

Adaptation Properties of Two Types of Sensory Neurons in a Spider Mechanoreceptor Organ

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Juusola, Mikko and Andrew S. French. Adaptation properties of two types of sensory neurons in a spider mechanoreceptor organ. *J. Neurophysiol.* 80: 2781–2784, 1998. The VS-3 slit-sense organs of the tropical wandering spider *Cupiennius salei* contain two types of mechanosensory neurons with similar morphology but different adaptation properties. We measured the changes in membrane potential produced by mechanical stimulation and by electric current stimulation in a large number of neurons of both types. No significant differences were found between the passive membrane properties of the two groups, but there were significant differences in the extent and time course of receptor potential adaptation between the two types of neurons. These data, combined with the responses to suprathreshold electrical stimuli, indicate that adaptational differences exist at several stages in these neurons but that active membrane conductances dominate the overall behavior. The passive membrane measurements also indicate that effective voltage clamp of the receptor current at the tips of the sensory dendrites is possible in these neurons.

INTRODUCTION

Mechanoreceptor neurons of vertebrates and invertebrates display a wide range of dynamic responses, which are generally characterized as rapidly or slowly adapting behavior (French 1992; French and Torkkeli 1994; Sinclair 1981). Some common examples in human skin are the rapidly adapting Pacinian corpuscle (Mendelson and Loewenstein 1964) and the slowly adapting Ruffini endings (Chambers et al. 1972). Although mechanical components can certainly contribute to this range of adaptational behavior, it is clear that electrical processes in the cell membrane are also important (French and Torkkeli 1994).

The VS-3 slit-sense organ of the tropical wandering spider *Cupiennius salei* provides a useful model for examining the contributions of different processes to mechanoreceptor adaptation. The organ consists of seven or eight closely apposed slits in the external cuticle, with each slit innervated by a pair of sensory neurons (Barth and Libera 1970). The neurons vary in size, from ~50–120 μm across the fusiform somata. This allows penetration with a microelectrode to observe the responses to electrical and mechanical stimuli (Juusola and French 1995; Seyfarth and French 1994). Intracellular recordings showed that each pair of neurons consists of one rapidly adapting and one slowly adapting neuron. The rapidly adapting neuron fires only one or sometimes two action potentials in response to a maximum mechanical

or current step. In contrast, the other neuron of each pair fires comparatively long bursts of action potentials in response to similar electrical stimulation (Seyfarth and French 1994). Here they are called single- and multiple-spike neurons.

There are no obvious morphological differences between the two types of neurons at the light microscope level, although differences in dendritic length at the terminal region near the slits can be seen in the electron microscope (Barth 1971). The exact positions of the neurons within the organ are also quite variable so that it is impossible to reliably identify the two types of neurons in a preparation before making an electrical recording.

Here we observed the responses of a large number of slit-sense organ neurons to both mechanical and electrical step stimuli. These data revealed detectable differences between the dynamic properties of the transduction step from mechanical stimulus to receptor potential in the two types of neurons, but also showed that the overall responses of the neurons are largely controlled by the electrical behavior of their action potential encoding systems. No significant differences were seen between the passive electrical properties of the two types of neurons, so the electrical differences are most probably located in voltage- or calcium-activated ion channels.

METHODS

Adult female spiders (*C. salei*) were taken from our laboratory colony. Legs were autotomized, and a concave piece of patellar cuticle containing the lyriform slit-sense organ VS-3 (nomenclature of Barth and Libera 1970) was dissected free and mounted with beeswax onto a custom-designed Plexiglas holder. The tissue was bathed in spider saline consisting of (in mM) 223 NaCl, 6.8 KCl, 8 CaCl₂, 5.1 MgCl₂, 11 sucrose, and 8 tris(hydroxymethyl)aminoethane, pH 8.2 (Maier et al. 1987). All chemicals were purchased from Sigma (St. Louis, MO). Details of the preparation were described previously (Juusola and French 1995; Seyfarth and French 1994). Borosilicate glass microelectrodes were made with a horizontal laser puller (P-2000, Sutter Instrument, Novato, CA), filled with 3 M KCl and coated with petroleum jelly to decrease stray capacitance (Juusola et al. 1997). Electrode resistances were 30–70 M Ω with time constants of 2–3 μs in solution. Microelectrodes were positioned with a Leitz-micromanipulator. The apparatus rested on a gas-driven vibration isolation table (Technical Manufacturing, Micro-g), and all experiments were performed at room temperature ($22 \pm 2^\circ\text{C}$).

