# Calcium-based Negative Feedback On Sensory Transduction In Spider Mechanoreceptors

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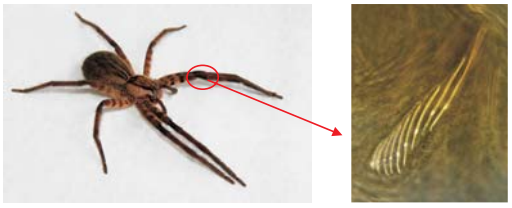
## 1. Introduction

Calcium ions pass through mechanotransduction channels in vertebrate hair cells and cause adaptation by reducing channel open probability (Beurg et al., Biophys. J. 94: 2639, 2008). However, little is known about the roles of calcium in transduction or adaptation of other 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. Calcium ions do not pass through the transduction channels, but enter the cells through low voltage activated $Ca^{2+}$ channels when action potentials fire. Intracellular calcium concentration, $[Ca^{2+}]_i$ is estimated to increase from ~70 nM to ~300 nM during rapid action potential firing.

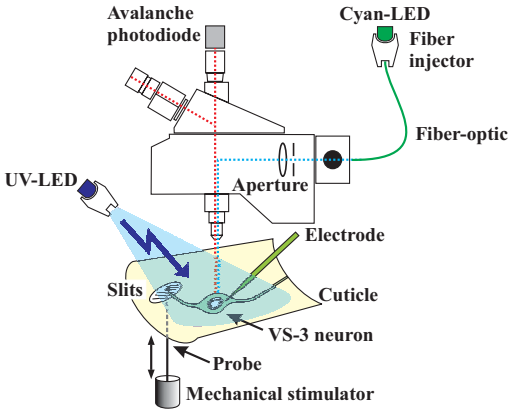
Here, we increased $[Ca^{2+}]_i$ by ultraviolet light release of caged calcium while simultaneously measuring calcium concentration with Oregon Green dye fluorescence and stimulating mechanically.

Increasing $[Ca^{2+}]_i$ by estimated concentrations of ~10 nM reduced receptor current, reduced receptor potential, slowed action potential firing and reduced action potential adaptation with time.



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.

## 2. Measuring and modulating internal calcium

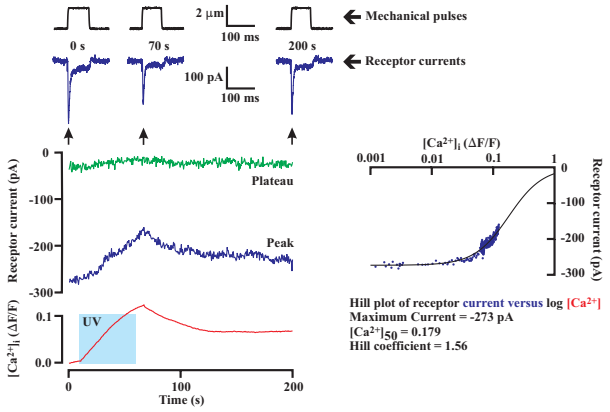



The $Ca^{2+}$ sensitive dye Oregon Green BAPTA-1 (OG488) was injected into VS-3 neurons iontophoretically through microelectrodes. Dye loaded cells were visualized by epifluorescence optics and a 40x water immersion objective, using high intensity Luxeon V Star Cyan LEDs as the excitation light source. 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. To minimize bleaching, cells were only illuminated during the brief times required to make fluorescence measurements.

Caged $Ca^{2+}$ (NP-EGTA) was co-injected with OG488, and controlled release of $Ca^{2+}$ was achieved by UV light flashes (100 ms pulses at 1 Hz for 50 s) from a pair of Nichia NCCU033 UV LEDs (peak output at 365 nm) driven by a constant-current power supply at 1 A.

