

INSTRUMENTS AND TECHNIQUES

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Recording from cuticular mechanoreceptors during mechanical stimulation

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Abstract It is technically demanding to make intracellular measurements of mechanoreception in intact arthropod cuticular receptors. Here we introduce a method for recording mechanically induced electrical events in a class of spider mechanoreceptors using single electrode voltage- or current-clamp. A concave piece of cuticle containing a mechanosensitive lyriform slit organ was dissected free and fixed with wax onto a specially designed holder. This holder-cuticle complex, filled with spider saline, allowed displacement of the slit membrane from below while simultaneously recording intracellularly from neurons of the organ through a thin saline film. Extracellular ion concentrations could be changed and ion channel blockers could be applied to the bath. The method promises to allow the investigation of the ion channels responsible for mechanically transduced receptor signals and spike encoding.

Key words Mechanoreceptors · Mechanical stimulation · Voltage-clamp · Spider

Introduction

The senses of touch, vibration, hearing and balance, as well as the functions of many vital systems are based on the transduction of movement or force into electrical signals, a process which probably involves specialized ion channels [6]. Unfortunately, the small size and inaccessible positions of most mechanoreceptors, as well as the lack of suitable recording methods, have precluded cellular studies of mechanotransduction and signal encoding, except for a few special cases such as

crayfish stretch receptors [10] and hair cells of the inner ear [4].

The surfaces of spider legs are provided with a large number of mechanoreceptors. Especially common are lyriform slit organs which detect strain in the exoskeleton. These organs consist of cuticular slits, each innervated by a pair of bipolar sensory neurons [1]. Lyriform organ VS-3 (nomenclature of Barth and Libera [2]) lies on the antero-ventral side of the leg patella [1]. It is formed from seven to eight cuticular slits, graduated in length from 15 μm to 120 μm long, and the corresponding pairs of spindle-shaped bipolar neurons which conduct the afferent information to the central nervous system [1]. According to Seyfarth and French [11] and Juusola et al. [8], the bipolar neurons can be grouped into two types by their responses to electrical and mechanical stimulation. The cells respond to depolarizing current steps and cuticular displacements by either producing one spike (a “single spiker”) or a short burst of spikes with decaying amplitude (a “multiple spiker”). This paper explains how to record intracellularly (with single electrode current- or voltage-clamp methods) from the neurons of the anterior lyriform slit organ of *Cupiennius salei* while mechanically stimulating the slit sense organ.

Materials and methods

Experimental animals and preparation

Adult (> 1 year old) tropical hunting spiders (*C. salei* Keys.) (ctenidae) from a laboratory colony were used in the experiments. The spiders were kept in plastic jars under controlled humidity at room temperature ($\approx + 21^\circ\text{C}$) and were fed with *Drosophila*, cockroaches and locusts. A leg was autotomized and a concave piece of cuticle containing lyriform organ VS-3 was dissected from the anterior patella and prepared for recording under a dissecting microscope. More detailed descriptions of this initial procedure are given by Seyfarth and French [11]. Here, it should be mentioned that cutting and preparing the slit sense organ preparation may release some of the natural tension existing in the patellar exoskeleton.

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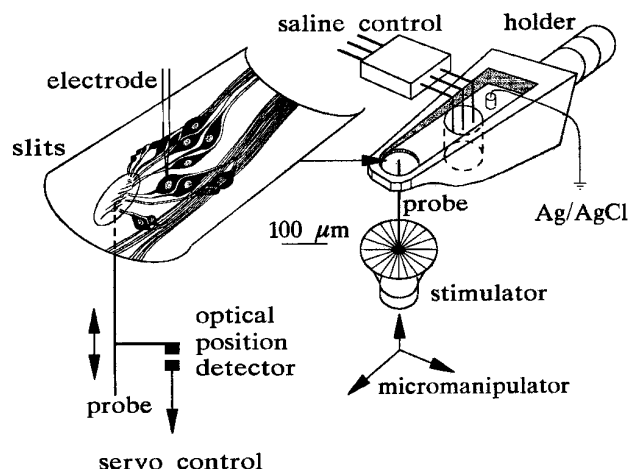
Preparing the set-up for mechanical stimulation

In order to provide mechanical stimuli to the slit organ or to the surrounding cuticle, several procedures were required during the recording set-up. Figure 1 displays some of the important features of the arrangement needed for successful recording.