Current-clamp measurements were performed with the discontinuous (switching) single-electrode method with an SEC-101 amplifier (npi Electronic, Germany). Switching frequencies of ~20 kHz and a duty cycle of 1/8 (current passing/voltage recording) were used in all experiments.

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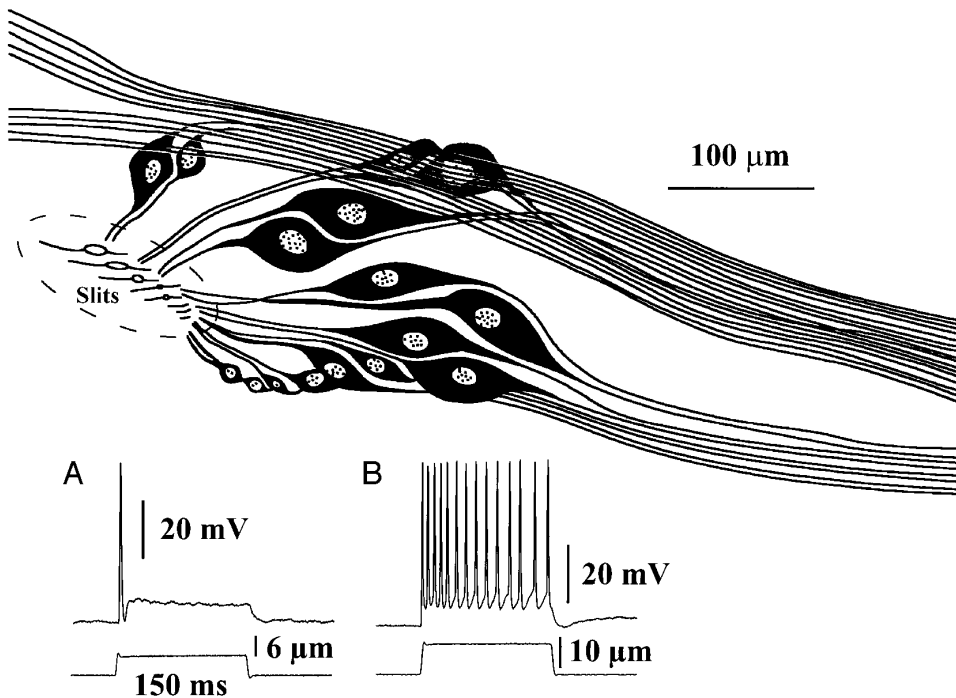


FIG. 1. Mechanosensory neurons of the VS-3 slit-sense organ from *Cupiennius salei*. *Top*: a camera lucida tracing of the peripheral regions of the neurons lying on and adjacent to the sensory nerve in the leg that carries their axons. Each slit is innervated by two neurons with different adaptational behavior [adapted from Seyfarth and French (1994)]. *Bottom*: typical intracellular recordings from single-spiking neurons (A) and multiple-spiking neurons (B) during mechanical indentation of the slits.

Mechanical stimulation was provided by a closed-loop electro-mechanical stimulator (Chubbuck 1966) with a bandwidth of 0–300 Hz. The stimulator was mounted on a three-dimensional micromanipulator to position the tip of the stimulating probe beneath the outer surface of the VS-3 slits (Fig. 1). The probe tip was a normal recording electrode, melted to produce a bead of $\sim 50\text{-}\mu\text{m}$ diameter. The tip could be clearly seen through the preparation and was placed on the slit innervated by the impaled neuron. All experiments were controlled by an IBM-compatible computer with ASYST 4.0 (Keithley) and 12-bit A/D and D/A converters (DT2821, Data Translation). Membrane potential was low-pass filtered at 33.3 kHz by the amplifier.

