Available online at www.sciencedirect.com
SCIENCE DIRECT®
Zoology 108 (2005) 195–200
ZOOLOGY
www.elsevier.de/zool

Sea snakes (*Lapemis curtus*) are sensitive to low-amplitude water motions

Guido Westhoff[a,*], Bryan G. Fry[b], Horst Bleckmann[a]

[a]*Institute of Zoology, University of Bonn, Poppelsdorfer Schloß, D-53115 Bonn, Germany*
[b]*Australian Venom Research Unit, University of Melbourne, Victoria 3010, Australia*

Received 4 April 2005; received in revised form 15 May 2005; accepted 5 July 2005

Abstract

The sea snake *Lapemis curtus* is a piscivorous predator that hunts at dusk. Like land snakes, sea snakes have scale sensillae that may be mechanoreceptive, i.e. that may be useful for the detection of water motions produced by prey fish. In addition, inner ear hair cells of sea snakes may also be involved in the detection of hydrodynamic stimuli. We generated water motions and pressure fluctuations with a vibrating sphere. In the test range 50–200 Hz evoked potentials were recorded from the midbrain of *L. curtus* in response to vibrating sphere stimuli. In terms of water displacement the lowest threshold amplitudes were in the frequency range 100–150 Hz. In this range peak-to-peak water displacement amplitudes of 1.8 μm (at 100 Hz) and 2.0 μm (150 Hz) generated a neural response in the most sensitive animal. Although this low sensitivity may be sufficient for the detection of fish-generated water motions, it makes it unlikely that *L. curtus* has a special hydrodynamic sense.

Keywords: Sea snakes; Mechanoreception; Evoked potentials; Electroreception

Introduction

Just like a number of freshwater snakes (e.g. *Erpeton* spec., *Eunectes* spec., *Natricinae*), sea snakes (Hydrophiinae) are highly adapted to an aquatic life (Heatwole, 1999; Ineich and Laboute, 2002). Most sea snakes are piscivorous predators that hunt during the day, at dawn or at night. To find their mobile prey in often turbid waters olfactory and visual cues may not be the best and only option. Like fish and many aquatic invertebrates (Kalmijn, 1988), sea snakes may also use cutaneous mechanoreceptors and/or inner ear receptors to detect weak water motions such as those generated by prey objects.

Various corpuscles (scale sensillae) have been found in the integument of snakes (e.g. Povel and Kooij, 1997). Single nerve fibers innervating cutaneous corpuscles of Texas rat snakes respond to mechanical stimuli (Jackson and Doetsch, 1977a, b). Similar to terrestrial snakes, sea snakes like *Lapemis hardwicki* (now called *Lapemis curtus*) and file snakes (Achrochordidae) also have scale sensillae that may be used for the detection of water displacements and, as has been speculated by Povel and Kooij (1997), for the detection of weak electric fields. Behavioral studies indicate that sea snakes (*Pelamis platurus)* are indeed sensitive to water motions caused by swimming fish (Heatwole, 1999). This assumption is in line with the observation that some sea snakes (*Pelamis platurus*) approach and eventually bite into a vibrating object (Heatwole, 1999).

Since there is no physiological evidence that sea snakes can sense low-amplitude water motions, we tried

*Corresponding author.
E-mail address: gwesthoff@uni-bonn.de (G. Westhoff).

0944-2006/$ - see front matter © 2005 Elsevier GmbH. All rights reserved.
doi:10.1016/j.zool.2005.07.001

to record action potentials from the scale sensillae while stimulating the snakes with sinusoidal water motions. In addition, we recorded evoked potentials from the midbrain of sea snakes while using the same stimuli.

Material and methods

For the experiments we used 15 sub-adult spine-bellied sea snakes *Lapemis curtus* (body length 60–80 cm, body weight 150–200 g). The snakes were caught near the coast of Weipa (Gulf of Carpentaria, Queensland, Australia) and transported to the Melbourne Aquarium where they were kept in a large saltwater tank. Experiments were carried out in accordance with the "Principles of Animal Care", Publication No. 86-23, revised 1985 by the National Institutes of Health (Permit WISP00269202; collection authorized by the Queensland Environmental Protection Agency).