The piece of cuticle containing the slit organ was placed on a custom-designed plexiglas holder and attached with beeswax (melting point $\approx 40^\circ\text{C}$) along its two long exterior sides. When placed properly, the slit sense organ lay at the bottom of the concave piece of cuticle facing the bottom centre. Because of the low melting temperature and the distance of 3 mm from the dry exterior sides to the neurons covered with cool spider saline ($+4^\circ\text{C}$; 223.0 mM NaCl, 6.8 mM KCl, 8.0 mM CaCl₂, 5.1 mM MgCl₂, buffered to 8.2 pH by 0.05 M Tris 7–9), attaching the preparation did not damage the neurons. Waxing was necessary to stop the preparation from moving while stimulating with pressure, and thus causing the electrode to slip out from a neuron. The dry exterior surface of the preparation faced downwards while its interior was continuous with the holder's 0.5-ml bath chamber. This arrangement allowed the spider saline of the preparation to mix freely with the bath solution. The bath was grounded with an indifferent electrode (Ag/AgCl). A micromanipulator was used to lower small rubber tubes into the holder's bath, to allow solution changes for chemical treatment or to adjust the saline level when necessary. Because of the small dimensions of the bath chamber, diffusion of oxygen from the air was found to be sufficient to maintain the electrical properties of the neurons, even with experiments lasting several hours.

The preparation-holder complex was firmly held in the air by a fixed stand and mechanical pressure was applied to the slit organ from below by a custom-made stimulator constructed from a small loudspeaker (diameter 7 cm). The stimulator, which had an

Fig. 1 Schematic drawing of the experimental arrangement for mechanical stimulation and intracellular recording. The piece of patellar cuticle containing the slits and their neurons (*left*) was waxed into position on the hole in the end of the plexiglas holder (*right*). The preparation fitted snugly in the holder along its sides. The interior of the piece of cuticle was continuous with the grounded bath solution, which could be controlled and exchanged via a series of small tubes. A microelectrode penetrated the neurons from above, while mechanical stimulation was applied to the dry exterior of the slits from below. The stimulator consisted of a fine insect pin connected to a small loudspeaker. The insect pin was grounded to reduce the electrical noise from the stimulator. The position of the pin was detected by a fixed infra-red transistor illuminated by an infra-red light-emitting diode mounted on the loudspeaker cone. The position signal was used to provide servo-control of the speaker with second-order frequency compensation



infra-red optical position indicator and servo-controlled position, was driven by computer-controlled voltage commands from a 12-bit digital to analogue convertor (DT2821, Data Translation). The infra-red sensitive diode (Radio Shack) was mounted next to the stimulus probe on the flat centre of the loudspeaker cone and faced a matched infra-red light-emitting diode mounted on the stimulator frame. This arrangement allowed direct detection of the vertical position of the stimulus probe. To eliminate horizontal resonances during vertical stimulation, the probe had a tapering structure with three components. From its bottom, the probe was connected to the loudspeaker cone by a plastic screw. Above it was the shank of a hollow syringe needle, and inside the needle a small insect pin. The tip of the pin, which applied displacements to the exterior surface of the preparation, extended 3 mm out from the needle. Several different pin tip forms were tested before selecting a sharp parabolic shape (tip diameter 15 μm) that gave the fastest response of the receptor potentials measured in this study. Electrical noise caused by the stimulator was prevented by grounding the upper portion of the syringe needle close to the pin tip. The exact stimulus location (i.e. the pin tip) was visible under illumination through the transparent cuticle and was controlled by a three-dimensional micromanipulator. To eliminate mechanical interference, the stimulator, holder and all other instruments were clamped together on a heavy metal plate and placed on an air-driven vibration-isolation table (Technical Manufacturing, micro-G) with added rubber cushioning.

