Regional calcium changes in spider mechanoreceptors during sensory transduction

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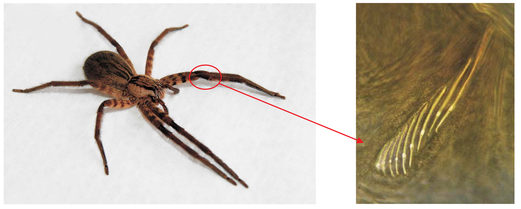
Introduction

Little is known about the roles of Ca^{2+} in transduction and action potential encoding of most mechanoreceptors. An isolated, but otherwise intact, preparation of the lyriform organ VS-3 of the spider, *Cupiennius salei*, allows intracellular recording from sensory neurons during mechanical stimulation and calcium imaging. Ca^{2+} enters these cells through low voltage activated Ca^{2+} channels, leading to a significant increase in free intracellular calcium $[Ca^{2+}]_i$ when the cells are firing action potentials. In previous studies $[Ca^{2+}]_i$ was estimated to be ~70 nM in resting VS-3 neurons, rising to ~300 nM during rapid action potential firing. Blockade of action potentials by TTX showed that Ca^{2+} does not enter through mechanotransduction channels.

Here, we examined the time course and amplitude of $[Ca^{2+}]_i$ changes in response to single action potentials in three peripheral regions (soma, axon, dendrite) of VS-3 neurons. We show a correlation between Ca^{2+} increase and low voltage activated Ca^{2+} channel distribution, and suggest that $[Ca^{2+}]_i$ modulates mechanotransduction.

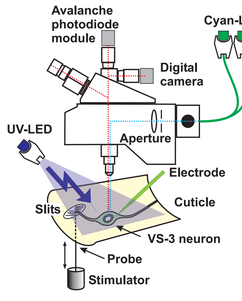


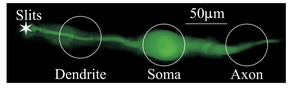
Female tropical wandering spider (*Cupiennius salei*) and VS-3 organ on the patella. Each of the nine cuticular slits is innervated by a pair of mechanosensory neurons.

Fluorescence measurements

The Ca^{2+} sensitive dye Oregon Green BAPTA-1 (OG488) was injected iontophoretically into VS-3 neurons through microelectrodes. Dye loaded cells were visualized by epifluorescence optics and a x40 water immersion objective, using high intensity Luxeon V Star Cyan LEDs as the excitation light source. Illumination of the preparation was restricted to a 50 µm circle by apertures in the light path. OG 488 fluorescence was detected and quantified by an avalanche photodiode module or imaged by a digital camera. To minimize bleaching, cells were only illuminated during the brief times required to make fluorescence measurements or obtain images.

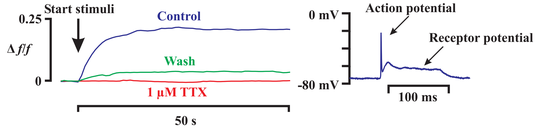
In some experiments caged Ca^{2+} (NP-EGTA) was co-injected with OG488, and controlled release of Ca^{2+} was achieved by UV light flashes (100 ms duration, 1 Hz)



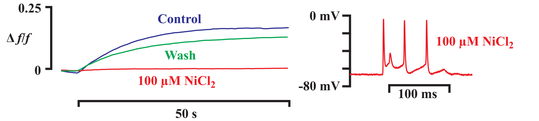


Composite image of a VS-3 neuron filled with OG488. Circles indicate areas on dendrite, soma, and axon where action potential induced changes in $[Ca^{2+}]_i$ were measured. The slit region, the area of mechanotransduction, is marked by an asterisk.

