Sequential Mechanical Stimulation Dynamically Regulates Calcium Response in DRG Neurons

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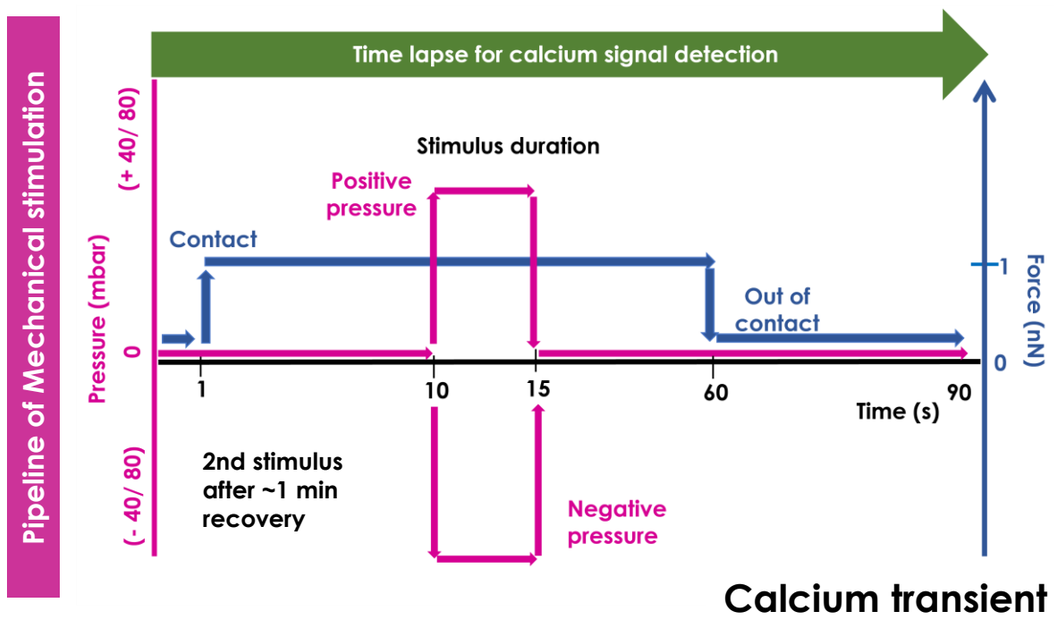
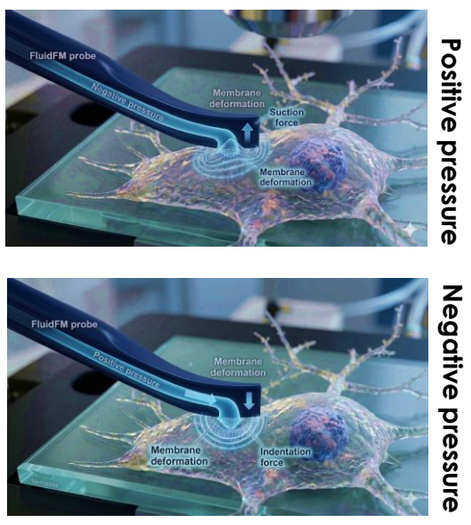
Mechanically-activated ion channels are central mediators of sensory transduction in dorsal root ganglion (DRG) neurons [1,2]. To better understand the force-dependent activation, adaptation, and dynamic regulation of sensory mechanotransduction, we investigated the activation of **mechanosensitive calcium channels** in **DRG** during repetitive mechanical stimulation, with sequential stimuli of opposite polarity.

DRG neurons subjected to sequential opposite-polarity pressure stimulation

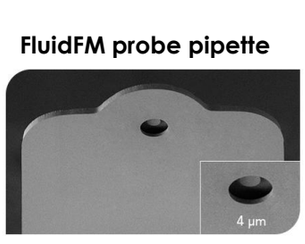
Sequential **negative-to-positive** and **positive-to-negative pressure** stimulation was applied to DRG using Fluidic force microscopy (FluidFM). Alternating pressure was delivered to the plasma membrane of individual neuronal soma through a microchanneled cantilever connected to a pressure-control system. A constant contact force of 1 nN was maintained by AFM feedback control, while mechanosensitive channel activity was monitored by Ca^{2+} imaging.

