Low-Voltage-Activated Calcium Current Does Not Regulate the Firing Behavior in Paired Mechanosensory Neurons With Different Adaptation Properties

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Low-voltage-activated calcium current does not regulate the firing behavior in paired mechanosensory neurons with different adaptation properties. J. Neurophysiol. 83: 746–753, 2000. Low-voltage-activated Ca\(^{2+}\) currents (LVA-\(I_{\text{Ca}}\)) are believed to perform several roles in neurons such as lowering the threshold for action potentials, promoting burst firing and oscillatory behavior, and enhancing synaptic excitation. They also may allow rapid increases in intracellular \(\text{Ca}^{2+}\) concentration. We discovered LVA-\(I_{\text{Ca}}\) in both members of paired mechanoreceptor neurons in a spider, where one neuron adapts rapidly (Type A) and the other slowly (Type B) in response to a step stimulus. To learn if \(I_{\text{Ca}}\) contributed to the difference in adaptation behavior, we studied the kinetics of \(I_{\text{Ca}}\) from isolated somata under single-electrode voltage-clamp and tested its physiological function under current clamp. LVA-\(I_{\text{Ca}}\) was large enough to fire single action potentials when all other voltage-activated currents were blocked, but we found no evidence that it regulated firing behavior. LVA-\(I_{\text{Ca}}\) did not lower the action potential threshold or affect firing frequency. Previous experiments have failed to find \(\text{Ca}^{2+}\)-activated K\(^+\) current (\(I_{\text{K(Ca)}}\)) in the somata of these neurons, so it is also unlikely that LVA-\(I_{\text{Ca}}\) interacts with \(I_{\text{K(Ca)}}\) to produce oscillatory behavior. We conclude that LVA-\(\text{Ca}^{2+}\) channels in the somata, and possible in the dendrites, of these neurons open in response to the depolarization caused by receptor current and by the voltage-activated Na\(^+\) current (\(I_{\text{Na}}\)) that produces action potential(s). However, the role of the increased intracellular \(\text{Ca}^{2+}\) concentration in neuronal function remains enigmatic.

INTRODUCTION

Calcium currents (\(I_{\text{Ca}}\)) that activate in a low voltage range (LVA), and often transiently (T type), have been observed in a variety of neurons from vertebrate and invertebrate central and peripheral nervous systems. These currents activate at potentials low enough to gate the activity of other depolarizing voltage-activated ion channels and therefore their putative functional roles include lowering the threshold for spike generation (reviewed by Huguenard 1996), contribution to the depolarizing envelope that underlies neuronal burst firing (Llinas and Yarom 1981) and promotion of intrinsic oscillatory behavior (Lewis and Hudspeth 1983; McCormick and Huguenard 1992). LVA-\(\text{Ca}^{2+}\) channels have been shown to exist in the dendrites of several central neurons where they can be activated by synaptic potentials and may therefore enhance the synaptic input leading to action potentials (Magee and Johnston 1995). One important putative function of LVA channels is to increase intracellular \(\text{Ca}^{2+}\) concentration, which could lead to various \(\text{Ca}^{2+}\)-dependent secondary responses (McCobb and Beam 1991).

The lyriform slit sense organ VS-3 in the patella of the spider, Cupiennius salei, (nomenclature of Barth and Libera 1970) consists of seven to eight slits each innervated by a pair of bipolar mechanosensory neurons. Because one neuron (Type A) in each pair usually fires only one or two action potentials after a step stimulus whereas the other (Type B) can fire a burst of up to several hundred milliseconds (Seyfarth and French 1994), the VS-3 organ provides an excellent model for examining the role of \(I_{\text{Ca}}\) in regulating excitability. The difference in spiking behavior can be explained only partially by differences in the time courses of the receptor potentials (Juusola and French 1998), and voltage-activated potassium currents do not contribute to spiking behavior (Sekizawa et al. 1999). We have discovered a large transient \(I_{\text{Ca}}\) in the somata of both types of VS-3 neurons, which activates in the range previously described for LVA currents. Here we examine the kinetics and pharmacological sensitivity of this current with the main focus being its possible functional significance in the spiking behavior of these two different types of neurons.

