CHAPTER 1

Mechanosensitive Ion Channels of Spiders: Mechanical Coupling, Electrophysiology, and Synaptic Modulation

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I. OVERVIEW

Arthropods have provided several important mechanoreceptor models because of the relatively large size and accessibility of their primary sensory neurons. Three types of spider receptors: tactile hairs, trichobothria, and slit sensilla have given important information about the coupling of external

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mechanical stimuli to the neuronal membrane, transduction of mechanical force into receptor current, encoding of afferent action potentials, and efferent modulation of peripheral sensory receptors. Slit sensilla, found only in spiders, have been particularly important because they allow intracellular recording from sensory neurons during mechanical stimulation. Experiments on slit sensilla have shown that their mechanosensitive ion channels are sodium selective, blocked by amiloride, and open more at low pH. This evidence suggests that the channels are members of the same molecular family as degenerins, acid-sensitive ion channels, and epithelial sodium channels. Slit sensilla have also yielded evidence about the location, density, single-channel conductance, and dynamic properties of the mechanosensitive channels. Spider mechanoreceptors are modulated in the periphery by efferent neurons, and possibly by circulating chemicals. Mechanisms of modulation, intracellular signaling, and the role of intracellular calcium are areas of active investigation.

II. INTRODUCTION

Humans inhabit a sensory world dominated by vision, but we also use mechanotransduction to provide the senses of hearing, vestibular sensation, touch, and vibration, as well as chemotransduction for the senses of taste and smell. In contrast to our visual world, a spider's life is dominated by vibration and other mechanical inputs, even in those spider species that have relatively good vision. Waiting for prey to land on a web, hunting along the ground or on a plant, and negotiating a vibratory mating ritual—in all their daily activities the mechanical senses are vitally important. In addition, both humans and spiders detect a variety of internally generated mechanical signals from their musculoskeletal systems and internal organs that allow feedback regulation of movement and many internal physiological processes.

Although mechanotransduction is such an important sense for humans, spiders, and most other animals, its fundamental mechanisms have been difficult to unravel, mainly due to the small size and complex morphology of most mechanoreceptor endings. Arthropods (insects, arachnids, and crustaceans) not only possess large arrays of different mechanoreceptors, but the relatively large sizes of some of their sensory neurons, and the close association of many mechanosensory neurons to the external cuticle have provided several model systems for investigating fundamental mechanisms of mechanotransduction.

The most crucial step in mechanotransduction is a change in cell membrane potential, the receptor potential, produced by the application of a mechanical stimulus to the cell. To study this phenomenon ideally requires a preparation where the electrical event can be directly observed during

accurately controlled mechanical stimulation. This is possible in several spider preparations, and the information thus obtained will be the major subject here.

III. TYPES OF SPIDER MECHANORECEPTORS

The hairiness of spiders is well known, but what are the functions of the thousands of hairs covering a typical spider? Many provide nonsensory functions. These include adhesion to the substrate via surface tension, combing of silk threads from spinnerets, supporting the air bubbles of water spiders, providing attachment sites for spiderlings clinging to a female, and deterring predators by intense skin irritation (reviewed by Foelix, 1996). However, most of the surface hairs are sensory structures. Two major types of sensory hairs are the trichobothria, or filiform hairs, and the shorter tactile hairs (Fig. 1). Each of these hair structures is innervated by multiple neurons, typically four in *Cupiennius salei*, although it is not clear that all these neurons are mechanically sensitive. This situation contrasts somewhat with insects, which typically have only one sensory neuron per hair, but the general structures are otherwise similar.

In addition to hairs that extend beyond the cuticle, embedded in spider cuticle are numerous mechanoreceptors of a type that is not found in other arthropods, the slit sensilla (Figs. 1 and 2). These are widely distributed in the exoskeleton, including the legs, pedipalps, and body (Barth and Libera, 1970; Barth, 1985, 2001; Patil *et al*., 2006). They detect mechanical events in the cuticle, primarily strains imposed by normal movements of the animal and vibrations due to predators, prey, and mates.

Spiders also possess a range of mechanoreceptors deeper within the animal, particularly the joint receptors and muscle receptors, but spiders apparently lack the chordotonal structures that are widespread in insects and crustaceans, serving particularly as vibration and auditory receptors (Seyfarth, 1985; Barth, 2001).