For the experiments the snakes were either anaesthetized with Tiletamine and Zolazepam (Zoletile® 6–9 mg/kg bodyweight) and/or immobilized with Pancuronium® (1.5 ml/kg bodyweight). For recordings, the snakes were transferred to an experimental tank ($320 \times 280 \times 140$ mm) of seawater and fixed with rubber bands on a holder with the dorsal head surface at least 1 cm below the water surface. For artificial breathing a PVC-tube (outer diameter 2 mm) was inserted into the glottis of the snakes. We ventilated the lungs of the snakes by mouth-to-mouth breathing once every 30–45 min via the PVC-tube. All experimental animals recovered from Pancuronium® and Zoletile® after 10–14 h, i.e. our simple method of lung ventilation was sufficient to keep the snakes alive.

Peripheral recordings: For recordings from the scale sensillae we used tungsten microelectrodes (1–3 MΩ), glass micropipettes filled with potassium chloride (< 1 MΩ), glass-sheathed indium alloy metal electrodes (< 1 MΩ; Dowben and Rose, 1953), or a chlorided silver wire (0.1 mm diameter, < 0.1 MΩ resistance) that – with the exception of the tip – was insulated with plastic tubing (Budelmann and Bleckmann, 1988). Scale sensillae could be viewed through a binocular (Zeiss Stemi V11). For recordings the tip of the electrode was placed on top of or close to the edge of a scale sensillum.

Central recordings: For central recordings a small part of the dorsolateral surface of the midbrain of the anaesthetized snakes was exposed. Prior to surgery and in addition to the general anaesthesia, we injected Xylocain® as a local anaesthetic under the skin of the snakes' head. A plastic ring of 1 cm diameter was glued on the head of the snakes to prevent seawater from entering the cranium. Tungsten microelectrodes (1–3 MΩ) were used for brain recordings. Neural responses were amplified (DAM 80, WPI, bandpass, filter setting 1–3000 Hz), displayed on an oscilloscope, and stored (sampling rate 10000 Hz) on a notebook (IBM, 1200 MHz). For off-line data analysis we used the software Igor Pro (Wavemetrics).

Water motions were generated with a plastic sphere (10 mm diameter) that was attached to the membrane of a loudspeaker (Sanyo S05S05, 8 Ω, × 0.3 W) by a plastic rod (8 cm long, 2 mm diameter). The sphere was placed next to the head ipsilateral (peripheral recordings) or contralateral (central recordings) to the recording site. Axis of sphere vibration was in the transversal plane, tilted at 20°, such that the upper limit was further from the snake than the lower limit of movement. The distance between the surface of the sphere and the skin of the snake was 2–3 mm. For threshold determination sinusoidal stimuli (50–200 Hz, duration 100 ms, rise/fall times 20 ms) were generated with a laptop (IBM, 1200 MHz), D/A converted (SoundMax AC'97), amplified (custom made amplifier) and fed into the loudspeaker. At a given frequency, the response threshold was defined as the peak-to-peak water vibration amplitude that caused an evoked potential whose amplitude was about twice as high as that of the noise. Evoked potentials were analyzed from records that represented the average of 32–64 responses. Sphere vibration amplitudes were measured with a capacitive displacement sensor (ADE-technology, resolution 0.2 μm). The corresponding water displacement amplitudes were calculated according to the equation (Harris and Bergeijk, 1962)

$$A = (R^3/D^3) \times A_0,$$

where A_0 is the displacement amplitude of the sphere, R is the radius of the sphere and D the shortest distance from the sphere center to the point of interest.

Electric field stimuli were presented as 10 Hz sine waves of 100 ms duration and 100–500 μV amplitude between two 4 cm carbon rod electrodes which were 10 cm apart and placed on both sides of the head of the snake. Field intensities were monitored with a pair of silver wire electrodes in the bath near the animal.

Results

With the tip of a microelectrode placed on the skin close to a receptor, extracellular neural responses have been recorded from the lateral line neuromasts of fish (e.g. Mohr and Bleckmann, 1998) and amphibians (Northcutt and Bleckmann, 1993), the epidermal lines of cephalopods (Budelmann and Bleckmann, 1988; Bleckmann et al., 1991b), the trichobothria (Reißland and Görner, 1978) and slit sensillae of spiders (Bleckmann and Barth, 1984), the infrared organs of pyrophilous beetles (Schmitz et al., 1997, 2000) and

the electroreceptors of amphibians (Northcutt and Bleckmann, 1993) and fish (Teunis et al., 1990). Using the same method, we tried to record neural responses from a total of 45 prefrontal, frontal, supraocular, parietal, nasal and supralabial scale sensillae of three spine-bellied sea snakes *L. curtus*. Since the scale sensillae could easily be recognized with a binocular, a precise placement of the electrode tip was always possible. We tried metal, glass capillary, indium alloy metal and silver wire electrodes (see Material and methods) as well as different recording sites (close to a scale sensillum, on top of a scale sensillum). Despite all our efforts we failed to get any neural activity from the scale sensillae of *Lapemis* to water motions.

