

# Use of automated patch clamp to record membrane currents in freshly isolated smooth muscle cells

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## Introduction

Automated patch clamp (APC) has been used to record membrane currents from heterologous expression system as well as cultured neuronal and cardiac cells. However, there are no reports on membrane currents being recorded by APC from freshly isolated smooth muscle cells. Here we report preliminary data on voltage-activated chloride channel currents recorded from freshly isolated smooth muscle cells from rat bladder.

Chloride channels (ClC) play a pivotal role in the physiological functions of smooth muscle cells, influencing processes such as electrical excitability, ion homeostasis, and transepithelial

transport. The ClC ion channels are integral in maintaining the delicate balance of chloride ions within the cells, which is crucial for the regulation of muscle tension and contraction.

ClC ion channels are essential components of smooth muscle cell physiology, contributing to the regulation of vascular tone and blood pressure. Their intricate regulation and diverse roles underscore the complexity of smooth muscle cell function and the importance of chloride channels in the maintenance of normal physiological states and the development of various diseases.

## Summary

- We have recorded large voltage-dependent chloride channel currents from rat bladder smooth muscle cells using the QPatch compact.
- The currents were outwardly rectifying and had slow kinetics of activation similar to recordings in guinea pig bladder smooth muscle cells (Yarotsky *et al.*, 2019) and heterologously expressed ClC-channel currents (Piccolo *et al.*, 2010; Kurita *et al.*, 2015).
- The currents were inhibited considerably by 100  $\mu$ M niflumic acid like the study in the guinea pig bladder (Yarotsky *et al.*, 2019).
- Niflumic acid produced considerable inhibition of contractile activity in strips of rat bladder.
- Future experiments will ascertain the pH dependence of this chloride channel.

## References:

- Piccolo A, Malvezzi M, Accardi A. Proton block of the ClC-5 Cl<sup>-</sup>/H<sup>+</sup> exchanger. *J Gen Physiol*. 2010 Jun;135(6):653-9. doi: 10.1085/jgp.201010428. PMID: 20513761; PMCID: PMC2888053.
- Kurita T, Yamamura H, Suzuki Y, Giles WR, Imaizumi Y. The ClC-7 Chloride Channel Is Downregulated by Hypoosmotic Stress in Human Chondrocytes. *Mol Pharmacol*. 2015 Jul;88(1):113-20. doi: 10.1124/mol.115.098160. Epub 2015 May 5. PMID: 25943117.
- Yarotsky V, Malysz J, Petkov GV. Properties of single-channel and whole cell Cl<sup>-</sup> currents in guinea pig detrusor smooth muscle cells. *Am J Physiol Cell Physiol*. 2019 May 1;316(5):C698-C710. doi: 10.1152/ajpcell.00327.2018. Epub 2018 Dec 19. PMID: 30566392; PMCID: PMC6580156.

## Methods

### Cell isolation

Approximately 20 mg of bladder tissue was placed in 1 mL isolation medium (LM-R1643/500) with 1% L-Glutamine (200 mM solution, G7513), and 1% penicillin-streptomycin (15140-122). The tissue was digested with 0.1% papain (10108014001), 0.1% bovine serum albumin (BSA) (A2153) and 0.1% DL-Dithiothreitol (DTT) and placed in a block heater (Vortemp 56, Labnet) for 20 minutes (37°C, 21 RPM). The tissue was washed twice with filtered PBS (fPBS) and then incubated with 0.1% collagenase (C9891,) 0.1% BSA and 0.1% DL-Dithiothreitol (DTT) (in isolation medium) in the block heater for 40 minutes. Isolated cells were pelleted using a micro-centrifuge (2.8x1000 RPM) for 10 minutes, then washed with fPBS and pelleted once more. The cells were resuspended in a final volume of 20  $\mu$ L of isolation media.

### Solutions

Cells were centrifuged and resuspended in extracellular recording solution and then applied to the QPatch 8 planar electrode plate. For recordings, extracellular solution contained (mM) 145 NaCl, 4 KCl, 2 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 10 HEPES, 10 glucose (pH 7.4, 300 mOsm) whilst the intracellular solution contained (mM) 120 KF, 20 KCl, 10 HEPES, 10 EGTA (pH 7.2, 300 mOsm).

### Whole cell patch clamp

The cells were centrifuged and resuspended in extracellular recording solution before applied to the QPatch 8 planar electrode plate. Giga seal and whole-cell conformation were obtained using an optimised, adaptive whole-cell protocol.