IV. MECHANICAL COUPLING

The first functional stage of any mechanoreceptor is mechanical coupling from the initial stimulus to the mechanically sensitive membrane of the sensory neuron. A large contribution to overall function is suggested, although not yet proven, by the wide range of accessory structures found in mechanoreceptors of both vertebrates and invertebrates, which are assumed

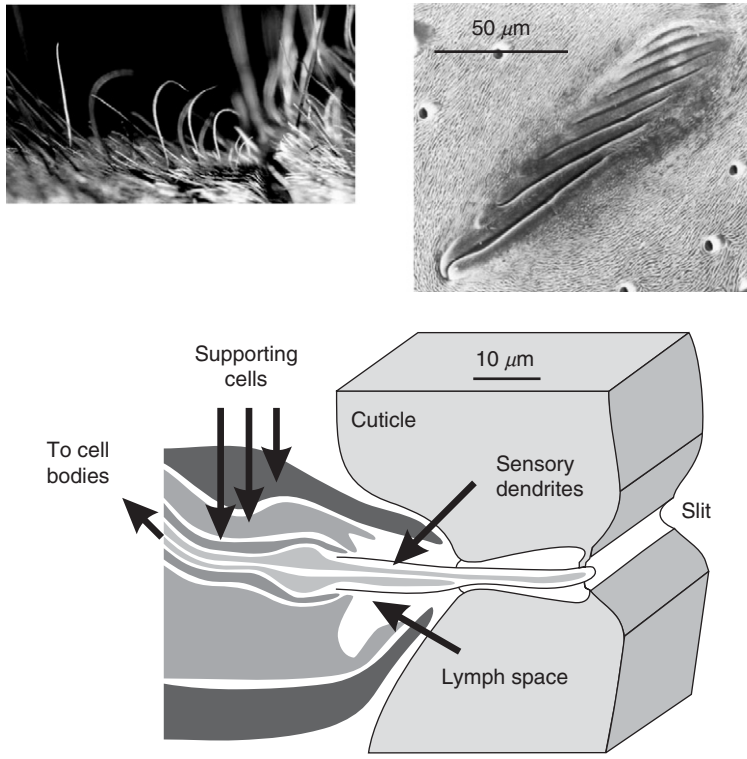

FIGURE 1 Major types of spider cuticular mechanoreceptors. Top left: hair sensilla at the joint between the tibia (left) and the femur of a leg of *Cupiennius salei*. Longer, vertical hairs are trichobothria, typically about 1-mm long, surrounded by numerous shorter tactile hairs. Top right: scanning electron micrograph of a lyriform organ consisting of approximately parallel slit sensilla from a leg of *C. salei*. Dark circles are the sockets of broken hair sensilla. Lower drawing shows the arrangement of sensory neurons and surrounding tissues at a typical slit sensillum. Pairs of sensory dendrites, up to 200-μm long terminate in a ciliary enlargement that leads to a tubular body surrounded by a dense dendritic sheath. Supporting cells produce a lymph space surrounding the terminal dendrites that has a different ionic composition than the normal extracellular fluid. One of the two sensory dendrites proceeds further into the slit structure, but the functional reason for this difference is unknown. On the basis of data from Barth, 2001, 2004; Widmer *et al.* (2005).

to serve a mechanical coupling role. Detailed quantitative understanding of this coupling function is limited by the relatively small sizes of most receptors and the unknown mechanical properties of the materials used to construct the structures surrounding the sensory endings. The dynamic properties of coupling structures are particularly difficult to elucidate because it is hard to

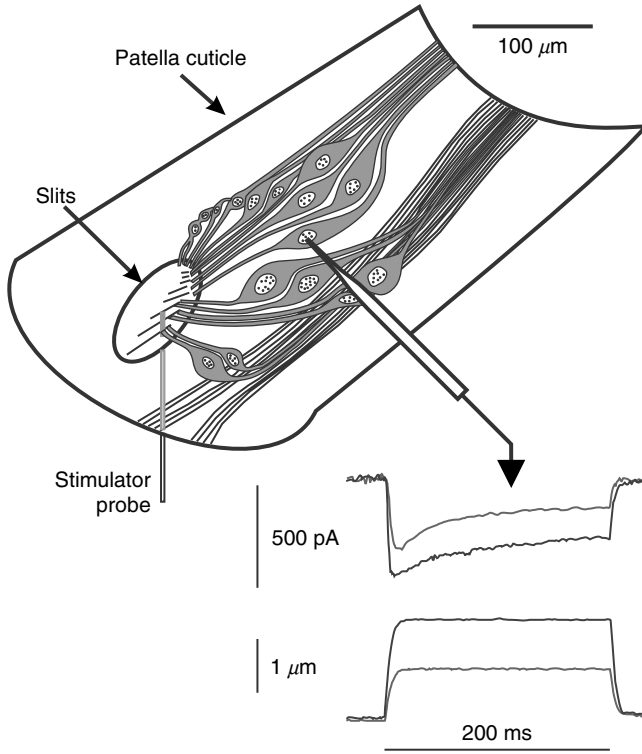

FIGURE 2 Intracellular recording from VS-3 neurons. The approximately tubular patella is split in two along its length and the muscle tissues removed to reveal the mechanosensory neurons lying in the hypodermal membrane. A glass microelectrode is used to penetrate the soma of a neuron while a mechanical probe is raised from below to indent the appropriate slit from the outside. Step indentations under voltage clamp produce inward receptor currents that saturate at a few micrometers. The receptor currents have an adapting component, but most of the current adapts relatively slowly and incompletely. On the basis of data from Höger *et al.* (1997).

measure the individual movements of each component as the sensillum is mechanically stimulated.