METHODS

Preparation

A laboratory colony of Central American wandering spiders, C. salei, Keys, was kept at room temperature (22 ± 2°C; mean ± SD). Legs from adult spiders of either sex were autotomized, and a piece of patellar cuticle containing slit sense organ VS-3 was dissected free in spider saline [which contained (in mM) 223 NaCl, 6.8 KCl, 8 CaCl\(_2\), 5.1 MgCl\(_2\), 5 sucrose, and 10 HEPES, pH 7.8] (Höger et al. 1997). The hypodermis preparation of VS-3 neurons described in detail by Sekizawa et al. (1999) was used in all experiments and is shown in Fig. 1. Briefly, the neurons were detached from the cuticle but remained embedded in an internal membrane (hypodermis), which then was spread onto a small coverslip that was either uncoated or coated with 5 μg/ml Collagen IV (Sigma, Oakville, ON). The coverslip then was placed on the preparation holder. The axons and dendrites were crushed at ~100 μm from the somata to improve the voltage-clamp conditions.

Recording and stimulation

Current- and voltage-clamp recordings were performed by the discontinuous single-electrode method (Finkel and Redman 1984) with an SEC-10 I amplifier (NPI Electronic, Tamm, Germany). The conditions for successful single-electrode voltage and current clamp...
have been described in detail by Torkkeli and French (1994), and the same methods were used previously in VS-3 neurons to study voltage-activated currents (Sekizawa et al. 1999). Borosilicate microelectrodes (1 mm OD and 0.5 mm ID) were pulled with a horizontal puller (P-2000, Sutter Instrument, Novato, CA). For current-clamp experiments, the electrodes were filled with 3 M KCl, and for voltage-clamp experiments 3 M CsCl was used in the pipette, in both cases the electrode resistances were 45–85 MΩ. Electrodes were coated with petroleum jelly to decrease stray capacitance (Juusola et al. 1997), and they had time constants of 1–3 μs in solution. Switching frequencies of 20–40 kHz and a duty cycle of 1/4 (current passing/voltage recording) were used in the experiments. All voltage-clamp experiments were performed by averaging three recordings with 5-s intervals.

The neurons were observed by an inverted microscope with bright field optics (Olympus, Tokyo, Japan). Neurons were impaled with high-frequency oscillation (“buzzing”), and they were allowed to stabilize for 15 min before the start of experiments. Only neurons with resting potentials of approximately −60 mV, action potential amplitudes of >40 mV and thresholds for firing action potentials of >0.75 nA were used for recording.

All current- and voltage-clamp experiments were controlled by an IBM-compatible computer with custom-written software (ASF Software, Halifax, NS). The computer provided voltage and current stimuli via a 12-bit D/A converter. Membrane potential was low-pass filtered at 33.3 kHz and current at 3.3 kHz by the voltage-clamp amplifier. In current-clamp experiments the input resistances were estimated by measuring voltage-responses to hyperpolarizing current pulses of 0.25 nA. The mean input resistance of Type-A neurons was 145.6 ± 124.6 MΩ (mean ± SD, n = 34) and of Type-B neurons 113.5 ± 59.6 MΩ (n = 32). In voltage-clamp experiments the input resistance was calculated from the linear part of the current-voltage curve at hyperpolarizing potentials, and this value was used for leakage subtraction. The mean values of input resistances after blockage of I_{Na} and I_{k} (see following text) were 259.0 ± 168.0 MΩ (n = 19) for Type-A neurons and 208.8 ± 151.1 MΩ (n = 19) for Type-B neurons. Similar differences in the input resistances between Type-A and Type-B VS-3 neurons have been found before (Sekizawa et al. 1999).

Statistical analyses were performed with the unpaired t-test for significantly different means assuming different variances.

### Chemicals

Pharmacological agents used to block membrane currents were dissolved in spider saline and freshly prepared for each experiment or kept frozen at the same or higher concentrations. To record I_{Ca} voltage-activated I_{Na} were blocked with 1 μM tetrodotoxin (TTX) and I_{k} with a combination of 25 mM tetrathyammonium chloride (TEA, Kodak) and 25 mM 4-aminopyridine (4-AP) in the bath (to adjust osmolarity sucrose was removed and NaCl concentration was reduced to 178 mM) and replacing the electrode filling solution with 3 M CsCl. With sharp microelectrodes, only a small amount of the pipette solution can reach the cell interior, and therefore the blockade of I_{k} was never complete at strong depolarization. Amplitude of outward current at higher depolarizations varied significantly between different neurons, indicating that CsCl dialysis was stronger in some neurons than others. The data used for analysis were collected from neurons that did not have outward currents at potentials of ≤60–80 mV from resting potential even when I_{k} was blocked with Cd^{2+}.