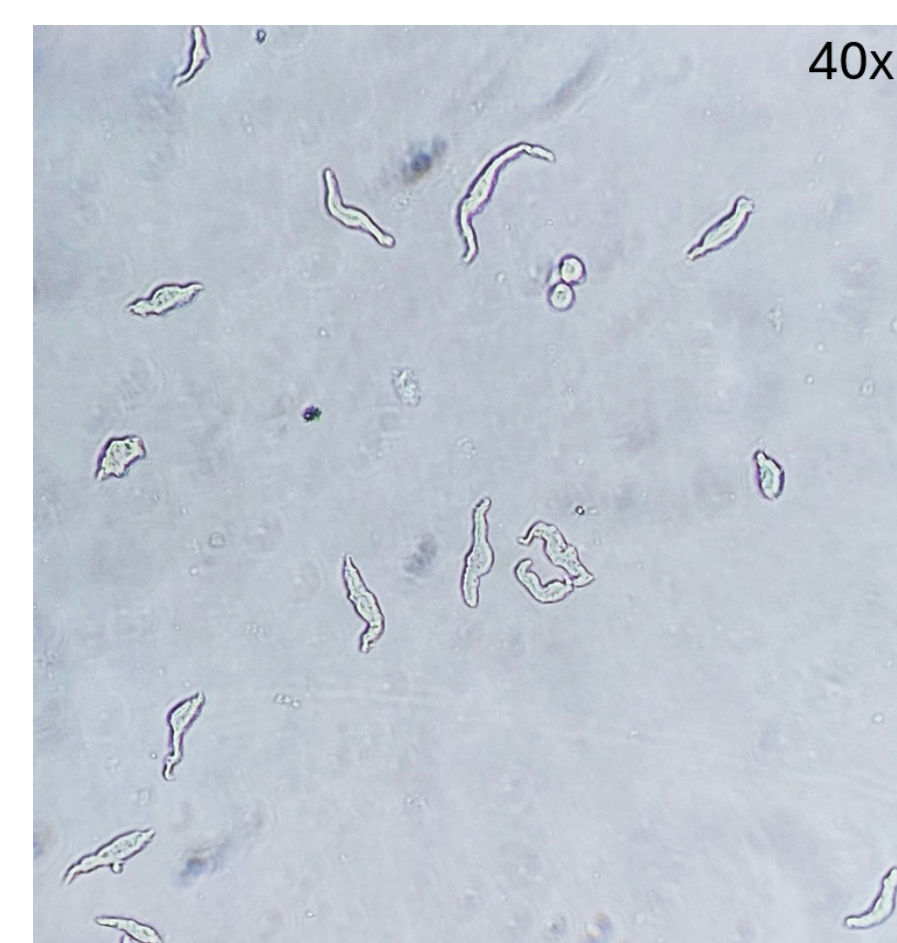


Fig. 1: Isolated smooth muscle cells from rat bladder tissue

### Voltage protocol

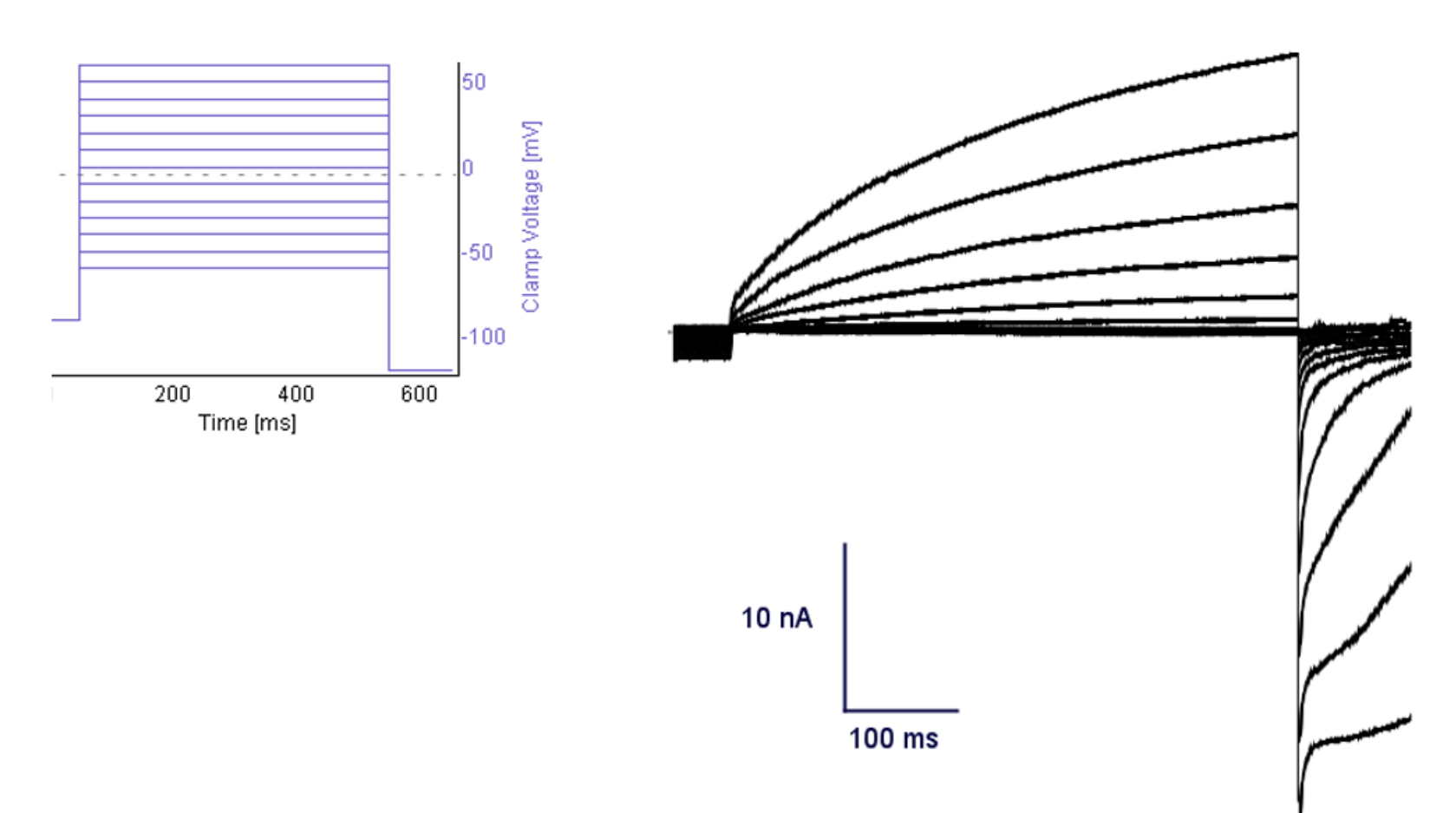


Fig. 2: Ion channel currents were evoked by 500 ms depolarizing pulses between -60 mV and 60 mV in 10 mV increments from a holding potential of -90 mV, followed by a hyperpolarizing step to -120 mV.

### Whole cell parameters

Avg (Cell C-slow)	SD (Cell C-slow)	Avg (Whole-cell Resistance)	SD (Whole-cell Resistance)
[pF]	[pF]	[M $\Omega$ ]	[M $\Omega$ ]
29.2	42.3	256.5	200.4

