Calcium concentration changes in spider mechanoreceptors during sensory transduction

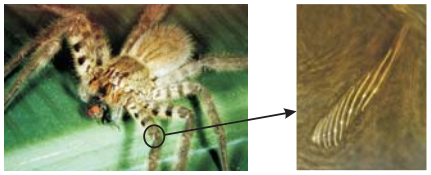
Ulli Höger, Päivi H. Torkkeli and Andrew S. French

Department of Physiology and Biophysics, Dalhousie University, Halifax, NS, Canada

CIHR IRSC NSERC CRSNG

Introduction

Little is known about the roles of Ca^{2+} in transduction and action potential encoding by most mechanoreceptors. Intracellular recordings can be performed from sensory neurons in the lyriform organ VS-3 of the spider, *Cupiennius salei*, during mechanical stimulation. These neurons have a low voltage activated Ca^{2+} current that can produce action potentials after K^+ and Na^+ currents are blocked, but whose function is unknown. Here, we examined changes in $[Ca^{2+}]$ during mechanical stimulation.



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

Calcium estimation

Neurons received repetitive mechanical pulses, causing one action potential per stimulus. $[Ca^{2+}]$ was assumed to follow a single compartment model (Helmchen and Tank 2000):

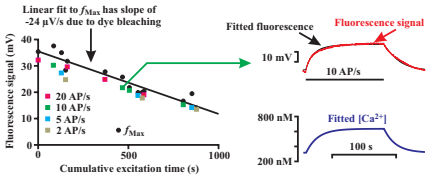
Before action potential firing: $[Ca^{2+}] = [Ca^{2+}]_{Min}$ $t<t_0$

During action potential firing: $[Ca^{2+}] = [Ca^{2+}]_{Min} + A(1 - e^{-(t-t_0)/\tau})$ $t_0<t<t_1$

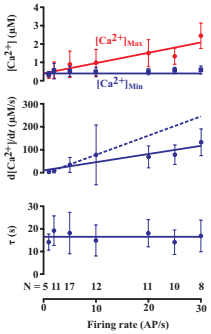
After action potential firing: $[Ca^{2+}] = [Ca^{2+}]_{Min} + Ae^{-(t-t_1)/\tau}$ $t_1<t$

Fluorescence was fitted to this model using: $f = ([Ca^{2+}]f_{Max} + K_D f_{Min})/([Ca^{2+}] + K_D)$

We used K_D = 206 nM (Sabatini et al. 2002). $R_f = f_{Max}/f_{Min}$ was measured *in vivo* (next box).



Resting and stimulated calcium levels



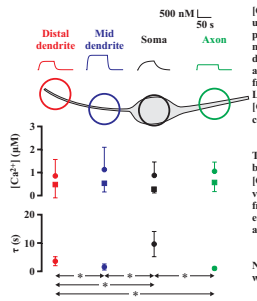
Linear fit of plateau calcium level, $[Ca^{2+}]_{Max}$, increased from 403 nM with no simulation to 2,095 nM at 30 AP/s (red circles and line). Resting calcium levels, $[Ca^{2+}]_{Min}$, are plotted with a constant line at the zero stimulation intercept of 403 nM (blue circles and line).

Rate of rise of $[Ca^{2+}]$ at the start of action potential firing. Slope of the linear fit to all the data was 3.55 nM/AP. Fitting only the initial data from 0-10 AP/s gave a slope of 8.38 nM/AP (dashed line). For an elliptical cell of 100 µm x 50 µm x 50 µm, this would give a charge flow of ~2 x 10^{-13} C/AP.

Time constant of rise and fall of $[Ca^{2+}]$ at the start and end of action potential firing. A horizontal line is drawn through the mean value of τ = 16.47 s.

All data shown as mean ± standard deviation. Total of 74 experiments on 11 different preparations.

Regional distribution of calcium levels



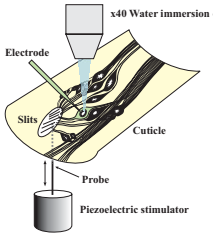
$[Ca^{2+}]$ at four regions of VS-3 neurons using mechanical stimulation to produce 10 AP/s. Circles show measurement apertures at: distal dendrite, mid-dendrite, soma and axon. Traces show $[Ca^{2+}]$ estimates from each region for one neuron. Lower graphs show mean ± SD $[Ca^{2+}]_{Min}$ and $[Ca^{2+}]_{Max}$, and time constant, τ, for the four regions.

