Ratiometric measurements of calcium concentration during sensory transduction in spider mechanoreceptors

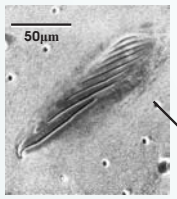
Ulli Höger, Päivi H. Torkkeli and Andrew S. French

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

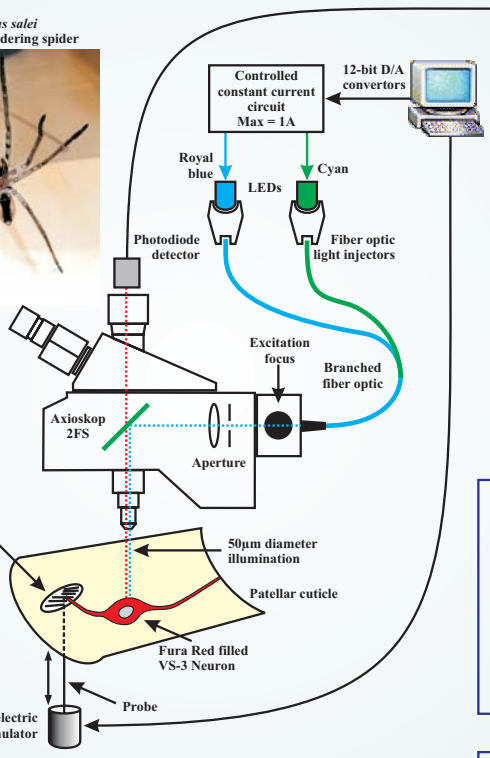
Introduction

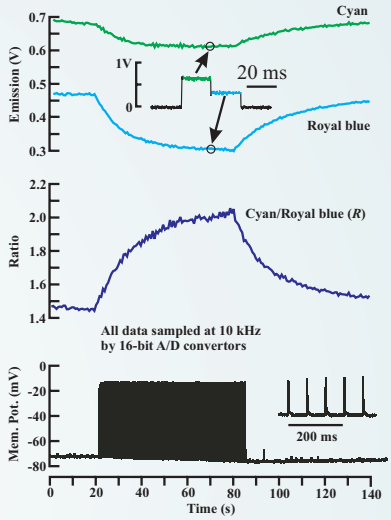
In a previous study using Oregon Green BAPTA-1 fluorescence we found that intracellular calcium concentration in spider mechanoreceptor neurons rose during mechanical stimulation. We also showed that calcium elevation required the opening of voltage-dependent calcium channels by action potentials, and could not be produced by the receptor potential alone. While evidence for mechanisms of calcium elevation in these neurons was clear, estimates of actual calcium concentration depended on properties of the fluorescent dye in the neuron cytoplasm that could not be verified. We have now developed a method for ratiometric estimation of calcium concentration in these neurons using Fura Red dye, excitation by two light emitting diodes (LEDs) of different wavelengths, and an avalanche photodiode fluorescence detector. The method is simple and economical to implement, allows concentration changes to be measured in the millisecond time range, and could easily be applied to a wide range of preparations.

Cupiennius salei
The tropical wandering spider



VS-3 Slit-sense lyriform organ





Methods

Spiders (*Cupiennius salei*) were taken from a laboratory breeding stock. Autotomized legs of adult females were dissected under spider saline (223 mM NaCl, 6.8 mM KCl, 8 mM $CaCl_2$, 5.1 mM $MgCl_2$, 10 mM HEPES, pH 7.8). Preparations carrying the intact lyriform organ VS-3 were mounted in a Plexiglass dish, allowing access to the neurons with a recording electrode from above and mechanical stimulation from below. The preparation was continuously superfused with spider saline, keeping the fluid level in the recording dish constant. Complete exchange of the fluid in the dish (~1 ml) took less than 30 seconds.

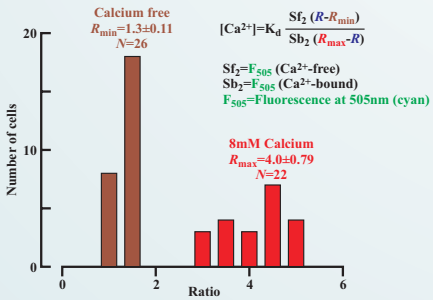
Electrical recordings were made with the switching single electrode technique in current clamp mode (duty cycle 1:8 stimulating to recording, switching frequency 20 kHz; SEC-10L amplifier, NPI Electronic). Borosilicate glass microelectrodes were positioned with a micromanipulator (PCS 5000, Burleigh Instruments).

Technical details

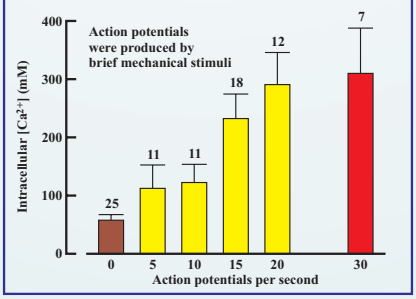
- LEDs - Luxeon V Star LXHL-LR5C Royal Blue (445-460 nm, peak 455 nm) and LXHL-LE5C Cyan (490-520 nm, peak 505 nm)
- Fiber optic light injectors - Luxeon FRAEN
- Dual branch fiber optic light guide - Dolan-Jenner #700007050012, randomized fibers
- Calcium indicator - Fura Red, peak emission at 670 nm
- Detector - Hamamatsu C5460-01 avalanche photodiode with thermal feedback, range 400 -1000 nm
- Epifluorescence optics - Omega Optical XF2009 dichroic beam splitter and XF3012 emission filter
- Mechanical stimulator - Physik-Instrumente P-841.10 translator, PZT Servo controller

Ratiometric calibration

Cells were pre-treated with 50 µM calcium ionophore



[Ca²⁺] vs firing rate



Summary

- Ratiometric estimation of $[Ca^{2+}]$ was performed in spider VS-3 neurons during mechanotransduction using Fura Red injection through sharp microelectrodes
- LED excitation allows brief illumination, which reduces bleaching and phototoxicity
- LEDs allow ratiometric $[Ca^{2+}]$ estimation with sub-millisecond time resolution
- LEDs combined with avalanche photodiode detection gave a signal range of ~4:1 (zero to saturated $[Ca^{2+}]$) with high signal-to-noise ratio
- Resting $[Ca^{2+}]$ in VS-3 neurons was ~ 60 nM
- $[Ca^{2+}]$ in VS-3 neurons rose to a maximum of ~300 nM at 30 action potentials per second
- The role of $[Ca^{2+}]$ elevation in the function of these mechanoreceptors is still unknown

Thanks to Shannon Meisner for technical assistance, and to:

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