## Results

### Relative gene expression

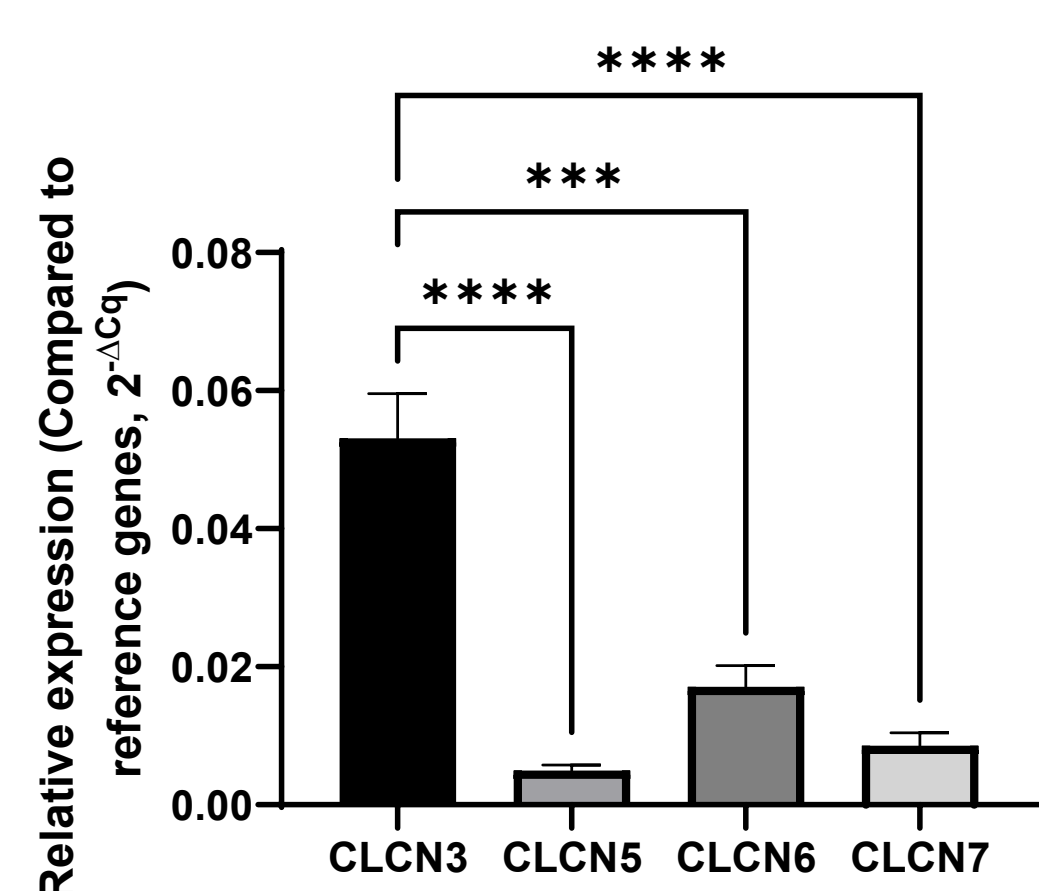


Fig. 3: Relative gene expression of various chloride channel isoforms from reverse transcription quantitative polymerase chain reaction (RT-qPCR) in male rat bladders. Expression was normalized to GAPDH and CYC1 housekeeping genes. N=4-5, One-Way ANOVA, error bars represent SEM.

### Current-voltage relationship

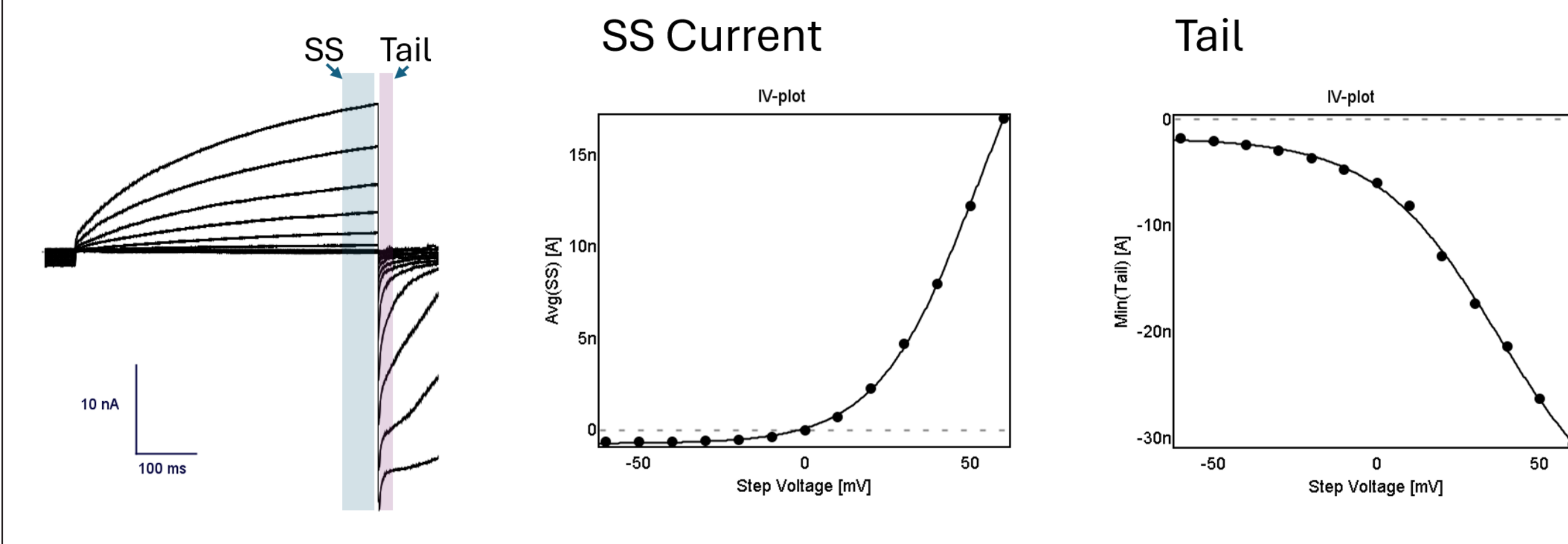


Fig. 4: Current-voltage relationship: A) 2 cursor intervals were used to measure steady state (SS, blue) activating currents, and peak tail currents (Tail, purple), both evoked by depolarizing pulses (Figure 1). B) IV plot of steady state (SS) activating currents demonstrating inward rectification. C) IV plot of peak tail currents.