In four sea snakes we finally tried to record responses from the midbrain. Two snakes were unresponsive to visual stimuli (the beam of a flash-light or the light of a photodiode that was switched on and off) and to vibrating sphere stimuli (50–150 Hz, duration 100 ms, peak-to-peak sphere displacement amplitudes up to 50 μm). The other two snakes responded with large evoked potentials to the beam of a flash light (see inset in Fig. 1), the light of a photodiode (not shown) and vibrating sphere stimuli (Figs. 1 and 2). Responses (evoked potentials) to the vibrating sphere consisted of an initial negative potential followed by a large positive and a large negative potential. No action potentials were recorded either to visual or to hydrodynamic stimuli.

Several lines of evidence suggest that the evoked potentials were no mechanical or electrical artifacts but represented biological events in response to local water movements: (i) evoked potentials could only be recorded if the sphere was in the water, (ii) the amplitude of the evoked potentials decreased with increasing stimulus repetition rate (Fig. 1), and (iii) at high stimulus intensities the responses reached saturation (for an example, see Fig. 2, inset).

In one animal we determined the vibration thresholds for the frequencies 50, 75, 100, 125 and 200 Hz. Peak-to-peak sphere displacement amplitudes (the distance between the sphere and the snake was 2 mm) sufficient to cause a neural response were 11.0 μm (50 Hz), 13.7 μm (75 Hz), 4.5 μm (100 Hz), 15.0 μm (125 Hz), 16.5 μm (150 Hz) and 20.0 (200 Hz). The other animal was

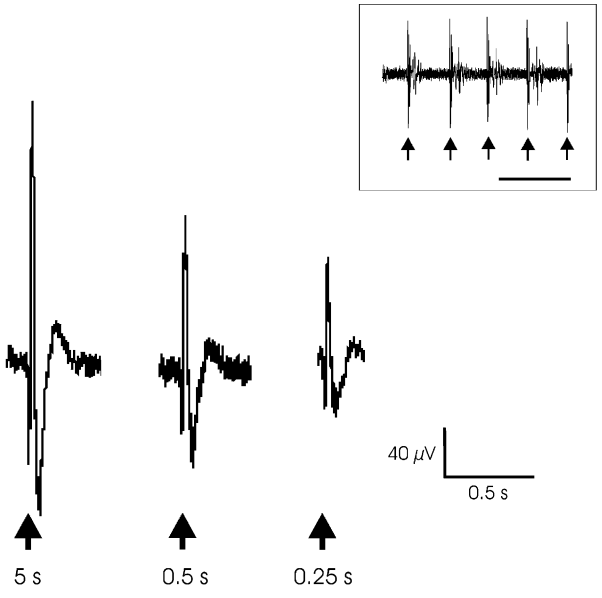

Fig. 1. Evoked potential responses in the midbrain of *L. curtus* to water motions caused by a vibrating sphere (vibration frequency 350 Hz, duration 100 ms, peak-to-peak vibration amplitude 50 μm) and to visual stimulation (inset). Stimulus intervals were 5, 0.5 and 0.25 s. Positive voltages are upward. Each record represents the average of 32 responses. Arrows indicate stimulus onset. Note the decrease in evoked potential amplitude with increasing stimulus repetition rate. Time bar in the inset = 1 s.

