Nociceptive Terminals *In-Vivo* Generate Ongoing Activity

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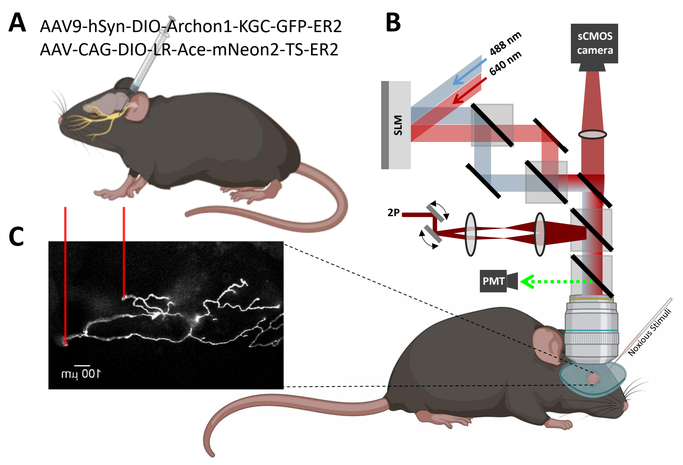
Abstract

Nociceptive terminals detect and transmit information regarding noxious stimuli, thus initiating pain sensation. Since the terminals are inaccessible to conventional electrophysiological recordings, little is known about their physiological properties. Here, we expressed the genetically encoded voltage indicators (GEVI) Archon1 and Ace-mNeon in primary pain-sensitive (nociceptive) neurons innervating the cornea. Using a high-speed microscope and holographic illumination, we monitored voltage dynamics in corneal nociceptive terminals in anesthetized mice in vivo. Strikingly, we discovered that nociceptive terminals demonstrate ongoing action potentials even without the application of noxious stimuli. This activity could be generated by the terminals or propagate antidromically from the Trigeminal Ganglion (TG) somata. To test the somatic contribution directly, we targeted the TG using TG cannulation and local sodium channel blocker application. Application of lidocaine directly to had no effect on ongoing terminal activity, indicating that it does not originate from the cell body, but from the terminals. Next, we will use the Optopatch technique to stimulate one terminal and record activity in connected terminals or axons, testing signal integration at terminals. These experiments aim to uncover mechanisms of nociceptive terminal activity, revealing the interplay between somatic and terminal origins in peripheral pain encoding.