### Activation and deactivation kinetics

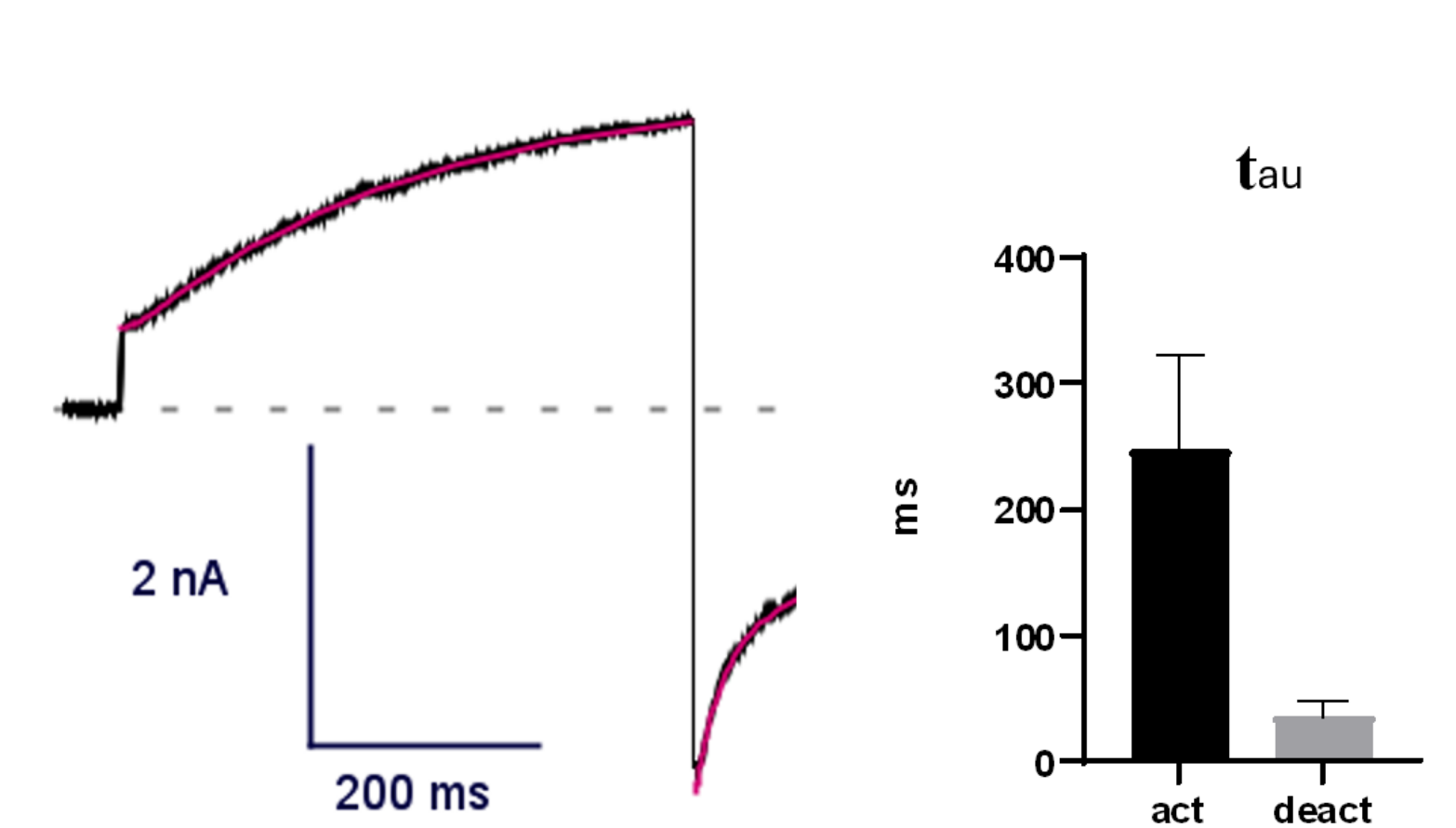


Fig. 5: Activation and deactivation kinetics: A) The time constant ( $\tau$ ) for activation and deactivation was found by a biexponential fit to a current trace evoked by a depolarizing pulse of +60 mV. The two fitted curves are here shown in red. B) The time constants for activation ( $t_{act}$ ) and deactivation ( $t_{deact}$ ) are  $247.2 \pm 30.6$  and  $5.6 \pm 35.3$  respectively (Avg  $\pm$  SEM).