In several experiments, extracellular CaCl$_2$ was replaced with equal (8 mM) concentrations of BaCl$_2$ or SrCl$_2$. We also tested several known blockers of I_{Ca} in current- and voltage-clamp experiments including CdCl$_2$, NiCl$_2$, nifedipine (initial dilution into dimethylsulfoxide as 100 mM) and ω-conotoxin-GVIA (ω-CgTX GVIA). The effect of blocking agents usually took place in 5–10 min. All chemicals were purchased from Sigma unless otherwise stated.

### RESULTS

The main goals of this study were to isolate currents carried by Ca$^{2+}$ in rapidly (Type A) and slowly (Type B) adapting mechanosensory neurons in the spider lyriform slit sense organ VS-3 and to determine their physiological functions. We examined the time- and voltage-dependent properties of I_{Ca} to relate it to the I_{Ca} described in other neurons and to learn if there was any difference in I_{Ca} between Type-A and Type-B neurons that could explain their different firing behaviors (Fig. 1). We also used well-known blockers to test for different components of I_{Ca} and determine their possible roles in the neurons’ dynamic behavior.

#### Voltage and time dependence of activation and inactivation of I_{Ca}

When voltage-activated I_{Na} and I_{k} were blocked (see methods), test potentials of −50–10 mV from a holding potential of

![Fig. 1](http://jn.physiology.org/)

**FIG. 1.** Intracellular recording from neurons in the VS-3 lyriform slit sense organ. VS-3 organs are located in the patella of each leg. Organ was dissected free from the cuticle with the neurons remaining in the hypodermis, which was then attached to a glass coverslip. VS-3 organ has 7–8 pairs of mechanosensory neurons. In each pair, 1 neuron (Type A) adapts rapidly to constant stimuli, whereas the other (Type B) fires bursts of action potentials of several hundred milliseconds. For voltage-clamp experiments, the axons and dendrites were crushed at −100 μm from the soma.

- **A**
  - 1 nA
  - 10 mV
  - -100 mV
  - 100 ms

- **B**
  - Type A
  - Type B
  - V [mV]
  - 0.5
  - 40
  - -80
  - -60
  - -40
  - -20
  - -10
  - 1
  - I/I_{max}

![Fig. 2](http://jn.physiology.org/)

**FIG. 2.** I_{Ca} in VS-3 neurons. A: currents elicited from a holding potential of −100 mV to potentials from −70 to 10 mV. B: peak currents (±SD) from 9 Type-A (●) and 9 Type-B (○) neurons plotted against test potentials. These I-V curves did not differ significantly between the 2 neuron types.
\[ I = I_0 \left( 1 - e^{-V/V_{50}} \right)^m e^{-t/t_1} \]
neurons, the peak amplitude was reached at curve to more negative potentials. In the case of Type-A


When peak and steady-state $I_{\text{Ba}}$ were plotted against membrane potential (Fig. 6


Ba$^2^+$ are slowly inactivating. That the decaying current was not flowing through a different group of Ca$^2^+$ channels than the peak current, but the same current did not reveal a different group of Ca$^2^+$ channels. In both cases, the recovery was complete in $\sim 100$ ms.


Ba$^2^+$ as the current carrier

When extracellular Ca$^2^+$ was replaced by an equimolar (8 mM) concentration of Ba$^2^+$, the inward current inactivated more slowly, revealing a steady component, but its amplitude did not change significantly (Fig. 6). Peak $I_{\text{Ba}}$ was $1.73 \pm 0.4$ nA for Type-A neurons and $1.74 \pm 0.4$ nA for Type-B neurons ($n = 8$ in both cases), but there was a shift in the $I$-$V$ curve to more negative potentials. In the case of Type-A neurons, the peak amplitude was reached at $-41.3 \pm 3.5$ mV ($n = 8$) and in Type-B neurons, it was at $-35.0 \pm 7.6$ mV ($n = 8$). When peak and steady-state $I_{\text{Ba}}$ were plotted against test potentials (Fig. 6B), they had very similar functional ranges, suggesting that Ba$^2^+$ did not reveal a different group of Ca$^2^+$ channels. When the inactivation time constants of $I_{\text{Ca}}$ and $I_{\text{Ba}}$ were plotted against membrane potential (Fig. 6C), the inactivation of $I_{\text{Ca}}$ was significantly different at all recording volt-