Action potentials cause calcium entry through voltage-activated calcium channels

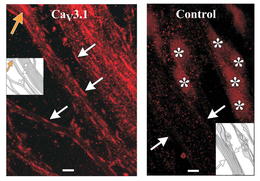


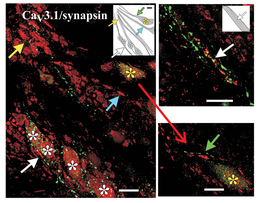
Mechanical step stimuli produced depolarizing receptor potentials and rapid action potentials in VS−3 neurons. In a neuron receiving regular, suprathreshold mechanical stimuli at a rate of 12 Hz, OG 488 fluorescence showed increasing $[Ca^{2+}]_i$. No increase in $[Ca^{2+}]_i$ was observed in response to mechanical stimulation when 1 µM TTX was used to block action potentials. Action potential generation and increase in $[Ca^{2+}]_i$ partially recovered on washing in normal saline.



Previous studies showed that $NiCl_2$ blocks low-voltage-activated calcium channels in VS-3 neurons. 100 µM $NiCl_2$ suppressed the increase in $[Ca^{2+}]_i$. It partially recovered after washing the neuron in normal saline. Application of $NiCl_2$ did not suppress action potentials.

LVA calcium channels in VS-neurons

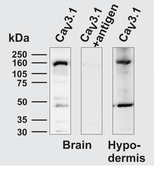




Left: Confocal two dimensional reconstruction of the main leg nerve, labeled with an antibody against the $Ca_V3.1(\alpha_{1G})$ isotype of low voltage-activated (LVA) Ca^{2+} channels. Axons (*white arrows*) and somata *(orange arrow*) of VS−3 neurons show intense striped labeling.

Right: When the $Ca_V3.1$ antibody was pre-incubated with control antigen no labeling was detected in VS-3 neurons (*asterisks somata, arrows axons*). Scale bars: 20 µm.

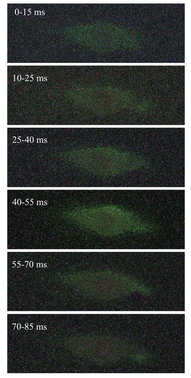
Double labeling of VS-3 neurons with anti-$Ca_V3.1$ (CY-3) and anti-synapsin (Alexafluor 488). $Ca_V3.1$ labeled stripes were abundant in dendrites (*yellow and blue arrows*) and somata (*asterisks*). Synapsin labeled efferent neurons surround all parts of the VS-3 neurons. In some areas $Ca_V3.1$ labeling was closely associated with anti-synapsin labeling. Scale bars: 20 µm. Synapsin antibody was generously provided by Dr. Erich Buchner, Würzburg.



Western blots of spider brain and leg hypodermis homogenates with anti-$Ca_V3.1$ antibody showed clear bands at ~160 kDa and ~45 kDa. These bands were not present when the antibody was pre-incubated with control antigen.

$[Ca^{2+}]_i$ change induced by single action potentials

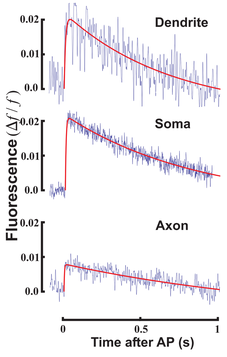
$[Ca^{2+}]_i$ rose in all regions of VS-3 neurons following mechanically-induced action potentials.



Photographic imaging of the time course of $[Ca^{2+}]_i$ change induced by a single action potential.

This series of time shifted images shows that the increase of $[Ca^{2+}]_i$ peaked ~50 ms after an action potential elicited by mechanical stimulation.

Time shifted images were obtained with a conventional digital camera in long time exposure mode (exposure time 5 min). During exposure the neuron was subjected to a series of 23 mechanical step stimuli (15 ms duration), each eliciting a single action potential. By varying the time delay between mechanical stimulus and a 15 ms pulse of excitation light, images at different time windows were captured. Background fluorescence was subtracted by creating control images using exactly the same exposure protocol, but without mechanical stimulation.



Quantitative imaging of the time course of $[Ca^{2+}]_i$ change induced by a single action potential.