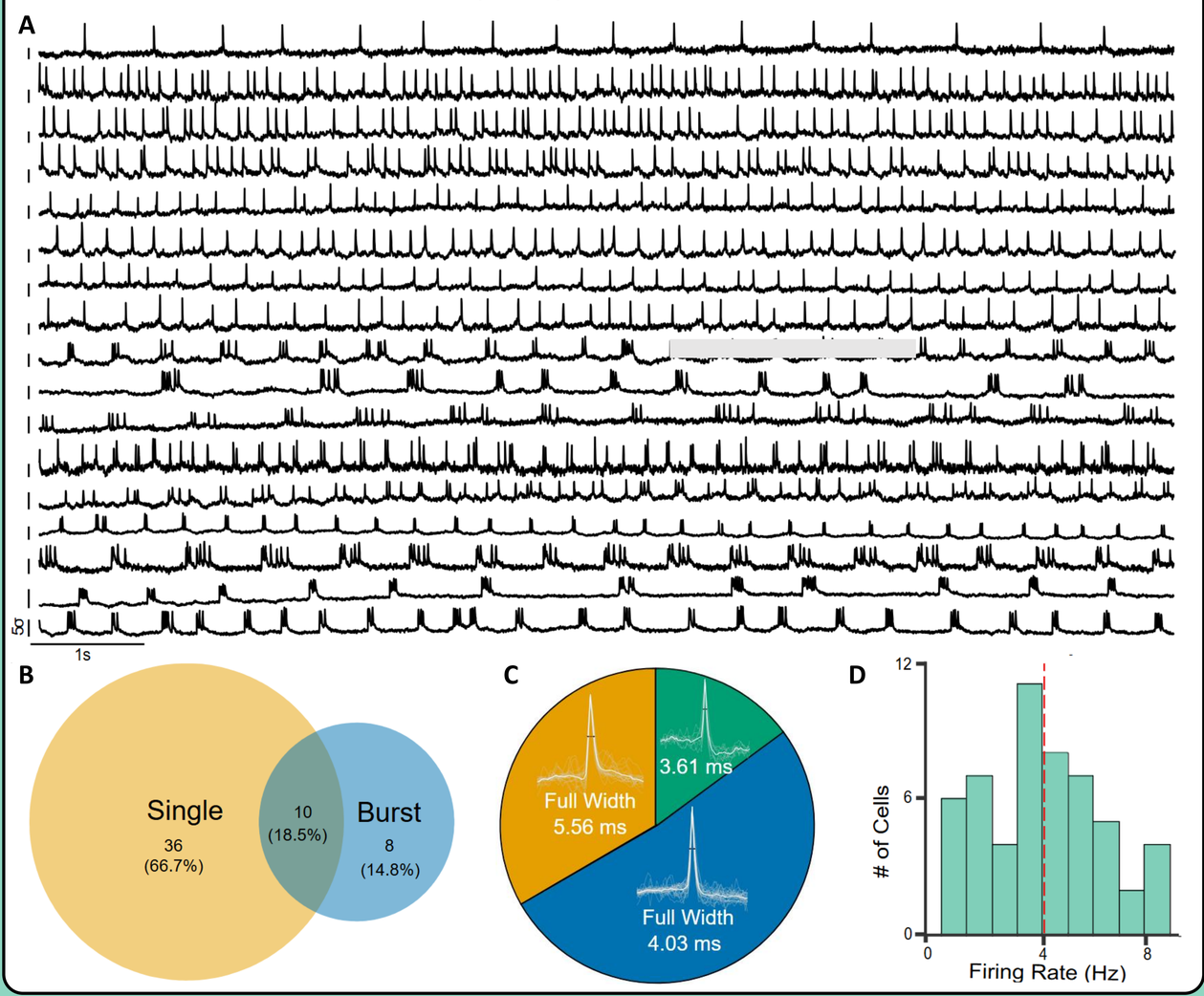
High-speed Voltage Imaging From Nociceptive Terminals *In-vivo*

A. Up: Archon1-GFP and Ace-mNeon constructs (up) stereotaclly injected to Trigeminal Ganglion (TG) (down). **Down:** Illustration of virus injection to the trigeminal ganglion. **B.** Experiment setup illustration. Eye-cup is fixed on mouse's eye; objective lens is above. noxious stimulus pipette is ready above terminal with tip in the SES solution. **C.** Epifluorescence image with wide-field green illumination of Archon1-GFP corneal nociceptive terminals *in-vivo* with Spatial Light Modulation (SLM).



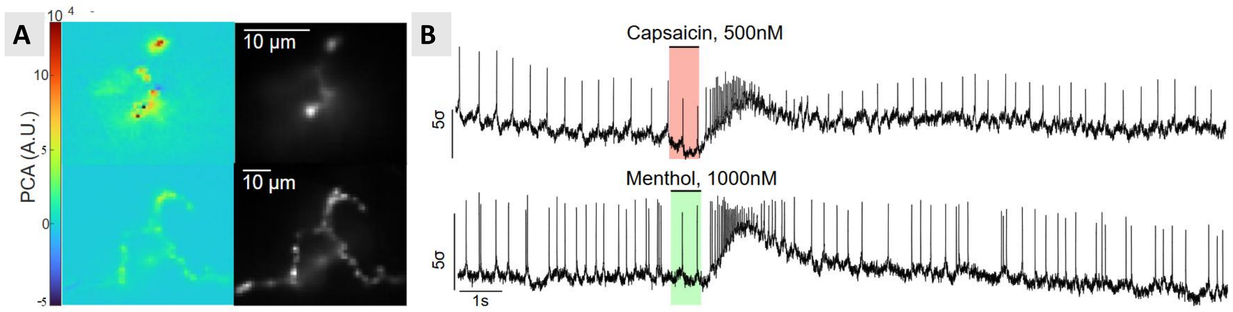
Nociceptive Terminals Show Heterogeneous Ongoing Activity Patterns

A. Representative traces of various ongoing activity patterns. **B.** Venn diagram showing the proportions of the three different ongoing firing types; single spike, bursts, and both. **C.** Pie chart demonstrating three different wave forms extracted using K-means. **D.** Firing Rate (FR) distribution across all recorded terminals. Mean FR (4.1 Hz), is marked in a dashed red line.