Statistical comparisons were made with the Student's two-tailed t -test for significantly different means, assuming different variances (Press et al. 1988).

RESULTS

The sizes of the mechanoreceptor neurons depend on their positions in the organ and can also vary significantly within a pair (Fig. 1). The slits are numbered beginning with the longest, with the neurons innervating slits 2–6 being generally larger and easier to penetrate, although those innervating slit 2 are often obscured by other axons. Most recordings shown here were from slits 3–5, but no attempts were made to compare measurements from neurons innervating different slits. Data were only recorded from neurons that demonstrated adequate sealing of the electrode into the cell membrane, as judged 5–10 min after penetration by a stable and adequate resting potential (less than or equal to -60 mV), membrane resistance ($\geq 30\text{ M}\Omega$), and action potential amplitude ($\geq 50\text{ mV}$). A total of 97 neurons satisfied these criteria and were used for further experiments.

In each case, neurons were tested with both mechanical and electrical stimulation and could be clearly divided into single-spiking (Figs. 1A and 2A) or multiple-spiking (Figs. 1B and 2B) types with either stimulus. Therefore, the domi-

nant adaptational behavior of the two types of neurons is primarily controlled by electrical properties of the cell membrane rather than any processes occurring between mechanical stimulation and the receptor potential.

Electrical stimulation with a range of hyperpolarizing and depolarizing currents revealed rather similar behavior, once the action potentials were suppressed with tetrodotoxin (Fig. 2). In general, the neurons all demonstrated strong, time-dependent outward rectification, with significantly smaller voltage responses to depolarizing currents than to hyperpolarizing currents. This outward rectification appeared at approximately the resting potential (Fig. 2, C and D), was a consistent feature, and did not vary with the adaptation properties of the neurons.

Passive membrane resistances (R_m) and time constants (τ) were estimated by measuring the voltage responses to a hyperpolarizing current of 0.1 nA (Seyfarth and French 1994). Mean values of these parameters (Fig. 2) were $R_m = 61.1 \pm 29.1\text{ M}\Omega$, $\tau = 6.3 \pm 3.7\text{ ms}$ (56 single-spike neurons) and $R_m = 70.8 \pm 26.9\text{ M}\Omega$, $\tau = 7.6 \pm 4.0\text{ ms}$ (41 multiple-spike neurons). These values did not differ significantly ($P > 0.05$) between the two cell types. Pooling these values gave a membrane capacitance of $0.11 \pm 0.05\text{ nF}$ ($n = 97$). Using a specific membrane capacitance of $1\text{ }\mu\text{F}/\text{cm}^2$ gives a mean membrane area of $11,000\text{ }\mu\text{m}^2$ and a mean specific membrane resistance of $1.6\text{ k}\Omega/\text{cm}^2$. The value for membrane area is in good agreement with the physical sizes of the neurons.

Responses to mechanical stimuli were obtained after suppressing action potentials with tetrodotoxin (Fig. 3). To standardize the responses, mechanical stimuli were increased in strength until a maximum electrical response was obtained. This maximum stimulus was usually $\sim 10\text{-}\mu\text{m}$ displacement. Receptor potentials displayed adaptation, with a peak response decaying to a plateau level over a period of $\sim 100\text{ ms}$. To quantify the adaptation we fitted the decays