When calibrating the stimulator, the probe displacements were measured under a compound microscope. Using this information the signals from the optical position indicator were calibrated on a micrometer scale. Additionally, the stimulator was tested by a semiconductor tension transducer (Endevco Pyxie) connected in a Wheatstone bridge configuration to another unstimulated transducer, to eliminate temperature sensitivity. During these tests the stimulator was driven by pseudorandomly modulated voltage commands (Gaussian amplitude distribution, power flat up 400 Hz). Peak-to-peak displacements were $<30 \mu\text{m}$, and the coherence function [3], calculated between the optical position reading and the current output of the tension transducer, was close to unity up to 400 Hz. This indicated that the servo-controlled stimulation system operated linearly over its frequency range when modulated even with fairly large displacements. The maximum operational displacement range of the stimulator was 0–100 μm , giving 1–2 μm for the resolution of the smallest step size. However, with the largest steps the displacement stimuli probably deviated from linearity, because of the inverse square relationship between the infra-red light intensity and distance.

The step displacement stimuli caused transient vertical resonances that were limited by the servo-control to the beginning and ending of the steps. With proper prefiltering of the step signal this ringing could be reduced. Further compensation of the step stimuli was found to be impractical, because a fast stimulus rise time was more desirable than the slower, oscillation-free performance that could be achieved by driving the step commands through a filter.

Recording procedures

Intracellular single electrode current- and voltage-clamp recordings from the mechanoreceptor neurons were performed via glass capillary microelectrodes (borosilicate glass; outer diameter 1 mm, inner diameter 0.7 mm; Hilgenberg, Germany) pulled with a laser-driven micropipette puller (P-2000, Sutter Instrument, USA). The general conditions for successful single electrode voltage- and current-clamp have been discussed previously by several authors [5, 7, 9, 12, 13], and are only briefly listed here. The crucial relationships are:

$$f_c > 3f_{sw} \text{ and } f_{sw} > 2f_s > 2f_r > f_m \quad (1)$$

where f_c is the upper cut-off frequency of the recording electrode; f_{sw} is the switching frequency of the time-sharing control of the voltage measurement and current injection (in voltage-clamp); f_s is the sampling frequency of the recording system; f_l is the upper cut-off frequency of the low-pass filter; f_m is membrane cut-off frequency. These conditions were all met during our experiments as indicated below.

The electrodes were lowered through a thin layer of saline by a micromanipulator while viewing through a dissecting microscope (Wild Heerbrugg M5A, Switzerland, magnification $\times 50$). Because of the tough glia and connective tissue shield surrounding the sensory neurons [8, 11], impalements were performed by gently tapping the electrode holder with a solid object. The resistances of the microelectrodes filled with 3 M KCl varied between 25 M Ω and 80 M Ω . The time constant of the electrode, τ_e in the tissue after dual capacitance compensation of the amplifier (SEC-1L, NPI Electronic, Germany; 1L headstage) was 1.5–3 μ s, corresponding to a high f_c of ≥ 60 kHz. After successful penetration (indicated by a ≈ 70 mV drop in potential that was often accompanied by a burst of action potentials), the plasma membrane of the neuron was allowed to seal around the electrode for a few minutes. Because of the difficulties with the covering glia, good penetrations of the neurons were rare ($< 25\%$). However, when successfully penetrated, some neurons provided several hours of recording.