Blocking effect of divalent cations Ni$^{2+}$ and Cd$^{2+}$

Divalent cations have been shown previously to function selectively on different types of Ca$^2^+$ channels. Ni$^{2+}$ usually blocks LVA channels more readily, whereas Cd$^{2+}$ is a more potent blocker of different types of high-voltage-activated (HVA) Ca$^2^+$ channels (Tsien et al. 1986). However, both cations do block all types of Ca$^2^+$ channels when applied at sufficiently high concentrations. We did not find clear differences between the effects of Cd$^{2+}$ and Ni$^{2+}$ on the $I_{\text{Ca}}$ of VS-3 neurons. In four experiments with 100 $\mu$M Ni$^{2+}$, $\sim 30\%$ of the inward current remained, and the peak and steady-state currents were reduced similarly, as shown in Fig. 7A where the Ni$^{2+}$ effect on $I_{\text{Ba}}$ is illustrated. With long (>20 min) incubation times, the same concentration of Ni$^{2+}$ blocked all of the inward current. The Ni$^{2+}$ effect was always stronger at more negative potentials (Fig. 7B), and the reduction of inward current was smaller at more depolarizing test voltages. The effect of 50 $\mu$M Cd$^{2+}$ on $I_{\text{Ca}}$ is demonstrated in Fig. 8A and B. In this cell, $I_{\text{Ca}}$ did not have a clear steady-state component, but when Ba$^{2+}$ was used as the current carrying ion in other experiments, there was a steady-state current and we did not see any difference between the effects of Cd$^{2+}$ on peak and steady-state currents. The average amplitude of remaining inward current after 10 min in Cd$^{2+}$ was 40% of control, and as with Ni$^{2+}$, most of the current was blocked by application times of 20 min.

Because LVA-$I_{\text{Ca}}$ has been suggested to be an important factor in the regulation of action potential thresholds and the regulation of action potential firing frequencies (Llinas and Yarom 1981), we tested the effects of Ni$^{2+}$ and Cd$^{2+}$ on the voltage responses at several different holding potentials in both Type-A and Type-B neurons. Figure 7C illustrates the small effect that Ni$^{2+}$ had on one Type-B neuron at a holding potential of $-80$ mV. In this typical experiment, the number of action potentials was not affected by application of 100 $\mu$M Ni$^{2+}$ and the threshold current was not significantly changed either, although the potential at which action potentials were fired was slightly more positive in the Ni$^{2+}$-treated cell than in the control situation. Figure 8C illustrates the effect of 50 $\mu$M

\begin{figure}[h]
\begin{center}
\includegraphics[width=\textwidth]{fig6.png}
\end{center}
\caption{Using Ba$^{2+}$ as a charge carrier produced longer lasting currents. $A$: when Ca$^{2+}$ was replaced with equimolar Ba$^{2+}$ in the bath inactivation of the current became less evident. $B$: peak (○) and the decaying (●) $I_{\text{Ba}}$ activated at similar voltages, indicating that the decaying current was not flowing through a different group of Ca$^{2+}$ channels than the peak current, but the same current was slowly inactivating. $C$: inactivation time constants from 18 neurons where Ca$^{2+}$ was the current carrying ion (●) and 18 neurons with Ba$^{2+}$ as the current carrying ion (○) plotted against voltages. For this diagram, data from Type-A and Type-B neurons were pooled together.}
\end{figure}
Cd²⁺ on a Type-A neuron. In this recording, the neuron fired only one action potential after Cd²⁺ application, compared with two in the control situation. This was the strongest effect we ever saw after Cd²⁺ application. These results indicate that I_Ca does not affect the threshold of VS-3 neurons nor does it change the firing behavior in these neurons, because if this was the case, the threshold for action potentials would increase significantly after I_Ca is blocked. Although we did see small changes in the threshold in some experiments, they were in both directions. Similar changes often occur during long recordings, when the membrane seals better around the recording electrode or when the electrode clogs slightly.