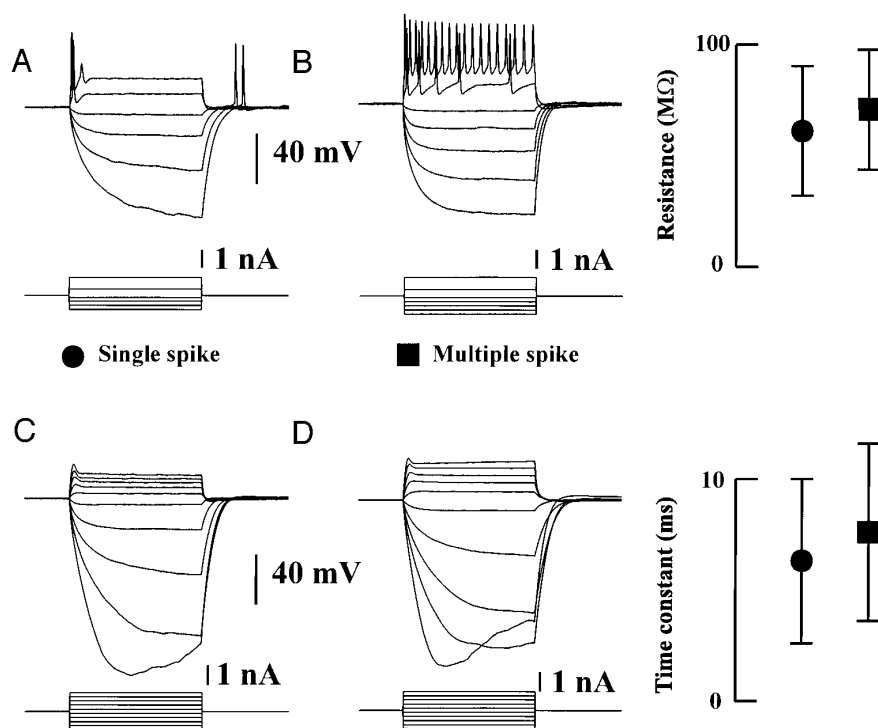


FIG. 2. General electrical properties of the two types of VS-3 mechanosensory neurons. *A*: intracellular recordings from a single-spiking neuron during a series of depolarizing and hyperpolarizing step currents of 150 ms duration. *B*: similar recordings from a multiple-spiking neuron. *C* and *D*: recordings from the same neurons after treatment with tetrodotoxin to suppress action potentials. *Right*: summary data for membrane resistance and membrane time constant obtained from small hyperpolarizing current steps in single-spiking ($n = 56$) and multiple-spiking ($n = 41$) neurons.

with single exponential functions (Fig. 3, *C* and *D*) and measured the fitted peak and final plateau potentials. Ranges and mean values for all the fitted parameters are shown in Table 1. There were no significant differences between the peak and plateau values in the two types of cells, but their ratios and the decay time constants were significantly different.

DISCUSSION

The utility of this preparation for studying receptor currents during mechanical stimulation depends on the quality

of the voltage clamp available at the dendrite tips, where the transduction is assumed to occur. The mean membrane time constant of all the neurons was ~ 7 ms (Fig. 2), and electron microscopy indicated that the diameters of the sensory dendrites generally exceed $2 \mu\text{m}$ (Seyfarth et al. 1995). Assuming a specific membrane capacitance of $1 \mu\text{F}/\text{cm}^2$ and an intracellular resistivity of $100 \Omega\text{cm}^2$ (Hille 1992) gives a space constant of $\sim 600 \mu\text{m}$. The length of the sensory dendrites varies from ~ 50 – $150 \mu\text{m}$ (Fig. 1), giving a range of 78–92% for the control of a steady potential at the sensory tips, which indicates that useful information can be obtained