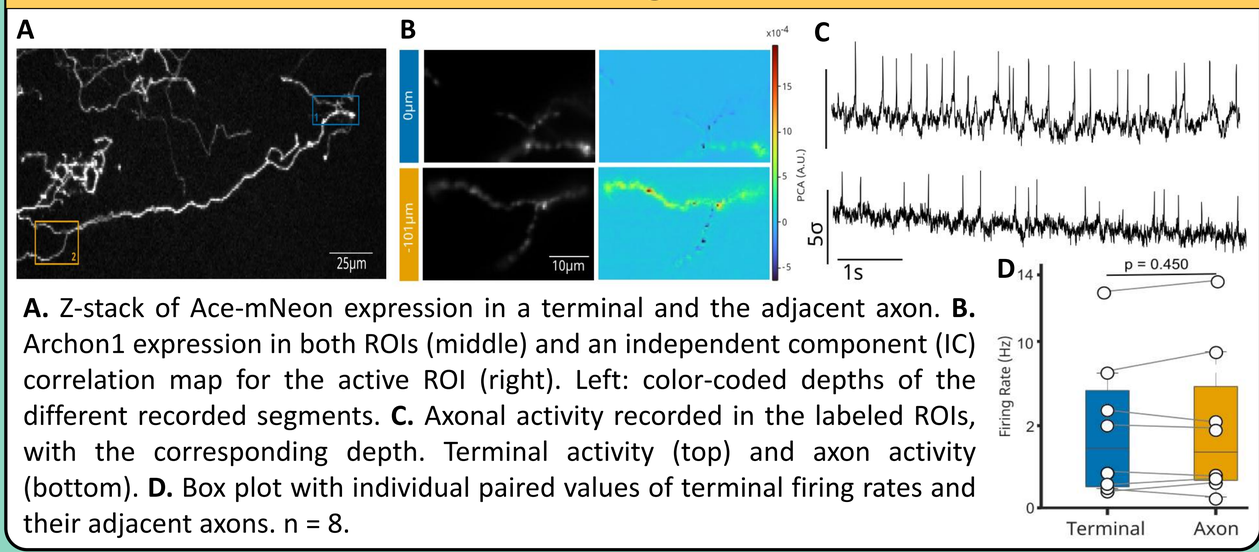


Nociceptive Terminals *In-vivo* Shows Ongoing Activity

A. Correlation map showing pixel-wise correlation values (color-coded), extracted using a PCA–ICA pipeline (left). Archon expression in the terminal's ROI. Image represents the mean across all recorded frames (right). **B**. Terminal activity before and after local application of 0.5µM capsaicin (Highlighted in red) (up). Terminal activity before and after local application of 1µM menthol (Highlighted in green) (down).