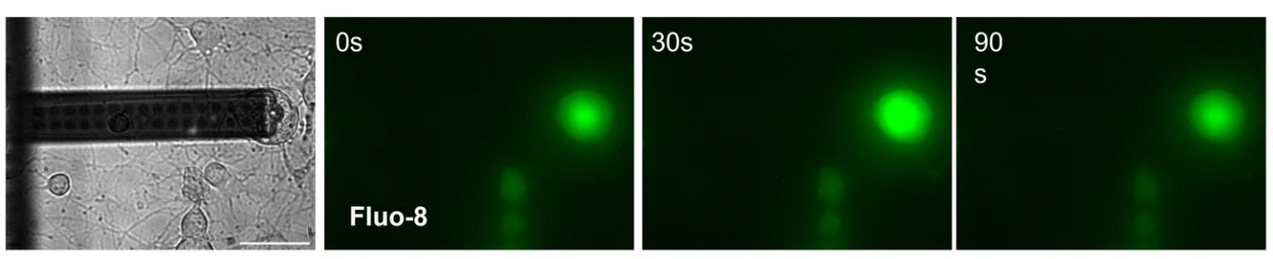


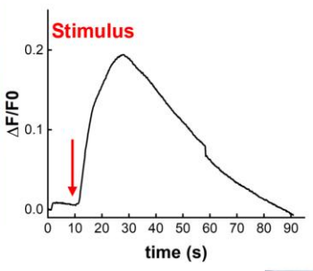
Expected membrane deformation



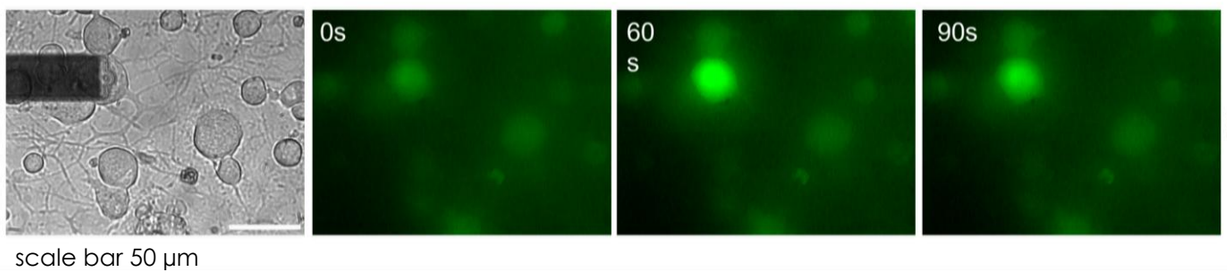
Calcium transient of DRG at stimulation of soma



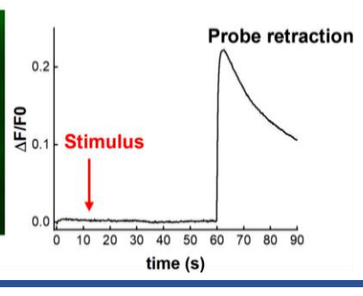




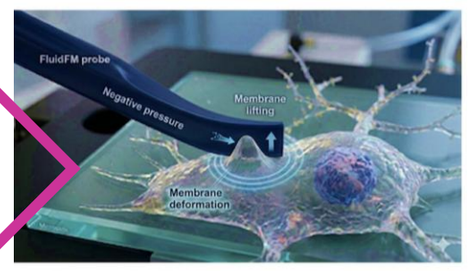
Calcium transient of DRG at retraction of FluidFM probe