The time course of $[Ca^{2+}]_i$ change following action potentials in three different regions of a VS-3 neuron. Fluorescence traces ($\Delta f/f$) were obtained by averaging the fluorescence changes in responses to individual action potentials (n= 30-100 trials). Each averaged data set was fitted (red solid line) with the following function:

$$\Delta f/f = a(e^{-t/c} - e^{-t/b})$$

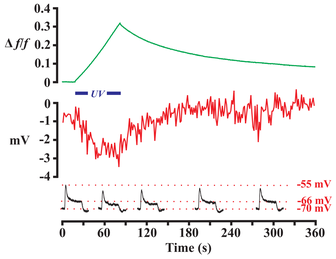
where t = time, a = amplitude constant, b = time constant of fluorescence increase following a stimulus, c = time constant of fluorescence decrease after stimulus induced peak.

Mean values of fitted parameters indicate similar time courses of $[Ca^{2+}]_i$ change in the dendrite, soma, and axon. The only significant difference ($P < 0.05$, unpaired t-test) was found in the peak amplitudes measured in dendrite and soma, probably due to the different volume to surface area ratios.

Parameter (mean ± SD)	Dendrite	Soma	Axon
b (ms)	6.4 ± 5.9	5.4 ± 2.6	4.6 ± 2.01
c (s)	1.04 ± 0.54	1.30 ± 1.30	1.24 ± 0.49
Peak amplitude	0.015 ± 0.001*	0.009 ± 0.006*	0.011 ± 0.009
Time to peak (ms)	19.2 ± 14	22.7 ± 11.0	16.4 ± 5.7
Number of cells	14	52	10

Modulation of receptor potential by $[Ca^{2+}]_i$

In a neuron co-loaded with OG 488 / caged Ca^{2+} (NP-EGTA) and treated with 1 µM TTX to prevent action potential generation and Ca^{2+} entry through voltage activated Ca^{2+}-channels, UV light flashes (100 ms, 1 Hz) were used to release caged Ca^{2+} and to increase $[Ca^{2+}]_i$. Mechanically induced receptor potentials (80 ms duration) were recorded during the UV induced increase of $[Ca^{2+}]_i$ and the subsequent recovery to baseline.



The upper trace shows the increase in $[Ca^{2+}]_i$ by release from caged Ca^{2+}. The lower trace shows changes in the receptor potential amplitude in close correlation with the increase of $[Ca^{2+}]_i$. The lowest traces show actual recordings of the receptor potential before, during, and after the release of caged Ca^{2+}.

There was a decrease in the receptor current amplitude, both peak and plateau, as $[Ca^{2+}]_i$ increased.

Conclusions

Peak values of $\Delta f/f$, determined by quantitative imaging, correspond to an increase of ~1 nM $[Ca^{2+}]$ per action potential in the soma, compatible with rises seen previously in response to rapid action potential firing, and with measured calcium currents.

Elevations of $[Ca^{2+}]_i$ in the dendrite, soma, and axon following an action potential follow a time course that can not be explained by simple diffusion from a localized entry site.

Instead, extracellular Ca^{2+} probably enters the dendritic, somatic, and axonal areas through voltage activated Ca^{2+} channels, which open when action potentials travel rapidly from the distal dendrite throughout the entire neuron.

A widespread distribution of $Ca_V3.1$ isotype of LVA Ca^{2+} channels in VS-3 neurons suggests that these channels allow Ca^{2+} entry during action potential firing.

Close proximity of $Ca_V3.1$ and synapsin labeling suggests that these channels may also play some role in the complex peripheral efferent modulation previously described in VS-3 neurons.

Mechanotransduction and action potential initiation occur in the sensory dendrites of VS-3 neurons. Location of voltage activated Ca^{2+} channels in the dendrites, the rapid rise of Ca^{2+} concentration during excitation, and the reduction of receptor potential by elevated intracellular $[Ca^{2+}]$, all indicate that changes in intracellular Ca^{2+} regulate receptor sensitivity by one or more negative feedback mechanisms.

See this poster again at: http://asf-pht.medicine.dal.ca