Barth (2001, 2004) has discussed in depth the available evidence about mechanical coupling of spider trichobothria, hair sensilla, and slit sensilla. This work also builds on a substantial base of comparable studies in insect cuticular sensilla. Tactile hairs, as the name implies, are thought to serve as touch detectors. They can bend, as well as rotate within their sockets, providing a reduction of movement estimated to be about 1:750, so that relatively

large external movements can be detected without damaging the hair. The longer trichobothria are specialized to detect air movements, and their varying lengths appear to be tuned to the fluid dynamics of air flow over the spider surface, especially considering the boundary layer effect. Estimates of their sensitivity indicate that they can detect movements carrying energy equivalent to a single photon of visible light and that they operate close to the level of baseline thermal noise. They seem designed optimally to detect turbulent air flow produced by rapidly moving prey, such as flying insects, and their varying lengths and diameters provide tuning to different stimulation frequencies.

Slit sensilla are distributed in a wide range of patterns over the spider body, from single, isolated slits to complex arrangements of multiple slits, forming lyriform structures (Fig. 1). It is clear that slit sensilla respond to strain in the exoskeleton, produced by the animal's movements or by vibrations conducted through the substrate. Measurements in models of spider leg cuticle indicate that the slits are optimally positioned to detect strain at the locations where it is maximized by normal loading and that slit orientations are matched to the directions of maximum natural stress. Most compound lyriform organs occur near the leg joints, while individual slits are often found at points of muscle attachment to the cuticle (Barth, 2001). The fine structure of an individual slit allows cuticular stress to apply a levered compression to the tips of the sensory dendrites. This arrangement has some similarities to the campaniform sensilla of insects, which seem to serve a similar stress-detecting function but use singly innervated, circular structures.

The varying lengths of the slits in a lyriform organ (typically 8 to 200 μm long by 1 to 2 μm wide) immediately suggest tuning to different temporal frequencies, as in the eponymous lyre. There is some evidence that this occurs, but the varying lengths may also serve functions such as measuring the relative intensity of the strain by progressive recruitment of different slits as strain increases (Barth, 2001).

V. MECHANOTRANSDUCTION IN SLIT SENSILLA

Spider slit sensilla have provided important experimental preparations for research into mechanotransduction because of the following advantages. (1) Their mechanical structures, while complex, are approximately two-dimensional and relatively amenable to analysis and stimulation. (2) The exposed location of the sensory neurons inside the surface cuticle has allowed the development of preparations in which simultaneous mechanical stimulation and stable intracellular recording, including voltage-clamp

recording can be conducted. (3) The sensory neurons are located within a hypodermal membrane that allows them to be removed from the animal intact. This has been particularly useful for studying their voltage-activated conductances. (4) A complex efferent innervation of the peripheral parts of sensory neurons promises to shed new light into understanding how mechanosensation is modulated.

The remainder of this chapter will focus on major findings about mechanotransduction, sensory encoding, and efferent modulation of these processes that have emerged from research on spider lyriform organs and trichobothria.

A. The Ionic Selectivity of Spider Mechanosensitive Channels

Intracellular recording during mechanical stimulation has been achieved in two spider leg lyriform organs, VS-3 on the patella (Juusola *et al.*, 1994) and HS-10 on the metatarsus (Gingl *et al.*, 2006). In each case, all neurons innervating the slits were found to be mechanosensitive. Voltage-clamp recording from the neuron cell bodies of VS-3 revealed an inward, depolarizing receptor current with both adapting and long-lasting components that saturated with slit indentations of about 3 μm (Fig. 2). Note that the slit indentation used in these experiments does not represent a natural stimulus. Although the major functions of VS-3 remain unclear, normal slit compression is presumably produced by cuticle strains. However, more natural stimulation of HS-10 was achieved by moving the tarsus and this gave very similar results to the VS-3 slit indentation.

The receptor current in VS-3 neurons could not be reversed, even with strong depolarization, and was completely eliminated when external sodium was replaced by choline (Fig. 3). Further tests with the common monovalent and divalent cations showed that, other than sodium, only lithium ions had detectable, but much lower, permeation (Höger *et al.*, 1997). These experiments indicate that spider mechanosensitive channels are highly selective for sodium ions.

Further support for this selectivity comes from measurements of the ionic composition of the solution in the lymph space that surrounds the dendrite tips (Fig. 1). Comparable insect mechanoreceptors have a high concentration of potassium ions in this region, as well as a potential that is positive compared to the normal extracellular space (Thurm and Küppers, 1980; Grünert and Gnatzy, 1987), but in spiders this region not only lacks the high potassium and positive potential but also has a relatively high concentration of sodium ions (Rick *et al.*, 1976).