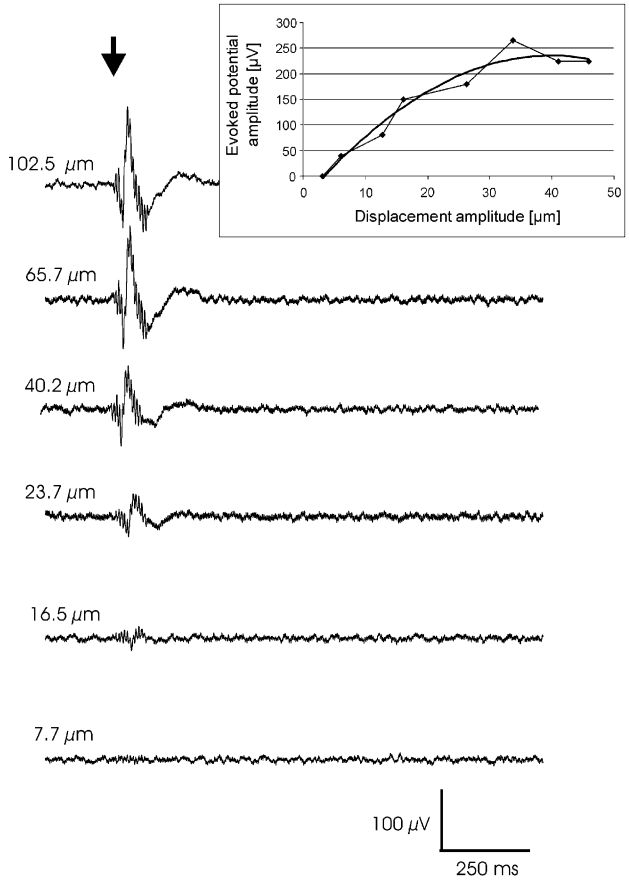

Fig. 2. Evoked potential responses in the midbrain of *L. curtus* to water motions caused by a vibrating sphere (150 Hz, 100 ms). Positive voltages are upward. Each record represents the average of 32 responses. Sphere vibration amplitudes are given to the left. Inset: peak-to-peak evoked potential amplitude as a function of sphere displacement amplitude. Note that the evoked potential amplitude reached saturation. Fit curve in inset is logarithmic.

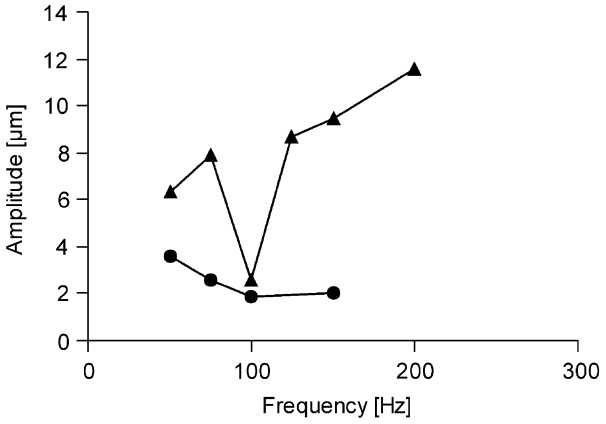

Fig. 3. Peak-to-peak water displacement amplitudes at the skin surface of the snake that elicited neural responses, plotted for the different stimulus frequencies. Triangles and dots indicate two different snakes.

stimulated with the vibration frequencies 50, 75, 100 and 150 Hz. In this animal peak-to-peak sphere vibration amplitudes of 6.3 µm (50 Hz), 4.5 µm (75 Hz), 3.2 µm (100 Hz) and 3.5 µm (150 Hz) were sufficient to cause a neural response. The corresponding water displacement amplitudes at the skin surface of the snake are shown in Fig. 3. In terms of displacement, frequencies around 75–150 Hz were most effective in eliciting a neural response.

Snakes were subsequently tested for responses to weak electric field stimuli. None of the two animals that responded to visual and mechanosensory stimuli showed any neural responses to weak electric fields. We used 10 Hz sine waves of 100 ms duration and the intensity of the field, measured as voltage gradient in the middle of the tank, was set to amplitudes of up to 500 µV/cm. Such stimuli would elicit evoked potentials in relevant parts of the brain in almost any electroreceptive fish (Bullock et al., 1983).

Discussion

Protozoa, bryozoa, coelenterates, ctenophores, flatworms, annelids, mollusks, crustacea, echinoderms, chaetognaths, urochordates, cephalochordates, fishes, and many aquatic amphibians can detect water motions (for a review, see Bleckmann, 1994). At least four amniote species also have this capability: seals (*Phoca vitulina*) (Dehnhardt et al., 1998, 2001) and water rats (*Hydromys chrysogaster*) (Meyer, Dehnhardt and Bleckmann, unpublished) can use their facial vibrissae to detect water displacements such as those generated by swimming fish. Crocodilians have dome pressure receptors for the perception of surface waves (Soares, 2002) and Florida manatees are covered with tactile hairs that they may use for the detection of water motions (Reep et al., 2002). Our physiological study suggests that sea snakes (*L. curtus*) also can sense water motions. The sensitivity of the snakes was, however, three orders of magnitude lower than the sensitivity of the fish and amphibian lateral line and one order of magnitude lower than the sensitivity of the epidermal lines of cephalopods (Bleckmann, 1994). On the other hand, it was similar to the behavioral sensitivity of seals (0.8 µm at 50 Hz) to water motions (Dehnhardt et al., 1998). The comparatively low sensitivity of sea snakes to water motions makes it unlikely that *Lapemis* has developed a special hydrodynamic sense. Instead, mechanoreceptors in the skin may function as touch receptors that also have some incidental sensitivity to water motions. Since we did not get any responses to electric stimuli, our results do not support the hypothesis of Povel and Kooij (1997) that the scale sensillae might have an electroreceptive function.