### Niflumic acid sensitive currents

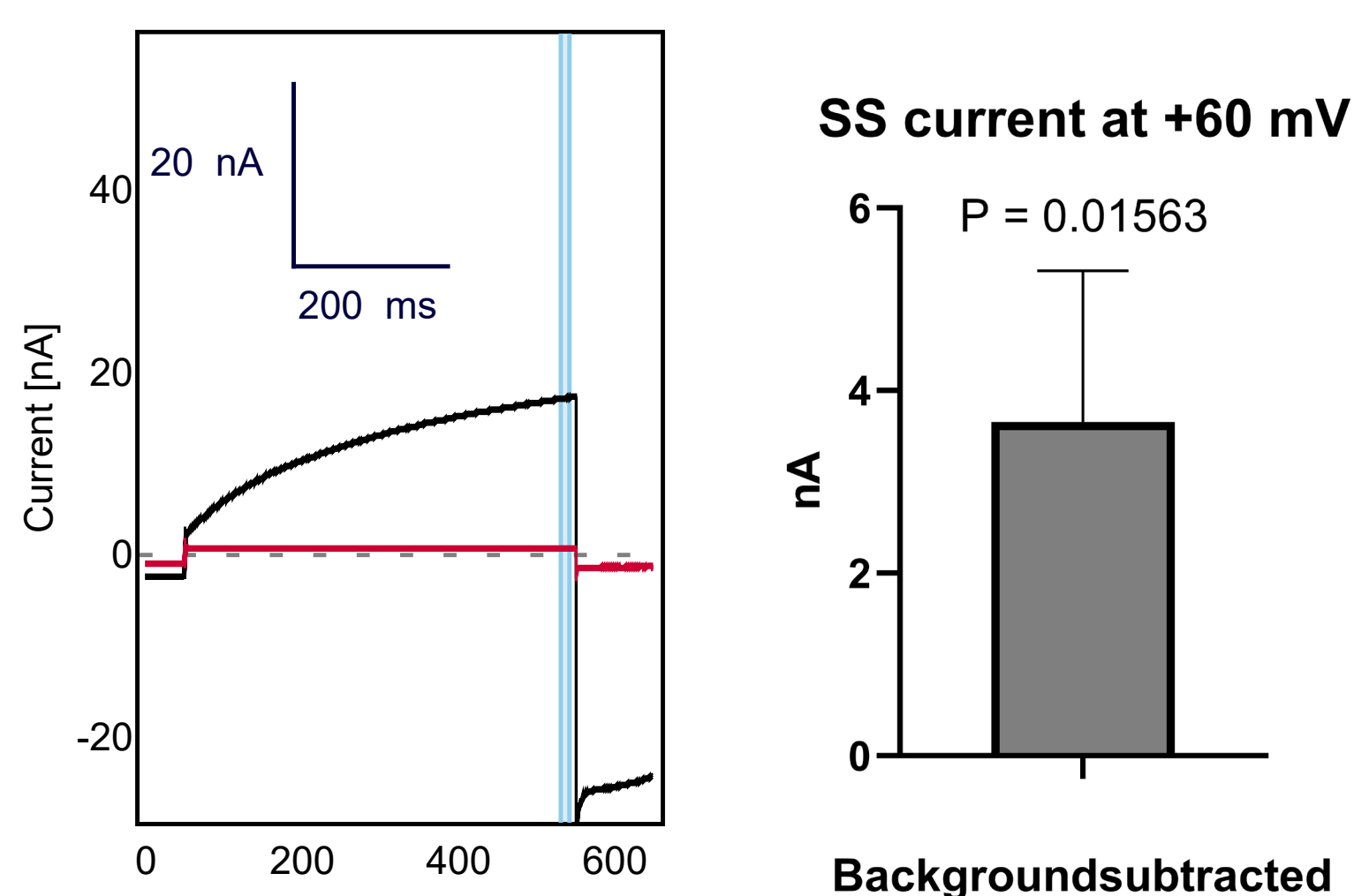


Fig. 6: A) Example trace demonstrating that a large fraction of the evoked currents was sensitive to 100  $\mu$ M Niflumic acid, indicating ClC-channel mediated current. B) Average Niflumic acid-sensitive current measured at steady state for a depolarizing pulse of +60 mV. Current size:  $3.7 \text{ nA} \pm 1.6$  (Avg  $\pm$  SEM).