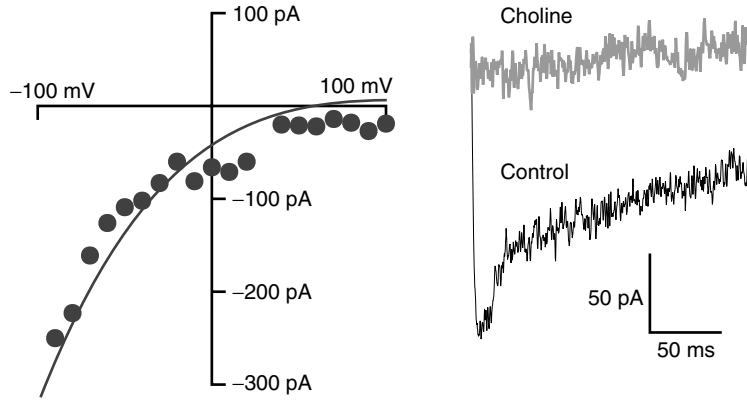

FIGURE 3 Receptor current is carried by sodium ions in VS-3 neurons. Graph shows typical peak receptor currents produced by step slit indentations of 3 μm while the neuronal membrane was held at different potentials. Note the failure to reverse, even at strong positive potentials. Replacement of the sodium ions in spider saline with the large choline cation completely eliminated the receptor current, but it returned when the normal saline solution was restored (control). On the basis of data from Höger *et al.* (1997).

B. The Location of VS-3 Mechanosensitive Channels

The bipolar structure of arthropod cuticular mechanoreceptor neurons (Fig. 2) has led to a long history of attempts to find the location of the mechanosensitive channels, as well as the location of the action potential-initiating region. Although the obvious location for transduction would seem to be at the distal tips of the dendrites because of the close apposition to the initial mechanical stimulus and the specialized electrochemical gradient of the lymph space (Fig.1), there have also been theories that transduction occurs near the ciliary basal body and that action potentials might arise in the axosomatic region (reviewed by French, 1988).

A direct test of the location of mechanotransduction was performed by applying small punctate stimuli to different locations along the dendrites of VS-3 neurons (Höger and Seyfarth, 2001). Only stimuli applied to the distal dendrites, close to the inner surface of the slits, produced electrical activity in the neurons, suggesting a distal location.

The general direction of signal flow in a sensory receptor from distal to proximal implies that transduction should occur either at the site of action potential initiation or possibly distal to it. Gingl and French (2003) used several techniques to locate the site of action potential initiation in VS-3 neurons, including the voltage jump method that measures collisions between

voltage waves started by the receptor potential and an artificially created potential step at the soma. These measurements all indicated that transduction and action potential initiation both start at the distal end of the dendrite. More recent work has directly observed action potentials flowing along the dendrite from the distal tips (Gingl *et al.*, 2004).

Although all these experiments support a distal location for the mechanosensitive channels, they cannot provide a more accurate position than somewhere within about 50 μm from the end of the dendrite. The basal body occurs at the distal end of the dendritic enlargement in VS-3 neurons (Fig. 1), which is close to the lymph space. More accurate localization will probably have to wait for better anatomical evidence such as antibodies to the mechanosensitive channels.

C. Mechanosensitive Channel Conductance, Density, and pH Sensitivity

Single-channel recordings of the mechanosensitive channels have not yet been achieved. Patch clamp recording from VS-3 neurons is complicated by their location within a hypodermal membrane and extensive glial wrappings. The probable location of the channels near the tip of the sensory dendrite adds further difficulty. An alternative approach is to measure the variance, or noise, of the total receptor current to estimate the single-channel conductance and number of channels (Traynelis and Jaramillo, 1998). This approach requires current variance measurements over a range of different current amplitudes, which can be achieved by varying the stimulus used to open the channels being investigated. In VS-3 neurons the receptor current adapts slowly after a step indentation of the slit, and this natural change in current was used to estimate the mechanosensitive channel properties.

For a single group of identical ion channels, the total variance, σ^2, of the current flowing through a membrane is given by:

$$\sigma^2 = \sigma_0^2 + I(V - E)\gamma - I^2/N \tag{1}$$

where σ_0^2 is the background variance due to other sources, I is total membrane current, V is the voltage across the membrane, E is the equilibrium potential of the ions flowing through the channel, γ is the single-channel conductance, and N is the number of channels in the membrane. Given the single-channel conductance and number of channels, the open probability of the channels can be calculated from:

$$P_o = \frac{I}{N(V - E)\gamma} \tag{2}$$

Höger and French (1999a) showed that the mechanosensitive channels were almost completely open at the start of a step indentation, but then closed with several time constants over a period of several minutes (Fig. 4). Their single-channel conductance estimate was about 7 pS and the number of channels per neuron was about 470. Neither of these parameters was sensitive to pH (Höger and French, 2002). However, acid conditions significantly raised the open probability of the channels, and hence the overall receptor current.