Ni²⁺ and Cd²⁺ also would block I_K(Ca) under the same conditions as were used in the current-clamp experiments, which would be expected to accelerate firing. We did not observe any such effect. This result supports our observations with specific blockers of I_K(Ca),iberotoxin, charbdotoxin, and apamin (results not shown), which have no effect on the dynamic behavior of these neurons. In addition, these neurons do not show the afterhyperpolarization at the end of a spike train that is usually produced by I_K(Ca).

**Ca²⁺ spikes**

VS-3 neurons fire Na⁺ spikes when they are bathed in normal spider saline (Seyfarth and French 1994). When 1 µM TTX is added to the saline, these neurons completely cease firing, but a small rapidly decaying depolarization is seen with high depolarizing voltages (e.g., Juusola and French 1998). When we blocked voltage-activated K⁺ currents with TEA and 4-AP, action potential amplitudes increased significantly and even Type-A neurons started firing several action potentials as shown in Fig. 9. This is a typical effect of blockade of repolarizing outward currents that is observed in many neurons. In all eight experiments here, the threshold for firing action potentials decreased when 4-AP and TEA were added to the saline. When we also added 1 µM TTX in addition to the K⁺-channel blockers, Type-A and Type-B neurons still fired one large and wide action potential, but neither of them generated repetitive responses. The threshold for this action potential was always higher than for the Na⁺ spikes, but still lower than in control recordings. Although Ca²⁺ spikes in the presence of K⁺-channel blockers are not a new finding (reviewed by Hagiwara and Byerly 1981), their uniformity in VS-3 neurons suggests that the difference in normal spiking behavior is not caused by I_Ca.

**Effect of toxins on I_Ca**

To further explore the identity of I_Ca in the VS-3 neurons, we used two specific blockers of Ca²⁺ channels, nifedipine and ω-CgTX GVIA, in several concentrations. These blockers have been shown to act specifically on HVA-Ca²⁺ channels, and ω-CgTX GVIA even more specifically on the N-type channels commonly found in neurons (Swandulla et al. 1991). There are no specific blockers of LVA-I_Ca, but their identification is commonly based on exclusion. ω-CgTX GVIA at 3 and 5 µM concentrations did not have any effect on either current or voltage responses of VS-3 neurons confirming that no part of the I_Ca belonged to the N-type HVA group.

Nifedipine did not have any effect on I_Ca in VS-3 neurons when applied at low (10–50 µM) concentrations, but when 100 µM concentration was used it did block approximately one-half of the I_Ca as shown in Fig. 10, and in most experiments, this effect was at least partially reversible. In several experiments, 100 µM nifedipine also caused a loss of voltage control in the voltage-clamp experiments; this may have occurred because of increased activity. Nifedipine had a clear effect on

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**FIG. 7.** Effects of 100 µM Ni²⁺ on current and voltage responses. A: Ni²⁺ blocked more than half of the inward current. Test pulse in both traces was −30 mV, and the current carrying ion in the control recording was Ba²⁺. Both the peak and steady-state current were affected by Ni²⁺. B: I-V plot from the recording shown in A at different test potentials. Ni²⁺ blocked the current more strongly at negative voltages than when the test potentials were close to 0 or positive. C: effect of Ni²⁺ on the voltage response was insignificant. In this typical recording from a Type-B neuron, the number of action potentials remained similar, and there was no change in the current amplitude needed to produce action potentials. Only a small increase in the level of depolarization was seen in the Ni²⁺ recording. Holding potential in this experiment was −80 mV and action potentials were produced by a 500-pA stimulus.

**FIG. 8.** Effect of 50 µM Cd²⁺ on current and voltage responses. A: Cd²⁺ blocked more than half of the I_Ca. Test pulse in both traces was −20 mV. B: I-V plot from the recording shown in A at different test potentials. Blocking effect of Cd²⁺ was as strong at all potentials. C: typical recording from a Type-A neuron before and after 50 µM Cd²⁺ was added into the bath. This neuron was only able to fire 1 action potential after Cd²⁺ application, but there was no change in the amplitude of current needed for spike production and the depolarization level remained the same. Holding potential in this experiment was −80 mV, and voltage responses were produced with a 750-pA current pulse.
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Voltage response, decreasing the threshold for action potentials and increasing firing frequency as shown in Fig. 10C. This effect was more pronounced when neurons were held at hyperpolarizing voltages. This effect cannot be explained by a blockade of \( \text{Ca}^{2+} \) channels but could have been caused by agonist effects on them.