Experiments were started by characterizing the electrical properties of a neuron by injecting depolarizing and hyperpolarizing current steps. This was followed by applying increasing displacement steps (range: 1–20 μ m) to the slits or to the adjacent cuticle. Depending on the electrode resistance, we used f_{sw} values of up to 15–20 kHz when current- or voltage-clamping the neurons. In some experiments the shape of the receptor potential and current were studied after blocking voltage-sensitive Na⁺ channels and spike production by adding 10 μ M tetrodotoxin (TTX) to the spider saline. The resulting voltage and current responses were observed by the SEC-1L high-impedance amplifier and low-pass filtered at 2 kHz. During each experiment the paired input and output signals were monitored on an oscilloscope, sampled at 2–4 kHz (f_s), digitized with a 12-bit A/D converter (DT2821, Data Translation, USA) and stored on the hard disk or in the memory of a computer (IBM-compatible). After treatment with TTX the receptor potential and current recordings were averaged. Data processing was performed later using custom-written software.

Results and discussion

In this section we examine typical responses to both electrical and mechanical stimulation and discuss possible factors that can influence the recordings.

Examples of responses to electrical stimulation

Figure 2A illustrates the electrical responses of a typical "single-spiker" neuron to 100-ms current steps of different polarities. Single-spikers produced only one, or occasionally two, spikes to an electrical step [8, 11]. The amplitudes of the responses to hyperpolarizing current steps showed large variability, corresponding to peak input impedances of from 60 M Ω to 300 M Ω . This variability may be related to the quality of the electrode seal and to the cell size. In stable recordings, the spike amplitudes of the cells varied from 45 mV to 80 mV and the resting potentials from -60 mV to -75 mV.

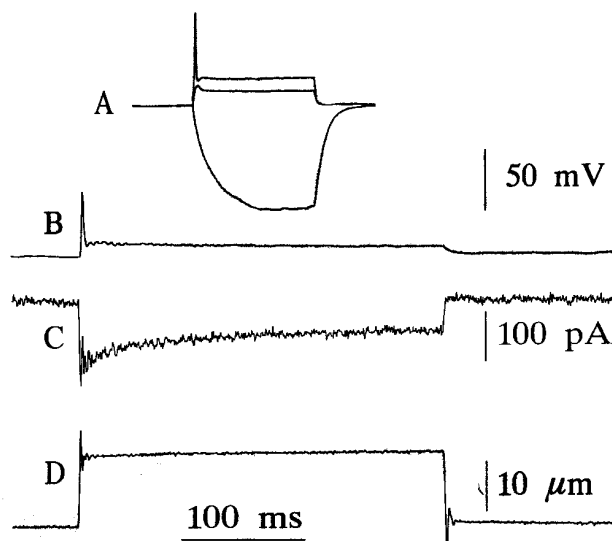


Fig. 2A–D Responses of slit sensilla neurons to electrical and mechanical stimulation. **A** A single-spiking neuron was stimulated with 100-ms current steps of -1.0 , $+0.5$ and $+2.5$ nA amplitude. **B,C** Voltage and current responses of another neuron to a 300-ms mechanical step. **D** The receptor current was observed 2 min after treatment with tetrodotoxin. **C,D** Averaged (10 times) traces

Displacing the slits of the slit sense organ

Figure 3 shows a scale drawing of the exterior surface of the slit sense organ and the tip of the probe. The probe was first elevated with the aid of a micromanipulator to be close to the slits and then advanced further by computer commands until it touched the slits. This was seen as touch-induced spiking. Next, the probe was delicately lowered to the point where the neuron's resting potential did not show any stretch-related activity, and the corresponding displacement reading was taken as the zero level. The vertical 20- μ m bar indicates the amplitude of the maximum step stimulus and thus the slit displacement. With this displacement range the slit sense organ neurons evoked stable responses to repeated stimuli indicating that the probe contact did not destroy the fine, mechanically sensitive structures of the organ.

Examples of responses to mechanical stimulation

Figure 2B demonstrates the typical response of a single-spiking neuron to a displacement step. Direct stimulation of the slits by the electromechanical pusher revealed very similar discharge patterns to the depolarizing current steps. Also note that mechanical stimulation gave a steady depolarizing receptor potential.

