systems may have played a role in the cuttlefish's detection of the local water movements.

The third possibility is that the cuttlefish could have visually detected either the movement of the sphere itself or ripples on the water surface. Electroretinograms and brain recordings have shown that the cuttlefish eye's fusion frequency to flashes of light is between 20 and 60 Hz (Hamasaki 1968; Bullock & Budelmann 1991); therefore, visual discrimination of frequencies could have been involved only for stimuli around or below 60 Hz.

Whether the cuttlefish's ability to sense local water movements can be considered as "hearing" is a semantic issue and lies with the definition of underwater sound and underwater hearing (cp. Budelmann 1992). The cuttlefish receptor system for the detection of local water movements is most likely the epidermal head and arm lines (see above). Since these lines are analogous to the fish and amphibian lateral lines and those lines are not considered an organ for hearing (see Webster et al. 1992), it would be inappropriate to consider the cuttlefish's detection of local water movements via the epidermal lines as hearing. To date, no cephalopod sense organ is known that is specialized for (underwater) hearing, if hearing is narrowly defined as the reception of the pressure component of sound (cp. van Bergeijk 1964).

In summary, the present experiments provide the first behavioural evidence that cuttlefish are able to detect local water movements. This ability is most likely based on the function of the epidermal head and arm lines (see also Budelmann et al. 1991). Not all frequencies of the applied water movements elicited behavioural responses, suggesting that some but not all water movements are important in the life of the cuttlefish; however, these results must be investigated in greater detail before sources that could cause relevant water movements can be evaluated.

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