**DISCUSSION**

LVA-\( I_{\text{Ca}} \) has been suggested to modify neuronal firing patterns by lowering the threshold for action potentials and inducing bursting behavior. The presence of LVA-\( I_{\text{Ca}} \) in the paired spider mechanoreceptor neurons led us to hypothesize that this current was responsible for the difference between the rapidly and slowly adapting members of each pair. However, our results do not support this hypothesis. On the contrary, we found that LVA-\( I_{\text{Ca}} \) in VS-3 neurons does not play any part in shaping the voltage response. What other functions could this prominent current have in these neurons? LVA-\( \text{Ca}^{2+} \) channels often are localized at major sites of synaptic input; dendrites and somata (e.g., Huguenard 1996; Kaneko et al. 1989) where excitatory synaptic input can activate \( I_{\text{Ca}} \). VS-3 neurons are peripheral sensory neurons but they have extensive synaptic connections from the CNS (Fabian-Fine et al. 1999a,b). Some of these synaptic connections are probably excitatory, but LVA-\( \text{Ca}^{2+} \) channels in VS-3 neurons are more likely to open in response to depolarizing receptor potentials and especially during action potentials, increasing intracellular \( \text{Ca}^{2+} \) concentration during firing for other cellular purposes.

**Kinetic properties of \( I_{\text{Ca}} \) in VS-3 neurons**

\( I_{\text{Ca}} \) in VS-3 neurons had rapid kinetics of activation and inactivation, and it also recovered from inactivation faster than in most other neurons. \( I_{\text{Ca}} \) activated at potentials positive to about \(-45 \text{ mV} \) and reached maximum amplitudes at \(-27.8 \) and \(-25.6 \text{ mV} \) in Type-A and Type-B neurons, respectively (Fig. 2). These values are in the range previously outlined for LVA-\( I_{\text{Ca}} \) (Gu and Spitzer 1993; Huguenard 1996; Kaneko et al. 1989; Liman and Corey 1996; Moolenar and Spector 1979; Rennie and Ashmore 1991; Yoshii et al. 1998). The rate of activation of \( I_{\text{Ca}} \) in both types of VS-3 neurons was voltage dependent and varied from 0.3 to 2 ms (Fig. 4A), somewhat faster than values of 2–50 ms previously reported for LVA-\( I_{\text{Ca}} \) (Huguenard 1996; Tsien et al. 1986). Inactivation time constants (Fig. 4B) in Type-A (6–38 ms) and Type-B (71–114 ms) neurons were similar to values obtained from other sensory and central neurons, where they vary between 10 and 400 ms (Bossu and Feltz 1986; Huguenard 1996; Liman and Corey 1996). When \( \text{Ca}^{2+} \) was replaced with Ba\(^{2+} \), the inactivation became significantly slower (Fig. 6), indicating its dependence on intracellular \( \text{Ca}^{2+} \) concentration. Although \( \text{Ca}^{2+} \)-dependent inactivation is a property of HVA-\( \text{Ca}^{2+} \) channels, it is not usually associated with LVA channels (Fox et al. 1987). However, it has been observed in some invertebrate neurons, e.g., transient \( I_{\text{Ca}} \) in cockroach dorsal unpaired median (DUM) neurons has \( \text{Ca}^{2+} \)-dependent inactivation (Wicher and Penzlin 1997).

Recovery from inactivation is the most variable feature of LVA-\( I_{\text{Ca}} \), with its time constant varying from \( \sim 100 \) ms to several seconds (Bossu and Feltz 1986; Carbone and Swandulla 1989; Huguenard 1996; Kaneko et al. 1989). In VS-3 neurons, the recovery was very rapid with a full recovery in \( \sim 100 \) ms and time constants of 20–30 ms, and there were no statistically significant differences between the two neuron types.