The receptor current was studied by single electrode voltage-clamp 2 min after introduction of TTX into the bath. TTX hyperpolarized the resting potential by ≈ 10 mV (blocking the resting conductance of the fast voltage-dependent Na⁺ channels), and blocked the

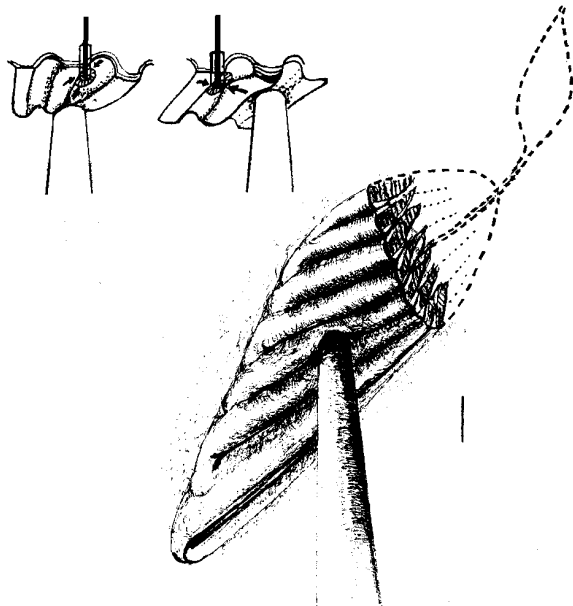


Fig. 3 The exterior face of the slit sense organ and the tip of the stimulus probe touching the slits. The *scale bar* (20 μm) illustrates the relationship between the dimensions of the slit organ and the stimulus probe, and indicates the amplitude of the maximum probe displacement. The *inset* shows various ways in which pressure applied to the slits could lead to monoaxial compression (arrows) of the coupling cylinder and deformation of the dendritic tip. The inset drawing is based on the structures given in [1]

spike production, but did not affect receptor current. Figure 2C shows the receptor current in the single-spiker of Fig. 2B. Since a significant portion of the receptor potentials and current survived the dendritic conduction, the dendritic length constant is probably large compared to the dendrite length. Of course, this value is further enhanced by treatment with TTX, which suppresses the voltage-dependent Na^+ channels.

The vigorousness of the spike response, and the amplitudes of the receptor potential and receptor current depended on the exact location of the probe's tip [8]. Usually the neurons were most sensitive to the displacements directed to the same slit that they innervated. With such direct stimuli, the interval from the beginning of the step to the first spike or peak receptor current was about $\approx 0.5\text{--}1.0$ ms. These findings may tell us something about the displacement-induced deformation of the slit structures. (1) The high repeatability, (2) the small delay between the displacement stimulus and the cell response and (3) the fact that maintained displacements of the probe induced maintained displacements of the slit membrane (as seen in receptor current) suggest that: (1) The coupling between the tip of the probe and the slit membrane is tight, and (2) the slit membrane behaves elastically with low viscosity. However, the applied displacements were large when compared to the displacements needed to stimulate vertebrate hair cells [4] and insect cuticular sensilla dendrites [6]. This implies that lever mecha-

nisms direct and attenuate the displacement to the dendritic ciliary bodies, where the mechano-electrical transduction is expected to take place [1]. Based on the EM-work of Barth [1] the ciliary structures of each neuron pair are encapsulated by a coupling cylinder in the middle of the narrowing slit groove. Such an organization would be suitable for transmitting monoaxial compressive forces to the ciliary bodies [1], but the suggested levering would depend on the visco-elastic properties of the slit structures. The inset in Fig. 3 illustrates how such displacement-induced deformation may occur when the probe presses different parts of the slit sense organ.

In conclusion, we have described a method that allows recording of voltage and current responses to mechanical stimulation from cuticular mechanosensory cells using single electrode current- or voltage-clamp. Since the method allows ion substitution and the use of channel blockers, it can be used for studying the receptor current and other membrane conductances.

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