

# Auditory and Vibratory Sense of Crickets



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## The Origin of Sound-Processing Elements in Ensifera Using Laser Vibrometry

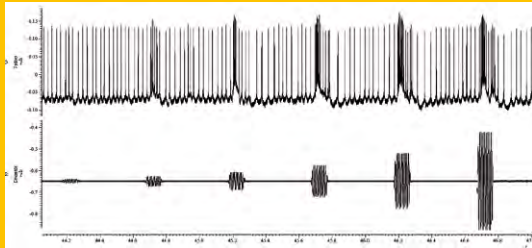
In the life of insects, vibrational signals mediate important information that is used in various contexts, from pair formation to detection of predators or finding prey. Therefore, insects are equipped with both extremely sensitive receptor organs in the legs for detection of substrate vibrations and the underlying neural network enabling recognition and localization of the signallers in a complex environment. Without the use of special equipment to detect those signals, the intriguing world of insect vibrations would remain hidden to humans, which mostly communicate by sight and sound.

### Introduction

Insects in the group called Ensifera produce sounds for communication. These insects, which include crickets and bush crickets, have evolved ears in their legs from the pre-existing vibratory organs. In our study, we investigated whether their auditory and vibratory sense may share a common origin at the level of the central nervous system. Therefore, we studied the network of vibration-sensitive interneurons in a primitive, deaf cave cricket (Fig. 1) and compared our results with well known results for auditory neurons of hearing Ensifera.



Fig. 1: The cave-dwelling cricket *Troglophilus neglectus* (Ensifera, Rhaphidophoridae) has organs specialized for detection of substrate vibrations located below the “knee” joint of all legs.



**Fig. 2:** The electrophysiological responses of an interneuron (top trace) to 100 Hz sinusoidal pulses of 100 ms duration, repeated 1/s, with intensities increasing in 5 dB steps from 0.02 -0.4 m/s<sup>2</sup> (bottom trace).



**Fig. 3:** Measuring the vibration stimuli using a laser vibrometer head with the laser focused on the mini shaker tip (lower image), in combination with the vibrometer controller (upper image).



**Fig. 4:** A photomicrograph of a vibratory interneuron, filled with fluorescent dye, in the prothoracic nerve chord segment (ganglion) of the cave cricket. Different focal planes are combined.

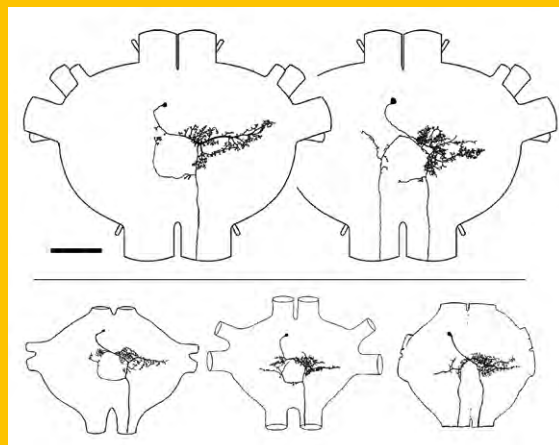
## Experimental Set-up

In our experiments, the first thoracic segment of the nerve chord in the cave cricket was penetrated by intracellular recording electrodes, while the insect's front legs were vibrated by sinusoidal pulses delivered by two mini shakers. By recording from a vibration-sensitive neuron, pulses of frequencies ranging from 50 Hz to 5000 Hz were delivered systematically with accelerations increasing from 0.01 m/s<sup>2</sup> to 22.5 m/s<sup>2</sup> (Fig. 2).

For calibration of the mini shakers, we used a Polytec OFV-353 Laser Vibrometer Head with an OFV-2200 Vibrometer Controller (Fig. 3) These are predecessors of Polytec's current generation of single-point vibrometers (editor's note). The amplitude of the produced vibrations was measured as peak velocity at the tip of the mini shaker cone – at the point where the leg of the animal was attached during the experiment. During the measurement, the input voltage of signals applied to the mini shaker was adjusted to deliver vibrations of equal peak acceleration value (4 m/s<sup>2</sup>) at all testing frequencies. The available output resolutions of the controller range from 5 to 125 mm/s/V and enable detection of the signals well above the noise level throughout the measurement range.

## Results

After a respective neuron was tested physiologically, it was filled iontophoretically with a fluorescent dye (Fig. 4). Since the homology of insects' neurons (those sharing the same ancestral precursor) can be inferred solely on the basis of morphological similarity, nine vibratory interneurons of the cave cricket could be clearly recognised as homologues to some of the sound-



**Fig. 5:** Schematic drawings of a vibratory interneuron from the prothoracic ganglion of two individual cave crickets (top drawings; scale bar represents 200 μm), compared to its putative homologues in the auditory system of different Ensifera with intact hearing (lower drawings; a hump-backed cricket and two cricket species, from left to right). Mod. from: Stritih and Stumpner, *Zoology* 112 (2009) 48–68.

processing elements from *Ensifera* with hearing (Fig. 5). In some of these neurons, a part of the arborizations appeared significantly modified compared to their presumed precursor neurons in the vibratory system. This modification indicates changes in synaptic connectivity during evolution. Nevertheless, the counterpart neurons express similar types of electrophysiological responses along with similar patterns of excitatory and inhibitory inputs received with similar latencies. These observations indicate their commonalities in intrinsic properties and the way of integration in the neural network. With respect to sensitivity of the vibratory neurons in the cave cricket, their acceleration response thresholds reached down to 0.005 to 0.01 m/s<sup>2</sup> (Fig. 6), making them among the most sensitive vibrational responses ever detected in animals.

### Outlook

The delivery of biologically relevant stimuli with accurately defined intensities is a significant problem for researchers investigating insect sensory systems. Non-contact laser vibrometry offers many advantages over contact methods of measuring vibrations including the accurate calibration of stimulus presenting devices used in the study of insect vibration sensing. By comparing these methods we determined that contact measurements may load the device under investigation, significantly changing not only its intensity but also its frequency (resonant) characteristics. The use of the laser vibrometry enabled us to precisely measure the stimuli applied to the insect directly and minimized any interference with the highly sensitive system under study.

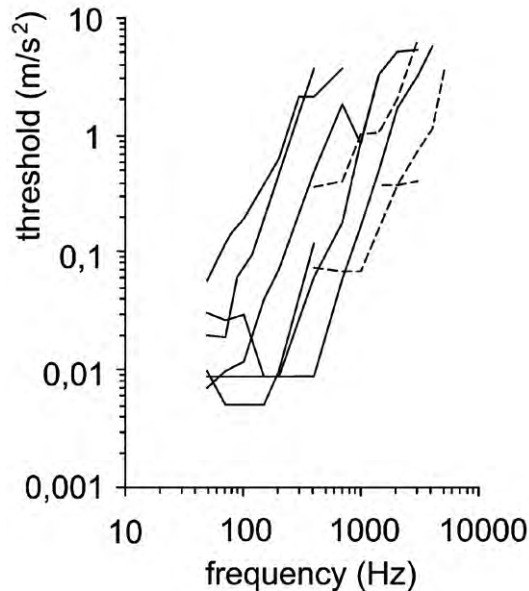


Fig. 6: Threshold tuning curves for a group of interneurons with the largest sensitivity to vibration frequencies found below 400 Hz. The dashed line indicates thresholds of their inhibitory inputs. from: Stritih, J. *Comp. Neurol.* 516 (2009) 519–532.

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