Steady-state inactivation of LVA-\( I_{\text{Ca}} \) in VS-3 neurons had slope factors close to 4 mV (Fig. 3), in the low range of values reported for central and sensory neurons (Gu and Spitzer 1993; Huguenard 1996; Liman and Corey 1996; Moolenar and Spector 1979; Tsien et al. 1986; Wicher and Penzlin 1997). However, \( V_{50} \) values of steady-state inactivation in Type-A and

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**FIG. 9.** Comparison of action potentials with and without the Na\(^+\) component. Control recording (—) was performed in normal spider saline (see METHODS). When repolarizing \( I_{\text{K}} \) were blocked with 25 mM tetraethylammonium chloride (TEA) and 25 mM 4-aminopyridine (4-AP) (· · ·), this Type-A neuron fired action potentials that had significantly higher amplitudes and slightly longer durations than the control. When 100 \( \mu \text{M} \) tetrodotoxin (TTX) was added (· · · ·), the same neuron was still able to fire 1 action potential that had an even larger amplitude than before TTX. Holding potential in all recordings was \(-70 \text{ mV} \), and action potentials were produced by a current stimulus of 250 \( \text{pA} \).

**FIG. 10.** Effect of 100 \( \mu \text{M} \) nifedipine on current and voltage responses. A: nifedipine blocked about half the inward current when applied in high concentrations. Control recording was done with \( \text{Ba}^{2+} \) as the current carrying ion and both current traces were produced with test potentials of \(-30 \text{ mV} \). B: \( I-V \) plot from the recording shown in A at different test potentials. Nifedipine effect was similar at all test potentials. C: application of nifedipine reduced the threshold and increased the number of action potentials. This effect was more pronounced when neurons were held at hyperpolarizing potentials during current stimulation. For this recording, a Type-B neuron was held at \(-80 \text{ mV} \) and stimulated with 500-pA current pulse. In the control situation, this cell did not produce action potentials when smaller stimuli were used, but the threshold decreased to 250 \( \text{pA} \) after Nifedipine.
Type-B VS-3 neurons were $-35.1$ and $-30.3$ mV, respectively, significantly more positive than the $V_{th}$ values of $-50$ to $-95$ mV reported for other neurons (Huguenard et al. 1996; Kaneko et al. 1989; Liman and Corey 1996; Moolenaar and Spector 1979; Yoshi et al. 1998), with the lower values being more common in sensory neurons.

The rapid kinetics of $I_{Ca}$ allows a fast neural response, a property that may be needed when VS-3 neurons respond to natural stimuli, such as substrate vibrations. Differences in the rate of inactivation and recovery from inactivation could be explained if Type-A neurons could respond more readily to high-frequency stimuli, whereas Type-B neurons would provide more time for Ca$^{2+}$ to enter at the cost of reduced high-frequency sensitivity.

**Ca$^{2+}$-channel blockers**

We found no evidence for Ca$^{2+}$-channel types other than LVA on the somata of VS-3 neurons. Peak and steady-state currents were equally sensitive to blockers, and their activation ranges were similar. $\omega$-conotoxin GVIA had no effect on VS-3 neuron $I_{Ca}$. At high concentrations, Ni$^{2+}$ and Cd$^{2+}$ block all types of Ca$^{2+}$ channels as well as other voltage-gated channels. At lower concentrations, Cd$^{2+}$ is less effective on LVA than HVA channels (Mogul and Fox 1991; Ozawa et al. 1989) and Ni$^{2+}$ acts more strongly on LVA channels (Hagiwara et al. 1989). In VS-3 neurons, the effect of Ni$^{2+}$ was slightly stronger on LVA-$I_{Ca}$ than that of Cd$^{2+}$, but both blocked the current completely when application times were long. Current-clamp experiments with Cd$^{2+}$ and Ni$^{2+}$ indicated that $I_{Ca}$ does not decrease the threshold of VS-3 neurons (Figs. 7C and 8C). Ni$^{2+}$ and Cd$^{2+}$ also block K$_{Ca}$ channels, so it is impossible to draw firm conclusions about their physiological effects when they are tested on neurons that have $I_{K(Ca)}$ (e.g., Kawai et al. 1996). Because the somata of VS-3 neurons do not have $I_{K(Ca)}$, we could reliably show that although Ni$^{2+}$ and Cd$^{2+}$ both block $I_{Ca}$, the current does not control the spiking of these neurons.