From the estimated single-channel conductance and number of channels, total mechanosensitive conductance was calculated to be about 3.5 nS in a single VS-3 neuron. However, independent estimates of total charge flowing during a step indentation gave a significantly higher estimate of about 15 nS (Gingl and French, 2003). A possible cause of this difference lies in the cable properties of the sensory dendrite. The measured length constant of the sensory dendrites is about 200 μm, which is comparable to the physical length of the dendrites (Gingl and French, 2003). Although the noise measurements were made at the neuronal resting potential to minimize the current requirements of the voltage clamp, it is possible that the current flowing through the mechanosensitive channels at the dendrite tip could depolarize the membrane beyond the control of the voltage clamp in the soma. This would reduce the estimated receptor current and its variance.

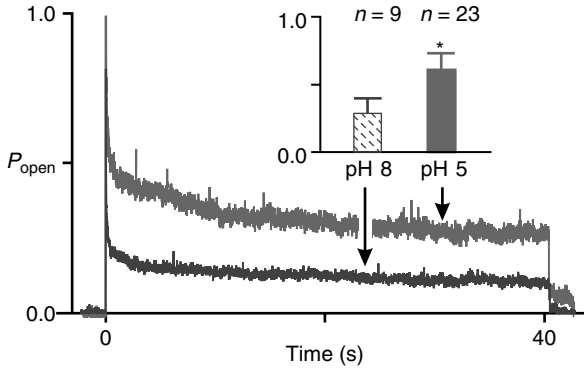

FIGURE 4 Noise analysis and pH sensitivity of VS-3 receptor current. Step indentations of the slits lasting 40 s produced a slowly adapting receptor current. Noise analysis was used to estimate the number of mechanosensitive ion channels, single-channel conductance, and channel open probability (P_{open}) during the step. Traces show P_{open} for a typical neuron at pH 8 (approximately normal conditions) and at pH 5. Inset shows mean values of P_{open} at 36 s after the step under normal and acid conditions. Asterisk indicates $p < 0.05$. On the basis of data from Höger and French (2002).

Therefore, the single-channel conductance of the mechanosensitive channels could be 20 pS or more. This would be in better agreement with estimates from mammalian auditory hair cells based on single-channel recordings, which are as high as 100 pS (Fettiplace *et al*., 1992).

D. Temperature Sensitivity of Mechanosensitive Channels

Mechanotransduction has been found to be more thermally sensitive than would be predicted from simple ion channel conductance in a range of vertebrate and invertebrate sensory receptors (reviewed in Höger and French, 1999b). Most of these measurements were made on the action potential signals from sensory receptors so that the location of temperature sensitivity could not be clearly established. The VS-3 organ provided the first direct measure of temperature sensitivity in the receptor current (Höger and French, 1999b). These data were well-fitted by the Arrhenius rate equation to give a mean activation energy of 23 kcal/mol (97 kJ/mol or $Q_{10} = 3.2$ at 20°C). This is the highest activation energy measured for mechanotransduction, although close to measurements in other systems (Höger and French, 1999b). It confirms the general finding that mechanotransduction involves a significant energy barrier, comparable to the energy required to break a covalent chemical bond. The reason for this relatively high activation energy is not clear but is probably associated with the mechanism that links mechanical stimulus to channel opening. It is much higher than the activation energy required for ionic movement through a water-filled channel or for the production of action potentials by voltage-activated ion channels.

E. Molecular Characterization of Spider Mechanosensitive Channels

Two major groups of ion channel molecules have been associated with sensory mechanotransduction. Members of the transient receptor potential (TRP) family of channels have been implicated in a range of sensory functions of both vertebrates and invertebrates, including phototransduction, thermal transduction, mechanotransduction, pain, and osmosensation (Minke and Cook, 2002; Corey, 2003; Maroto *et al*., 2005; Montell, 2005; Dhaka *et al*., 2006; Kwan *et al*., 2006). TRP channels have been strongly linked to hearing and touch in *Drosophila* (Kim *et al*., 2003; Gong *et al*., 2004) and to touch in *Caenorhabditis elegans* (Goodman and Schwarz, 2003; Li *et al*., 2006). TRP1 channels have been found in vertebrate pain receptors (Kwan *et al*., 2006), as well as mouse, bullfrog, and zebrafish inner ear hair receptors (Corey, 2003), appearing at the same embryonic stage as sound

sensitivity in mice (Lewin and Moshourab, 2004). However, a knockout mouse lacking TRP1 had an impaired response to painful stimuli but its hair cell transduction was not affected (Kwan *et al.*, 2006). None of the evidence yet gives clear proof that these channels are the primary source of the receptor current.