### ClC channels: Niflumic acid sensitive current

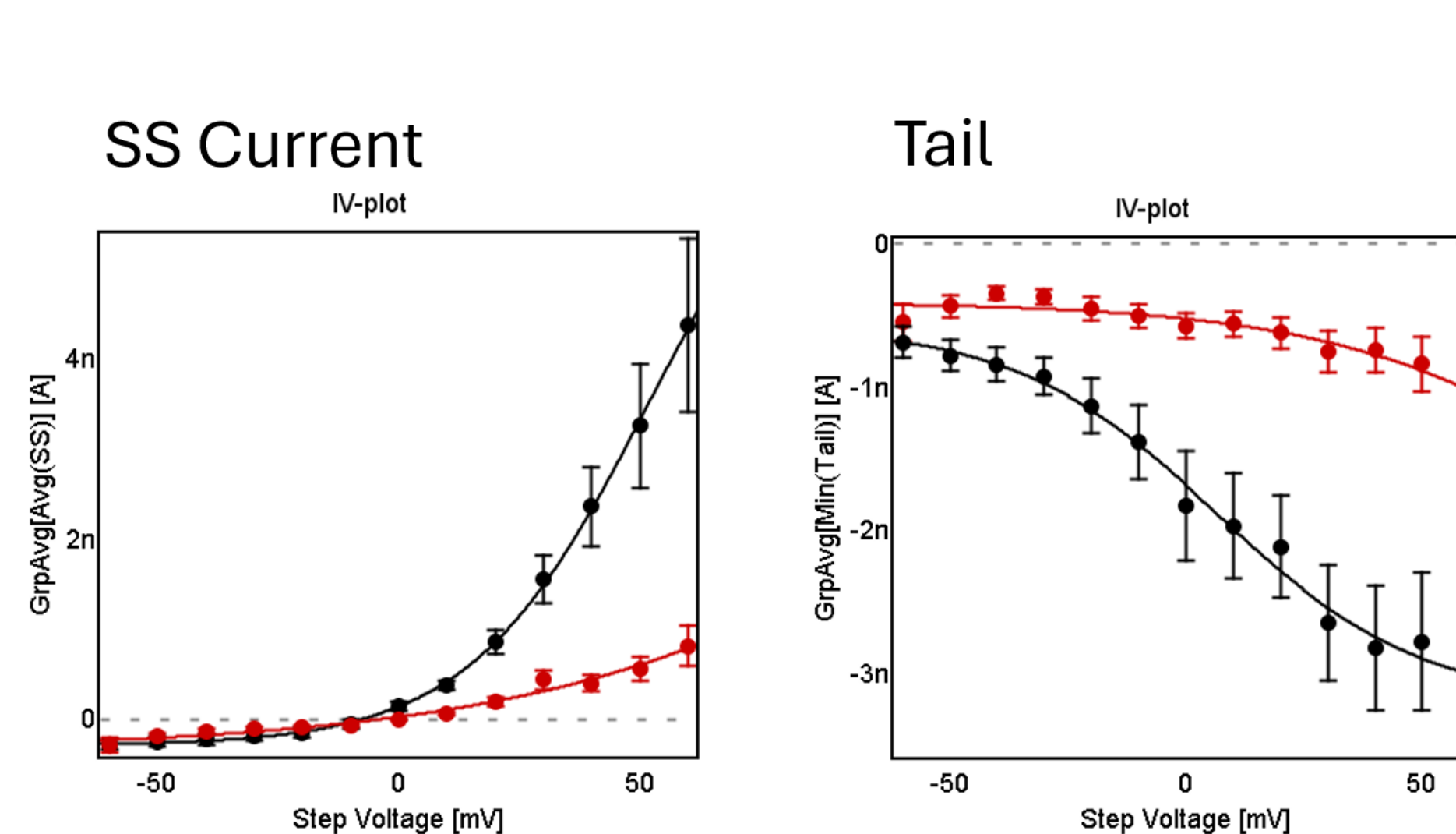


Fig. 7: Average Current-voltage relationship of Niflumic acid-sensitive currents: A) IV plot of steady state (SS) activating currents and B) IV plot of peak tail currents.