Nifedipine like other dihydropyridines (DHP) is a blocker of HVA-$I_{Ca}$ (Tsien et al. 1986). However, at high concentrations DHPs can have nonspecific effects on Ca$^{2+}$ and also on Na$^{+}$ and K$^{+}$ channels (reviewed by Carbone and Swandulla 1989). DHP block is more efficient at depolarizing potentials, with hyperpolarizing potentials tending to remove the block. This might explain the excitatory effect of nifedipine on the voltage response of VS-3 neurons at hyperpolarizing potentials. DHP-sensitive LVA-Ca$^{2+}$ channels are found in e.g., rat hypothalamic neurons (Akaike et al. 1989).

**Ca$^{2+}$ spikes**

Ca$^{2+}$ spikes are common in invertebrate muscle cells (Fatt and Katz 1953) and in developing neurons (Hagiwara and Byerly 1981). When quaternary ammonium ions are present, many vertebrate neurons also are able to fire Ca$^{2+}$ spikes (Fain et al. 1977; Horn 1978; Koketsu et al. 1959). Although both VS-3 neurons could fire one Ca$^{2+}$ spike when TEA and 4-AP were present (Fig. 9), this spike had higher threshold than Na$^{+}$ spikes under similar conditions and repetitive firing did not occur. In most other neurons, Ca$^{2+}$ spikes also have higher thresholds than Na$^{+}$ spikes (e.g., Linas and Yarom 1981).

**Functional significance of LVA-$I_{Ca}$ in VS-3 neurons**

$I_{Ca}$ activated and peaked at slightly more negative potentials in Type-A than Type-B neurons, but this difference was not statistically significant. In Type-A neurons, the depolarization level at which the cells fired action potentials was about $-31$ mV and in Type-B neurons $-39$ mV. A similar, statistically significant, difference was found before when threshold depolarizations of large numbers of VS-3 neurons were studied under similar conditions (Sekizawa et al. 1999). These findings argue against the hypothesis of $I_{Ca}$ lowering the threshold for action potentials because in that case, $I_{Ca}$ in Type-B neurons would activate at lower voltages.

The consequence of lowering the threshold for Na$^{+}$ spikes in vertebrate central neurons is assumed to be promotion of burst firing (Huguenard et al. 1996). However, LVA-$I_{Ca}$ is not only found in bursting neurons. In fact, its existence is not well correlated with firing behavior. For example, LVA-$I_{Ca}$ is present in mouse vomeronasal chemosensory neurons that fire tonically (Liman and Corey 1996) and in mouse retinal bipolar neurons (Kaneko et al. 1989) that do not fire action potentials. However, it is not found in cultured rat olfactory neurons (Trombley and Westbrook 1991) that only fire one action potential in response to a steady stimulus or amphibian olfactory neurons that fire phasically (Liman and Corey 1996). Here, we found LVA-$I_{Ca}$ in two phasic sensory neurons.

In vertebrate saccular hair cells, $I_{Ca}$ and $I_{K(Ca)}$ interact to enhance the frequency tuning to mechanical stimuli. However, in contrast to VS-3 neurons, $I_{Ca}$ in hair cells flows through HVA channels, and these neurons do not fire action potentials (Hudspeth 1986). The rapid activation and inactivation kinetics of $I_{Ca}$ in VS-3 neurons seem likely to allow fast cellular responses, which may be significant when there are high stimulus frequencies. Using action potential waveforms as stimuli, McCobb and Beam (1991) demonstrated that LVA-$I_{Ca}$ activated early during the rising phase of the action potential and peaked after the spike peaks. This behavior makes it possible to rapidly increase intracellular Ca$^{2+}$ concentration during the brief spike.

In contrast to earlier suggestions that LVA-$I_{Ca}$ is an important regulator of neuronal firing properties, we found no evidence that LVA-$I_{Ca}$ is involved in controlling the firing patterns of Type-A and Type-B mechanosensory neurons in the spider VS-3 organ. This leaves two important questions that we will address in the future: what is the function of this prominent current in these sensory neuron pairs and what is the origin of their different firing patterns? These questions and their answers seem certain to have wider implications for other neurons.

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Calcium Current in Spider Mechanoreceptor Neurons