The other channel family associated with mechanotransduction are the degenerin/acid-sensitive/epithelial sodium channels (DEG/ASIC/ENaC), best known for the amiloride-blockable epithelial sodium channels that conduct sodium flux through a wide range of epithelia (Bianchi and Driscoll, 2002). In *C. elegans*, two of the four proteins found only in mechanoreceptor cells are DEG molecules that have been proposed to form the core of the mechanotransduction channel, and the receptor current was carried by sodium ions (Goodman and Schwarz, 2003; Syntichaki and Tavernarakis, 2004). A DEG gene family was also associated with mechanosensitivity in *Drosophila* larvae (Adams *et al.*, 1998). In rodents, several members of the DEG family have been found in dorsal root ganglia and in fine nerve endings surrounding tactile hairs (Price *et al.*, 2000). Knockout animals for one channel, BNC1, showed reductions, but not elimination, of mechanosensation (Price *et al.*, 2000), and none of these molecules have yet been identified in well known skin mechanoreceptors, such as Pacinian corpuscles or Ruffini endings.

Although the molecular evidence favors TRP channels in *Drosophila* mechanosensation (Kim *et al.*, 2003), all the data from spider slit sensilla is more supportive of ASIC channels. The receptor current is highly selective for sodium and blocked by amiloride (Höger *et al.*, 1997). Mechanosensitive channel open probability is strongly increased at low pH (Höger and French, 2002). These are all characteristic properties of ASIC channels. In contrast, TRP channels are quite strongly associated with calcium signaling, and at least some sensory TRP channels are calcium permeable (Montell, 2005), whereas spider mechanosensitive channels are probably not permeable to calcium (Höger *et al.*, 2005).

Two other commonly proposed features of sensory mechanically activated channels are heteromeric construction and connections to extracellular and intracellular structural proteins. Evidence from several preparations indicates that multiple proteins are required to form functioning eukaryotic mechanically activated channels, and this may explain the difficulty of demonstrating mechanosensitivity from proteins expressed in oocytes or other systems (Hamill and McBride, 1996; Emtage *et al.*, 2004; Syntichaki and Tavernarakis, 2004). Mechanical connections to cytoskeletal and extracellular matrix structures have been proposed by several lines of evidence, including the amino acid sequences of proposed channel molecules (Emtage *et al.*, 2004). It has also been argued that lipid membrane alone could not

provide enough force to open a protein channel (Sachs, 1997). Microtubules are often prominent in mechanoreceptor endings, and in some cases have been suggested to form a cytoskeletal anchor (Gillespie and Walker, 2001). Spider slit sensilla, like other arthropod cuticular mechanoreceptors, contain prominent arrangements of microtubules in the sensory dendrites that extend to the distal tips, but mechanotransduction in VS-3 neurons and some insect cuticular mechanoreceptors persists after pharmacological destruction of microtubules (French, 1988; Höger and Seyfarth, 2001).

VI. DYNAMIC PROPERTIES OF MECHANOTRANSDUCTION AND ACTION POTENTIAL ENCODING

Recordings of action potentials from spider tactile hairs and trichobothria show neurons that are normally silent, signaling brief touching or vibration (Barth, 2004). Slit sensilla neurons are also silent in their resting condition and respond preferentially to rapid changes. Each slit is innervated by two neurons that have different dynamic properties. Type A neurons are very rapidly adapting, giving only one or two action potentials at the start of a step indentation, while Type B neurons give a longer burst of action potentials (Fig. 5). This pattern has been observed in both VS-3 and HS-10 lyriform organs (Seyfarth and French, 1994; Gingl *et al.*, 2006) so it probably generalizes to most or all of the slit sensilla.

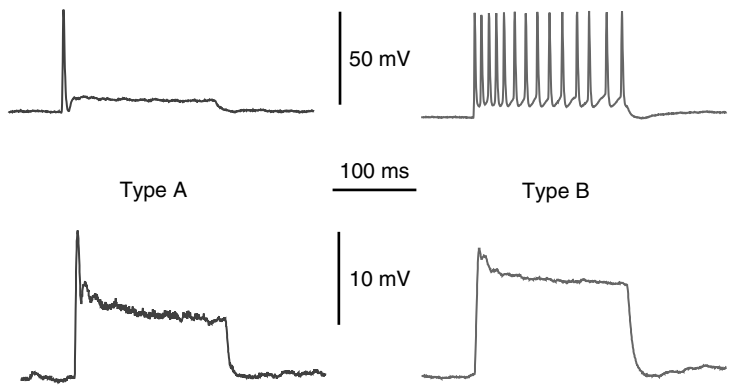

FIGURE 5 Spider slit sensilla are innervated by pairs of functionally different neurons. Intracellular recordings are shown from the two neuron types in a VS-3 preparation receiving step indentations of 150-ms duration. Upper traces show normal action potential responses from Types A (left) and B (right) neurons. Lower traces show receptor potentials produced by similar steps after action potentials were blocked by treatment with tetrodotoxin. On the basis of data from Juusola and French (1998).