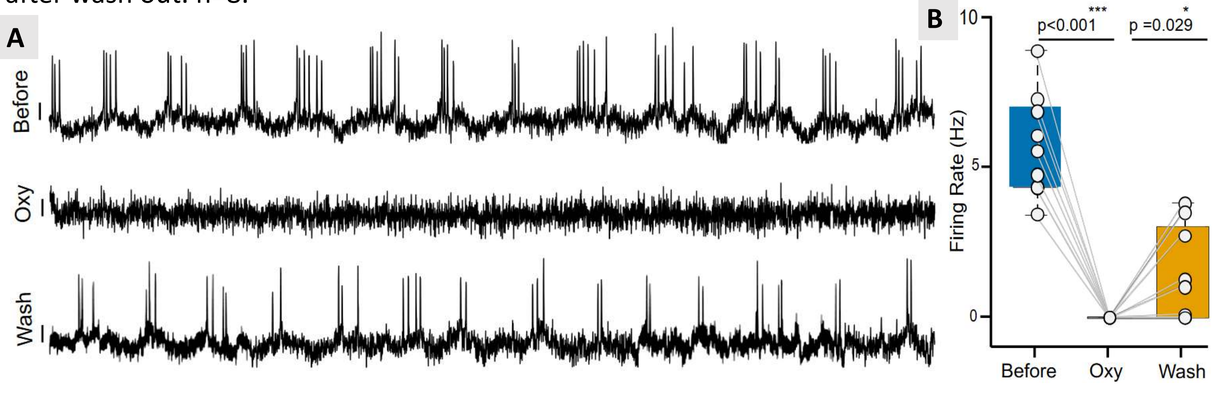
Axon and Terminal Firing Rates are Correlated



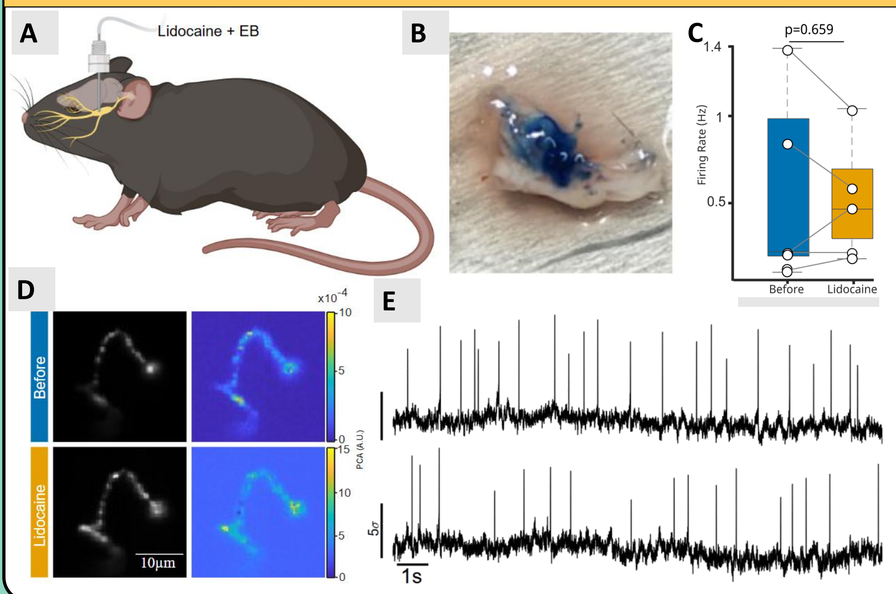
A. Z-stack of Ace-mNeon expression in a terminal and the adjacent axon. **B.** Archon1 expression in both ROIs (middle) and an independent component (IC) correlation map for the active ROI (right). Left: color-coded depths of the different recorded segments. **C.** Axonal activity recorded in the labeled ROIs, with the corresponding depth. Terminal activity (top) and axon activity (bottom). **D.** Box plot with individual paired values of terminal firing rates and their adjacent axons. n = 8.

Ongoing Activity Consists of Sodium-Dependent Action Potentials

A. Representative terminal: Spontaneous changes of fluorescence levels measured in a terminal (top), spontaneous changes of fluorescence levels measured in the same terminal after application of the sodium channel blocker oxybuprocaine (0.4%) (middle), Fluorescence levels measured in the same terminals after washing out the oxybuprocaine (bottom). **B.** Box plot and individual paired values of the ongoing firing rate, before and following the application of oxybuprocaine (0.4%) and after wash out. n=8.



Ongoing Activity Is Generated Locally, Not in the Soma



A. Cannula implementation location in the TG. **B.** Presence of lidocaine (2%) + EB in TG. **C.** Ace-mNeon expression in ROI before and after lidocaine (middle) and an independent component (IC) correlation map for the active ROI (right). **D.** Ongoing activity in the terminal before (top) and after (bottom) lidocaine. **E.** Box plot with individual paired values of terminal firing rates before and after lidocaine. n = 5.