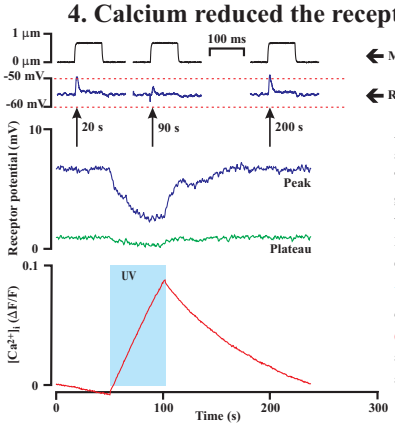
Neurons were stimulated mechanically by 80 ms steps (typically 1-3 µM amplitude) from a piezoelectric stimulator (P-841.10, Physik Instrumente).

## 3. Calcium reduced the receptor current




A neuron co-loaded with Oregon Green and caged $Ca^{2+}$ was treated with 1 µM TTX and voltage-clamped at the resting potential (-75 mV). Mechanical pulses (2 Hz, 80 ms duration) produced a receptor current. UV light flashes (100 ms, 1 Hz) released caged $Ca^{2+}$, which increased $[Ca^{2+}]_i$ (lower trace). Peak (middle trace) and plateau (upper trace) receptor current were reduced by increased $[Ca^{2+}]_i$.

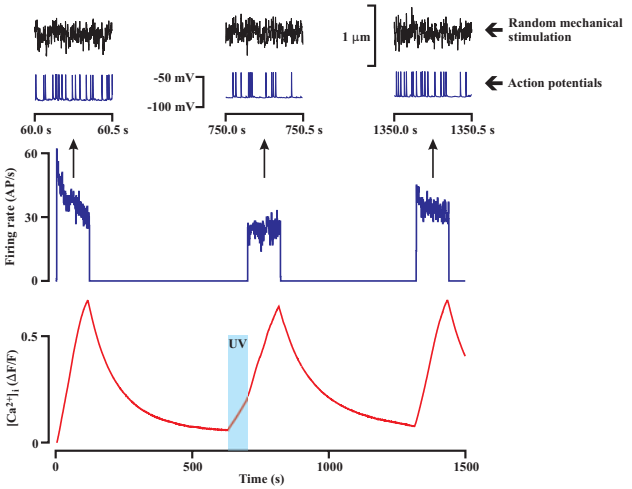
## 4. Calcium reduced the receptor potential




A neuron co-loaded with Oregon Green and caged $Ca^{2+}$ was treated with 1 µM TTX to prevent action potential generation and $Ca^{2+}$ entry through voltage activated $Ca^{2+}$-channels. Mechanical pulses (2 Hz, 80 ms duration) produced receptor potentials.

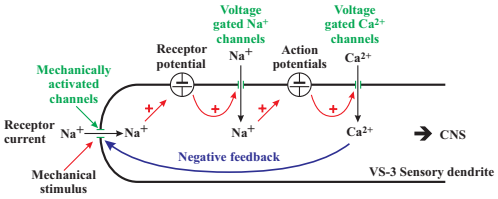
UV light flashes (100 ms, 1 Hz) released caged $Ca^{2+}$, causing increased $[Ca^{2+}]_i$ (lower trace) and reduced both the peak and plateau receptor potential amplitudes.

## 5. Calcium reduced action potential firing




Random mechanical stimulation (uppermost inset traces) caused rapid action potential firing that adapted strongly (middle trace and inset traces) and raised $[Ca^{2+}]_i$ (lower trace). UV release of caged calcium before mechanical stimulation raised $[Ca^{2+}]_i$, reduced the response to random stimulation and removed the adaptation. The strong, adapting response to random noise recovered partially with time, even though $[Ca^{2+}]_i$ was still partly elevated.

## 6. Summary and conclusions




- Calcium acts as a negative feedback regulator of mechanosensation by reducing the current through mechanically activated sensory channels
- Mechanically activated channels are very sensitive to $[Ca^{2+}]_i$ - The changes here were about 5-10 nM, based on earlier ratiometric estimates of $[Ca^{2+}]_i \approx 70$ nm
- Negative feedback reduces the receptor potential and slows action potential firing
- Action potentials increase $[Ca^{2+}]_i$ enough to reduce receptor current - this probably explains the slow adaptation seen after the start of a random mechanical stimulus

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