Recordings of the receptor current (Fig. 2) or the receptor potential (Fig. 5) do not show such strong adaptation or such a difference between the two neuron types (Juusola and French, 1998). Receptor potential in the Type A neurons does adapt more rapidly than in Type B neurons, but the difference is less dramatic than the firing behavior. This difference in action potential encoding can also be seen with direct electrical stimulation of the neurons, and can be explained by differences in the inactivation properties of the voltage-activated sodium channels that cause the initial phase of the action potentials (Torkkeli and French, 2002).

The time course of the receptor current and potential must be controlled by the combination of mechanical coupling components and mechanosensitive ion channels. However, little is known about the dynamic properties of either. Somatic measurements indicate that the receptor current decays with at least two time constants (Fig. 2), and voltage jump experiments indicated that there are larger, very transient components occurring in the distal dendrites (Gingl and French, 2003). It is possible that these different time constants represent separate filtering by the mechanical components and the mechanosensitive ion channels. The existing evidence is compatible with the most parsimonious model of transduction, that is, that a single type of mechanosensitive channel is present in both Types A and B neurons.

VII. CALCIUM SIGNALING DURING TRANSDUCTION BY SPIDER MECHANORECEPTORS

The membranes of VS-3 neurons contain low-voltage-activated calcium-selective ion channels (Sekizawa *et al.*, 2000). Measurements of intracellular calcium concentration during mechanical stimulation of the slits showed that calcium rises from a resting level of about 400 nM to a maximum level of about 2 μM during rapid action potential firing (Höger *et al.*, 2005). These experiments failed to show any change in calcium concentration without action potentials, even when there was a receptor potential of 10 mV amplitude or more, confirming that the mechanosensitive ion channels are not significantly permeable to calcium. They also failed to show any release of calcium from internal stores. The amount of calcium entering during action potential firing was compatible with the estimated conductance via voltage-activated calcium channels.

These data raise the question of what role the elevation of calcium plays during normal sensory transduction. There are no known calcium-sensitive ion channels in VS-3 neurons, and blockade of calcium entry does not reliably affect action potential firing. Calcium rose by similar amounts throughout the VS-3 neurons, but with different time courses in different regions

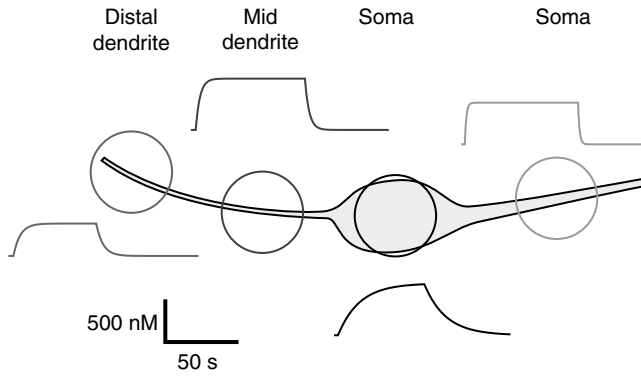

FIGURE 6 Calcium concentration rises significantly in VS-3 neurons when they are firing. Traces show calcium elevations in different regions during stimulation at 10 action potentials per second. Resting calcium concentration was about 400 nM in all regions and the increases in different regions were not significantly different. However, the time course of elevation was significantly slower in the soma, as shown by the traces. On the basis of data from Höger *et al.* (2005).

(Fig. 6), suggesting that calcium channels are distributed throughout the cells. One possible role for calcium would be regulation of the mechanosensitive channels. Calcium ions play major roles in controlling the dynamic properties of auditory hair cells, and at least some of the time constants involved seem to depend on intracellular actions of calcium on mechanosensitive ion channels (Ricci *et al.*, 2005).

VIII. SYNAPTIC MODULATION OF SPIDER MECHANORECEPTORS

An interesting feature of arachnid mechanoreceptors is that even their most peripherally located parts receive extensive and complex efferent innervation (Foelix, 1975; Fabian-Fine *et al.*, 2002), allowing an early modulation of the neuronal responses to mechanical stimuli. Several fine efferent fibers in the legs of *C. salei* extend along the sensory nerves all the way to the tips of the sensory dendrites. They form many types of synaptic contacts with the sensory neurons, the glial cells that enwrap the sensory neurons, and they also synapse with other efferents (Fabian-Fine *et al.*, 2002). The efferent fibers have been shown to contain a variety of transmitters, including γ-aminobutyric acid (GABA), glutamate, acetylcholine (ACh), and octopamine (Fig. 7; Fabian-Fine *et al.*, 2002; Widmer *et al.*, 2005), and the mechanosensory neurons respond to these transmitters (Panek *et al.*, 2002; Panek