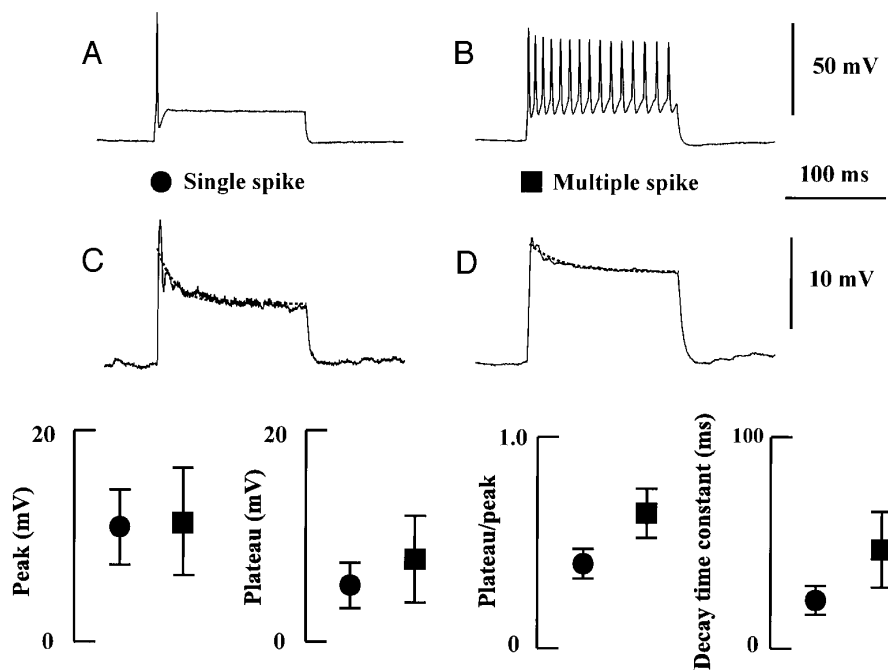


FIG. 3. Adaptation of the receptor potential in VS-3 mechanosensory neurons. Single-spiking (*A*) and multiple-spiking (*B*) neurons were identified by injecting 1-nA depolarizing current pulses. After tetrodotoxin treatment, receptor potentials were recorded in response to saturating mechanical steps ($\sim 10 \mu\text{m}$) in the same 2 neurons (*C* and *D*). Adaptation was quantified by fitting the potentials with single exponential decays (dotted lines in *C* and *D*) and calculating the peak and final plateau depolarizations together with their ratios and the exponential time constants (summary data shown in Table 1).

TABLE 1. *Adaptation of the receptor potential in VS-3 mechanosensory neurons*

	Single Spike	Multiple Spike	<i>P</i>
Peak range, (mV)	4.4–14.7	5.9–20.9	
Peak mean (mV)	10.8 ± 3.5	11.3 ± 5.0	>0.05
Plateau range (mV)	1.9–7.8	3.4–16.3	
Plateau mean (mV)	5.3 ± 2.1	7.8 ± 4.1	>0.05
Plateau–peak mean	0.47 ± 0.07	0.69 ± 0.11	<0.05
Time constant (ms)	22.2 ± 6.8	49.3 ± 26.7	<0.05

about the receptor current under voltage clamp. However, rapidly changing potentials would be less well clamped, particularly if voltage-sensitive currents were induced, and there is evidence that voltage-activated sodium channels are present in the dendrites (Seyfarth et al. 1995).

Although the adaptational behavior of the two types of mechanosensory neurons is profoundly different, we found no major differences between their passive properties. This follows previous findings that there were no significant differences between the resting membrane potentials or spike thresholds in the two cell types (Seyfarth and French 1994). Significant differences were found between the plateau–peak measurements of the receptor potentials and their adaptational time constants, although there were no significant differences between the actual peak or plateau values. This suggests that either extracellular mechanical components or the mechanically activated channels themselves contribute relatively weakly to the differences in adaptation. In contrast, the different responses to direct electrical depolarizations in the absence of mechanical stimulation indicate that the two types of adaptation behavior are primarily controlled by active membrane conductances.

A variety of active membrane processes were implicated in rapid sensory adaptation [reviewed in French (1992) and French and Torkkeli (1994)]. These include electrogenic sodium pumps, slow inactivation of voltage-activated sodium channels, and several types of voltage- and calcium-activated potassium channels. Exploration of the processes responsible for adaptation in the VS-3 organ should be particularly interesting because of the difference between the two types of neuron in the organ. Discovery of the mechanisms by which this difference in adaptation is achieved may

have wide implications for the control of adaptation in a range of sensory receptors.

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