### Tension recording of bladder strip response to Niflumic acid

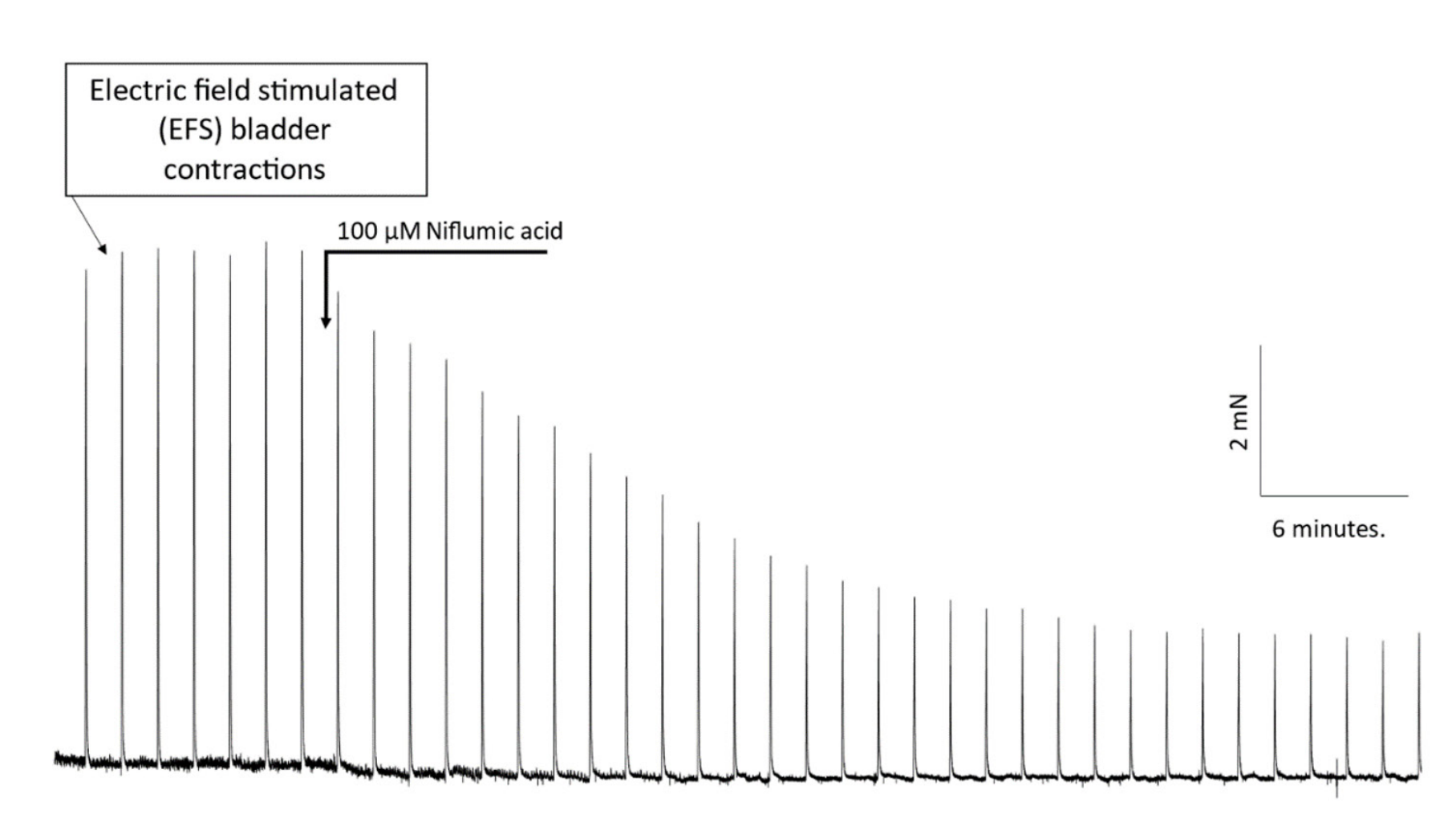


Fig. 8: Representative myography trace of an isolated rat bladder segment treated with Niflumic acid. Bladder strips mounted within the tissue chamber of a myograph were subject to electric field stimulation (20 Voltage, 0.3 milli-second pulse width, 20 Hz) to induce high amplitude contractions. Addition of 100  $\mu$ M Niflumic acid resulted in bladder relaxation by approximately 80%.



### QPatch Compact - semi-automated patch clamp

- Recording in up to 8 cells asynchronously in parallel
- Water-based temperature control at each measurement sites
- Manual liquid additions supported by light and audio guidance
- Fast and complete liquid exchange via microfluidic channels
- On the run changing of protocols
- Glass surfaces to prevent adherence of sticky compounds
- Ready-to-use individual electrode pairs – no need to ever re-chloride electrodes again