Since we failed to record any neural activity from the scale sensillae of *Lapemis* we cannot rule out that the evoked potentials recorded from the midbrain were mediated by inner ear receptors. The following results speak against this explanation: when we applied vibratory stimuli to the edge of the experimental tank that would have elicited evoked potentials in relevant parts of the brain of teleost fish (Echteler, 1985), evoked potentials were never recorded. Thus, the responses to the vibrating sphere probably were not mediated by vibration sensitive receptors in the inner ear. Hand clapping in the vicinity of the experimental animal also did not lead to neural responses. Therefore, the responses probably were not mediated by cochlear hair cells either. In any case, although the sensitivity of *Lapemis* to water motions was not very high, it is sufficient to detect the water motions in the wake of a swimming fish (Bleckmann et al., 1991a).

Sea snakes take up oxygen through their skin. This may explain why their skin is heavily suffused with blood vessels (Heatwole, 1999). The scale sensillae of snakes are innervated by fibers of the trigeminal nerve (Jackson and Doetsch, 1977b). Because we failed to get any neural responses from electrodes placed directly on or close to a scale sensillum we tried to expose branches of the trigeminal nerve for recordings. Unfortunately, the high density of blood vessels underneath the skin had the consequence that even the slightest cut caused heavy and long lasting bleeding that made any attempt to expose fibers of the trigeminal nerve impossible. Heavy bleeding and also an extraordinarily hard brain capsule made it very difficult to access the mid- and hindbrain. While it was comparatively easy to access the forebrain of *Lapemis*, the midbrain and especially the hindbrain was situated deeply in the skull underneath large maxillary muscles. Nevertheless, in four snakes we finally succeeded in exposing at least a small part (about

0.5×0.5 mm) of the dorsal midbrain surface. This allowed us to insert the electrode tip in the brain but did not permit us to precisely position the electrode in the torus semicircularis or in the tectum opticum, two midbrain areas that are known to process sensory information in snakes and other vertebrates (Hartline and Campbell, 1969; Hartline and Newman, 1981). A misplacement of the electrode tip thus may be the reason why we failed to record evoked potentials in two out of the four snakes investigated.

The short supply of animals and the difficulty in keeping the sea snakes alive made it impossible to extend the study. Due to the small number of snakes from which brain potentials were successfully recorded our results can only be viewed as preliminary. However, since this is the first and only study in which sensory evoked potentials have been recorded from the brain of sea snakes we feel that it is justified to publish our data. The study shows that submerged and immobilized sea snakes can be artificially ventilated with a simple method and that sea snakes are sensitive to low-amplitude water motions. More studies are needed to uncover the receptors that mediated the evoked potentials in *Lapemis*.

Acknowledgements

This work was aided by a grant of the German Science Foundation to H.B. and G.W. We thank S. Alastair and many other members of the Department of Pharmacology of the University of Melbourne for help and loan of equipment. We thank W. Böhme at the Alexander König Museum in Bonn for providing specimens for anatomical inspection. M. Hofmann deserves special thanks for technical support and computer programming and J. Mogdans for critically reading the manuscript. We would also like to thank the Australia & Pacific Science Foundation and the Melbourne Aquarium for supporting the sea snake research.

References

Bleckmann, H., 1994. Reception of hydrodynamic stimuli in aquatic and semiaquatic animals. In: Rathmayer, W. (Ed.), Progress in Zoology, first ed., vol. 41. Gustav Fischer, Stuttgart, Jena, New York, pp. 1–115.

Bleckmann, H., Barth, F.G., 1984. Sensory ecology of a semiaquatic spider. II. The release of predatory behavior by water surface waves. Behav. Ecol. Sociobiol. 14, 303–312.