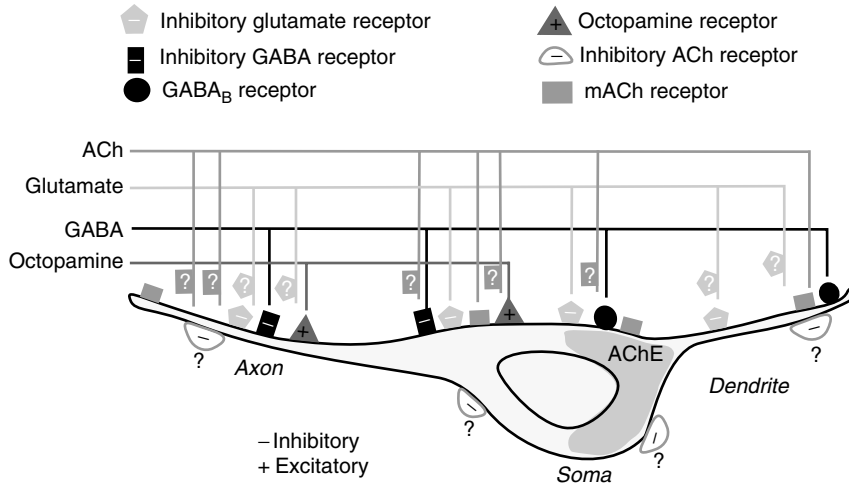

FIGURE 7 Schematic illustration of the arrangement of efferent neurons and transmitter receptors on a Type A spider VS-3 neuron based on immunocytochemical and electrophysiological evidence. The efferent fibers contain GABA, glutamate, octopamine, and ACh. The sensory neurons have inhibitory ionotropic GABA and glutamate receptors and excitatory octopamine receptors. Type A neurons also have inhibitory ionotropic ACh receptors and they express acetylcholine esterase (AChE) activity. In addition, metabotropic $GABA_B$ and muscarinic ACh receptors are found in all VS-3 neurons, but their physiological functions are unknown. Glutamate and mACh receptors are also present in the efferent fibers. On the basis of data from Fabian-Fine *et al.* (2002), Panek *et al.* (2002, 2003, 2005), Gingl *et al.* (2004), Panek and Torkkeli (2005), Widmer *et al.* (2005, 2006).

and Torkkeli, 2005; Widmer *et al.*, 2005, 2006). In addition, antibodies against transmitter receptors labeled specific sites on the sensory neurons (Panek *et al.*, 2003, 2005;Widmer *et al.*, 2005, 2006; Fig. 7).

GABA and glutamate both act on inhibitory ionotropic receptors that are Cl^--gated ion channels. Although both transmitters blocked VS-3 neurons' responses to mechanical stimuli, GABA had a significantly stronger effect than glutamate (Panek and Torkkeli, 2005). However, GABA only inhibited axonal action potentials while the glutamate effect involved both dendritic and axonal action potentials and it also reduced the receptor current amplitude (Gingl *et al.*, 2004; Panek and Torkkeli, 2005). Thus, glutamatergic efferents may control the cellular response to mechanical stimuli at earlier stages than GABAergic efferents. The VS-3 neurons also have metabotropic $GABA_B$ receptors, concentrated on the most distal parts of the cell bodies and on the dendrites (Panek *et al.*, 2003). Agonists of these receptors modulated voltage-activated calcium and potassium currents, allowing a longer lasting modulatory effect.

Application of octopamine, the invertebrate analogue of noradrenaline, enhanced trichobothria neuron sensitivity to mechanical stimuli (Widmer *et al.*, 2005). Immunocytochemical evidence indicated that one efferent fiber containing octopamine innervated each mechanosensory neuron in the spider leg and that octopamine receptors were concentrated at and close to the axon hillock (Widmer *et al.*, 2005). These findings suggest that octopamine acts as a transmitter rather than a neurohormone on spider mechanoreceptors, controlling each sensory neuron individually.

These recent findings only unravel a small part of the complex synaptic mechanisms that control the sensitivity and gain of spider mechanosensory neurons. For example, we still know very little about the cholinergic innervation that involves both muscarinic ACh receptors and ionotropic inhibitory receptors and is distinctly different in the two different types of VS-3 neurons.

IX. CONCLUSIONS

Spider mechanoreceptors have yielded a great deal of information about their mechanosensitive ion channels and their mechanisms of activation and modulation. However, much remains to be discovered. The electrophysiological data from slit sensilla suggest that the channel molecules are related to ASIC channels and they are probably located near the tips of the sensory dendrites. The relatively low numbers of channel molecules per cell are one reason why molecular characterization has so far proved elusive as it has in other mechanoreceptor systems. However, the spider preparations should continue to provide useful models for identifying the molecular basis of mechanosensation and this knowledge can be expected to assist the broader investigation of this crucial sense in animals and humans.

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