There were no significant differences between the values of $[Ca^{2+}]_{Min}$ or $[Ca^{2+}]_{Max}$ at the four positions, but values of τ were significantly different from each other ($p<0.05$, asterisks), except between the mid-dendrite and axon regions.

Numbers of estimates at each position were: 10, 10, 11 and 8, respectively.

Fluorescence measurements

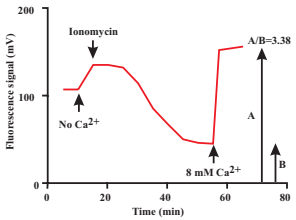
We measured $[Ca^{2+}]$ in VS-3 neurons by iontophoretic injection of Oregon Green BAPTA-1dye through microelectrodes. Cells were visualized by epifluorescence optics and a x40 water immersion objective. The excitation light source was a Luxeon V Star Cyan LED, and fluorescence was detected by an avalanche photodiode. To minimize bleaching, the cells were only illuminated during fluorescent measurements following mechanical stimulation.



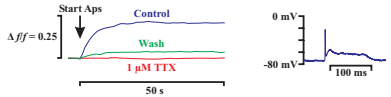
VS-3 neuron after dye injection. Dendrite and axon have been outlined for clarity.
An aperture in the light path restricted the measurements to a circle of 50 µm that could be moved to different cell regions.

Calibration of R_f

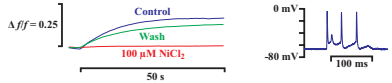
The preparation was initially superfused by spider saline (8 mM Ca^{2+}). Following dye injection the intracellular electrode was withdrawn (Time = 0) and fluorescence measured at 5 minute intervals. Changing the solution to zero calcium and 10 mM EGTA (first arrow) caused an increase in fluorescence, but addition of 100 µM ionomycin (second arrow) caused a large reduction. Returning to normal 8 mM Ca^{2+} (third arrow) now caused a large and rapid increase in fluorescence. $R_f = f_{Max}/f_{Min}$ was estimated to be 3.38 from the highest and lowest fluorescence values. The mean value was R_f=2.98±0.81 (n=4).



Action potentials cause calcium entry through voltage-activated calcium channels



Fluorescence increased in a VS-3 neuron receiving regular, suprathreshold mechanical stimuli at a rate of 1.25 AP/s (above left). 1 µM TTX suppressed the increase in $[Ca^{2+}]$. It partially recovered on washing in normal saline. Mechanical stimuli produce long depolarizing receptor potentials and rapid action potentials in VS-3 neurons (above right). TTX eliminated action potentials but not receptor potentials.



$NiCl_2$ blocks low-voltage-activated calcium channels in VS-3 neurons. 100 µM $NiCl_2$ suppressed the increase in $[Ca^{2+}]$ (above left). It partially recovered after washing the neuron in normal saline. $NiCl_2$ did not suppress action potentials (above right).

Conclusions

- Resting calcium concentration in VS-3 neurons was ~400 nM and increased to a maximum level of ~2 µM at high firing rates.
- Calcium enters through voltage-activated calcium channels opened by action potentials. Nickel blocks the channels and prevents the calcium rise.
- Calcium entry is abolished by TTX. No evidence was seen for calcium entry through mechanically-activated channels. The receptor potential is too small to open voltage-activated calcium channels.
- Thapsigargin (50 µM) had no effect on calcium levels (data not shown), indicating that calcium release from internal stores is not significant.
- Maximum calcium flow would carry charge of ~2 x 10^{-13} C/AP. Voltage clamp measurements of calcium currents predict ~6 x 10^{-13} C/AP.
- Calcium rise and fall was slower in the soma and the distal sensory dendrite. The soma has a relatively large ratio of volume-to-surface area.
- Resting and stimulated calcium levels were similar in all cell regions, suggesting that calcium channels and extrusion mechanisms are distributed throughout the cell.

References

Helmchen, F. & Tank, D.W. (2000) A single-compartment model of calcium dynamics in nerve terminals and dendrites. In: Yuste, R., Lanni, F. & Konnerth, A., (eds), *Imaging neurons, a laboratory manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp. 33.1-33.11.

Sabatini, B.L., Oertner, T.G. & Svoboda, K. (2002) The life cycle of Ca(2+) ions in dendritic spines. *Neuron* **33**, 439-452.