BARTH'S Myochordotonal Organ as an Acoustic Sensor in the Ghost Crab, *Ocypode*

Decapod crustaceans are well known as producers of air and substrate borne sounds[1–8]. For some species, these sounds have been shown to play an important role in intraspecific communication[4–7], but relatively little is known about how these signals are detected by the animals[9,10]. HORCH and SALMON[8] presented the first demonstration that crustaceans could hear air borne sounds. They showed that the ghost crab *Ocypode quadrata* responded to the air borne component of 3 kHz tones. This work was subsequently extended to other species of *Ocypode*[11]. An electrophysiological investigation of the basis for this sensitivity indicated that the reception of these sounds and of high frequency (> 1 kHz) substrate vibrations was a unique property of BARTH's organ[7]. This receptor is a myochordotonal organ situated near the origin of the accessory flexor muscle in the merus of the walking legs of decapod crustaceans[7,12]. In *Ocypode* its location is easily determined by the presence of a small, dome shaped 'window' in the exoskeleton at the site of termination of the dendrites of the sensory neurons[12].

Destruction of the exoskeletal window of BARTH's organ eliminated recordable responses in the supraoesophageal ganglion to air borne sounds and high frequency vibrations. Such responses were easily found prior to the operation, or after various pseudo-operations in which the organ was left intact. This implies that BARTH's organ may be the only receptor in these crabs capable of detecting such stimuli. More direct evidence for this hypothesis would come from a behavioral test of the importance of this organ in *Ocypode* for sensitivity to sounds and vibrations. I present here the results of such a test.

The experiments were done on Eniwetok, Marshall Islands, with the ghost crab *O. ceratophthalmus*. Animals were collected at night, and kept isolated for 2 or 3 days in small, opaque plastic containers with a small amount of fresh sea water. 1 or 2 days prior to being used, each animal was cooled in a refrigerator long enough to induce areflexia. The animals were divided into 2 groups. In the experimental group, the exoskeletal window of BARTH's organ was circumcised in each leg. The patch of exoskeleton was generally pulled inside the leg cavity by the tension of the tissue attached to it. The wounds were plugged with cotton. The control animals were pseudo-operated by removing a patch of exoskeleton 1 to 2 mm distal to the window of Barth's organ. Mortality was very low after the operation; the few animals which did succumb apparently did so because of excessive cooling. Animals from both groups maintained for some time after recovery showed apparently normal visual and feeding behavior.

The experimental approach is a modification of the startle response experiment first described by SALMON and ATSAIDES[13] in their study of the acoustic sensitivity of the fiddler crab *Uca*. Extensive work on both *Uca* and *Ocypode*[8,9,11] has shown that these animals, if motionless and attentive, will respond to the onset of a suprathreshold acoustic stimulus with sudden movements. The intensity of the movements, their latency from the onset of the stimulus, and the probability of a movement being made within a fixed period of time after the onset of the stimulus are all correlated with the magnitude of the stimulus. It is possible that some individuals react to acoustic stimuli by 'freezing', that is by remaining motionless. However, by using large numbers of animals and only testing each individual once or twice, startle responses have proven to be an effective measure of the perception of sounds by these crustaceans.

Tests were conducted in a room free from extraneous visual stimuli, but occasionally subject to noises from outside the building. Animals were placed individually in a plastic aquarium (bottom area about 30 cm × 40 cm) and a loudspeaker was then placed, facing down, over the top of the tank. The experimenter observed the animals through a small hole in an opaque screen. The crabs seemed unaffected by movements behind the screen.

Each crab was given one sound and one no sound (control) trial. For the sound trials, the stimulus consisted of 3 evenly spaced, 1 sec long, 2 kHz tone bursts. Each burst had rise and fall times of about 0.5 sec, and produced 92–103 db (re: 0.0002 μbar) air borne and 92–103 db (re: 10^{-3} cm/sec^2) bottom vibration components in the aquarium. (The range represents the variation from place to place in the tank.) Both components were more than 40 db above their corresponding background levels, and probably had some harmonic distortion. Purity of the frequency of the stimulus was unimportant for this test. In the control trials, no sounds were presented during the stimulus period. For both control and sound trials, the stimulus followed the first 10 sec pause in movements by the animal[8,11], or after 2.5 min if the animal did not move before that time. Any movement by the crab during the stimulus period was scored as a response. The order of presentations was balanced, so that about half of the animals in each group were presented with the sound trial first, and the remaining with the control trial first.