Bleckmann, H., Breithaupt, T., Blickhan, R., Tautz, J., 1991a. The time course and frequency content of hydrodynamic events caused by moving fish, frogs, and crustaceans. J. Comp. Physiol. A 168, 749–757.

Bleckmann, H., Budelmann, B.-U., Bullock, T.H., 1991b. Peripheral and central nervous responses evoked by water movements in a cephalopod. J. Comp. Physiol. A 168, 247–257.

Budelmann, B.-U., Bleckmann, H., 1988. A lateral line analogue in cephalopods: water waves generate microphonic potentials in the epidermal head lines of *Sepia* and *Lolliguncula*. J. Comp. Physiol. A 163, 1–5.

Bullock, T.H., Bodznick, D.A., Northcutt, R.G., 1983. The phylogenetic distribution of electroreception: evidence for convergent evolution of a primitive vertebrate sense modality. Brain Res. Rev. 6, 25–46.

Dehnhardt, G., Mauck, B., Bleckmann, H., 1998. Seal whiskers detect water movements. Nature 394, 235–236.

Dehnhardt, G., Mauck, B., Hanke, W., Bleckmann, H., 2001. Hydrodynamic trail-following in harbor seals (*Phoca vitulina*). Science 293, 102–104.

Dowben, R.M., Rose, J.E., 1953. A metal-filled microelectrode. Science 118, 22–24.

Echteler, S.M., 1985. Organization of central auditory pathways in a teleost fish, *Cyprinus carpio*. J. Comp. Physiol. A 156, 267–280.

Harris, G.G., Bergeijk, W., 1962. Evidence that the lateral line organ responds to near-field displacements of sound sources in water. J. Acoust. Soc. Am. 34, 1831–1841.

Hartline, P.H., Campbell, H.W., 1969. Auditory and vibratory responses in the midbrain of snakes. Science 163, 1221–1223.

Hartline, P.H., Newman, E.A., 1981. Integration of visual and infrared information in bimodal neurons of the rattlesnake optic tectum. Science 213, 789–791.

Heatwole, H., 1999. Sea Snakes. UNSW Press, Hong Kong.

Ineich, I., Laboute, P., 2002. Sea Snakes of New Caledonia. IRD, Paris.

Jackson, M.K., Doetsch, G.S., 1977a. Functional properties of nerve fibers innervating cutaneous corpuscles within cephalic skin of the Texas rat snake. Exp. Neurol. 56, 63–77.

Jackson, M.K., Doetsch, G.S., 1977b. Response properties of mechanosensitive nerve fibers innervating cephalic skin of the Texas rat snake. Exp. Neurol. 56, 77–90.

Kalmijn, A.J., 1988. Hydrodynamic and acoustic field detection. In: Atema, J., Fay, R.R., Popper, A.N., Tavolga, W.N. (Eds.), Sensory Biology of Aquatic Animals, first ed. Springer, New York, pp. 83–130.

Mohr, C., Bleckmann, H., 1998. Electrophysiology of the cephalic lateral line of the surface-feeding fish *Aplocheilus lineatus*. Comp. Biochem. Physiol. 119A, 807–815.

Northcutt, R.G., Bleckmann, H., 1993. Pit organs in axolotls: a second class of lateral line neuromasts. J. Comp. Physiol. A 172, 439–446.

Povel, D., Kooij, J.v.d., 1997. Scale sensillae of the file snake (Serpentes: Acrochordidae) and some other aquatic and burrowing snakes. Neth. J. Zool. 47, 443–456.

Reep, R.L., Marshall, C.D., Stoll, M.L., 2002. Tactile hairs on the postcranial body in florida manatees: a mammalian lateral line? Brain Behav. Evol. 59, 141–154.

Reißland, A., Görner, P., 1978. Mechanisms of trichobothria in orb-weaving spiders (Agelenidae, Araneae). J. Comp. Physiol. A 123, 59–69.

Schmitz, H., Bleckmann, H., Mürtz, M., 1997. Infrared detection in a beetle. Nature 386, 773–774.

Schmitz, H., Schmitz, A., Bleckmann, H., 2000. A new type of infrared organ in the Australian ''fire-beetle'' *Merimna atrata* (Coleoptera: Buprestidae). Naturwiss 87, 542–545.

Soares, D., 2002. An ancient sensory organ in crocodilians. Nature 417, 241–242.

Teunis, P.F.M., Bretschneider, F., Peters, R.C., 1990. Convergence ratio and transduction in catfish electroreceptive organs. Comp. Biochem. Physiol. 97A, 405–410.