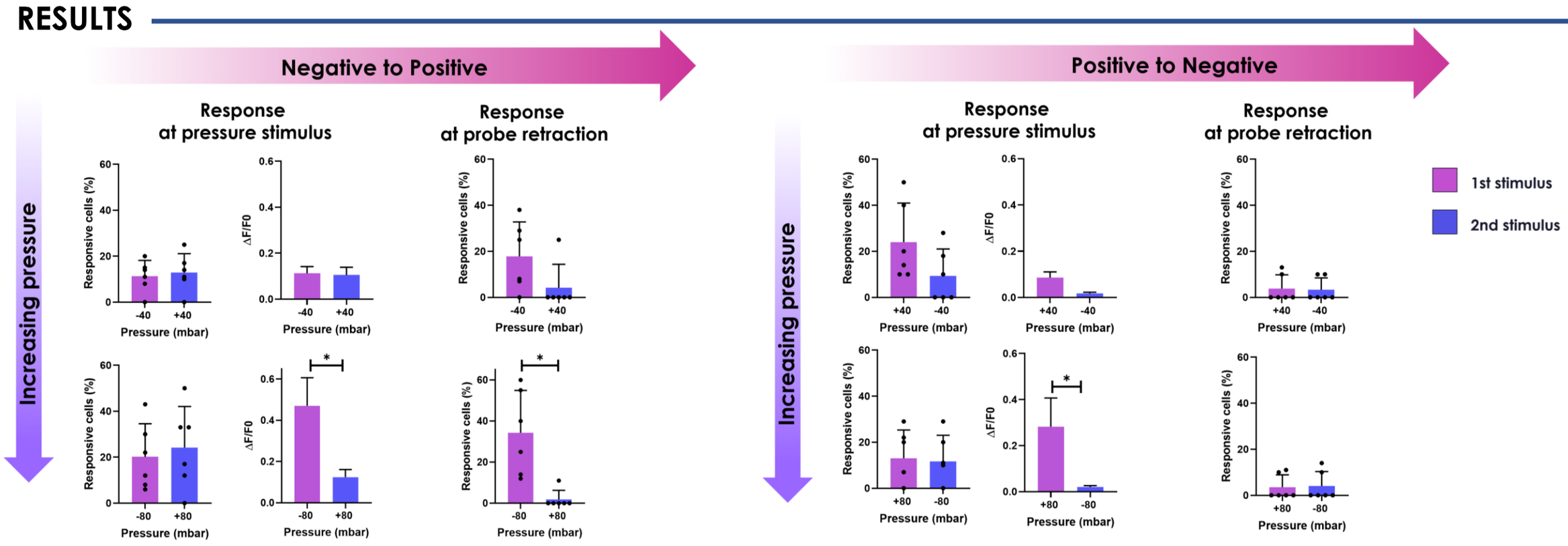
scale bar 50 µm



Possible scenario



RESULTS



Mechanically evoked calcium responses in DRG depend on stimulation sequence and resulting membrane deformation: DRG neurons showing transient calcium signal at -/+ 40mbar (n=61) and -/+80mbar (n=58); at +/- 40mbar (n=56) and +/- 80mbar (n=59) together with the fluorescence intensity response (each dot is percentage of an experiment); DRG exhibiting response at probe retraction (each dot is percentage of an experiment). All data derives from six independent experiments, n is the total number of cells stimulated. The data are shown as mean ± sem. Statistical analysis Wilcoxon test; data were considered statistically significant for $p < 0.05$

DISCUSSION AND NEW INSIGHTS

Using FluidAFM with bidirectional pressure and Ca^{2+} imaging, we observed that mainly at 80mbar, the first pressure pulse elicited the largest $\Delta F/F_0$ regardless of polarity, while, after ~1 min recovery, responses to a second pulse remained attenuated. This direction-dependent adaptation suggests a persistent local alteration of the membrane environment induced by the stimulus. Consistently, a probe retraction evoked a significant response only when suction was applied first, indicating incomplete relaxation of the membrane after pressure release.

These findings suggest that local membrane mechanical perturbations modulate the activation of mechanosensitive channels on a timescale of minutes, considerably slower than the rapid inactivation (ms/s) observed for PIEZO2. This slowly relaxing mechanical memory of the membrane may further regulate mechanosensitive channel activity in DRG neurons.

References

[1] E. Murthy Deciphering mechanically activated ion channels at the single-channel level in dorsal root ganglion neurons Gen Physiol (2023) 155 (6): e202213099.
[2]Murthy, S., Dubin, A. Patapoutian, A. Piezos thrive under pressure: mechanically activated ion channels in health and disease. Nat Rev Mol Cell Biol (2017) 18, 771–783

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