The results are shown in Table I. For both groups about 40% (13/30 and 10/25) of the animals responded during the control (no sound) trials. A X^2 test of 2 × 2 contingency The tested eye, following pupil dilation with phenylephrine HCl, was fitted with a protective contact lens,

Table I. Numbers of operated and pseudo-operated ghost crabs responding to a 4 sec period of sound and a 4 sec period of no sound

	Operated	Pseudo-operated
Number tested	30	25
Number responding during sound trials	16	19
% of total	(53)	(76)
Number responding during control (no sound) trials	13	10
% of total	(43)	(40)

Each animal was tested with one sound and one control trial.

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tables[14] applied to this data showed that the responses of the pseudo-operated and operated animals to the control trials were essentially identical ($X^2 = 0.0006$, $p > 0.1$). Thus, destroying the integrity of BARTH's organ apparently did not grossly affect the level of activity in these animals under the conditions of the experiment. When the tones were presented, only 53% (16/30) of the operated animals responded, but 76% (19/25) of the pseudo-operated animals responded. The McNemar test was used to assess the significance of this difference[14]. Table II shows that there was no difference in the behavior of the operated animals between the control and sound trials ($X^2 = 0.235$, $p > 0.1$). The pseudo-operated animals responded significantly more often in the sound trials than in the control trials ($X^2 = 4.923$, $p < 0.05$). This means that the operated animals seem to have failed to react to the stimulus, while the pseudo-operated animals did react to it.

Table II. Data for the McNemar statistic

I. Operated animals ($N = 30$)

	control trials	
Sound trials	*R*	*NR*
NR	7	7
R	6	10

II. Pseudo-operated animals ($N = 25$)

	control trials	
Sound trials	*R*	*NR*
NR	2	4
R	8	11

R number responding; *NR* number not responding.

This result and the electrophysiological evidence are both consistent with the view that BARTH's organ is the only receptor in *Ocypode* sensitive to high frequency vibrational and acoustic stimuli. As such, it is analogous to the subgenual organs of insects[15–17], but is possibly more amenable to neurophysiological study than the latter. Although much less studied in this regard than insects, many decapod crustaceans communicate with acoustic signals[4–7, 11]. BARTH's organ probably is the receptor used for detection of these signals, at least for those with frequencies above 1 kHz, and thus may be crucial for intraspecific communication among crustaceans.

Zusammenfassung. Nachweis des Barth'schen Myochordotonal-Organs bei der Krabbe *Ocypode*, als sensorieller Apparat für hochfrequente, akustische Signale.

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Microsomal Na^+-Stimulated NADH- Cytochrome c Reductase: Could it be Involved in Sodium Transport?

During the last few years, there has been much discussion about the possibility of the existence of more than one sodium pump in absorbing epithelia. The most convincing evidence has been obtained with kidney proximal tubular cells[1] but research with various different epithelial cells has suggested that the dichotomy is widespread. One of the weak points of the hypothesis remains the fact that, whereas it is generally accepted that sodium-for-potassium exchange is mediated by a ($Na^+ + K^+$)-stimulated ATPase[2], no indication has been forthcoming as to the biochemical nature of the second pump. A plausible candidate for such a mechanism appeared to be afforded by the Na^+-stimulated NADH-cytochrome c reductase system of microsomal fractions, first described by SIEKEVITZ[3]. The present preliminary survey was undertaken in an attempt to discover any link between this enzyme and the sodium-pumping system of the kidney. Of the various criteria listed by SKOU[4] for the identification of an enzyme system with a pumping mechanism, we have concentrated on its cytological location, its sensitivity towards cationic

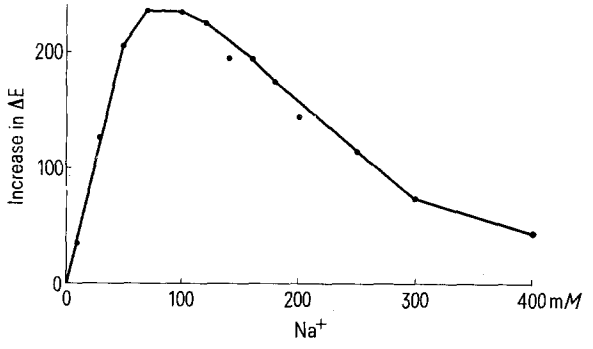

Fig. 1. Sensitivity of dog renal cortex microsomal NADH-cytc. reductase to sodium ions. Results express the increase in enzyme activity in the presence of different concentrations of sodium ions (in the form of NaCl) with reference to the basal level in the absence of sodium.

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