SOPHION BIOSCIENCE | Discover more

Advancing Automated Electrophysiology: Single-Channel Recordings in the Cell-Attached Configuration

Kadla Rosholm[1], Daniel Sauter[2], Mads Korsgaard[1]
[1]Sophion Bioscience A/S, Denmark [2]Sophion Bioscience, Inc., USA

Introduction

Automated patch clamp (APC) platforms are widely used in ion channel research for robust and scalable measurements of macroscopic currents. While APC excels at measuring ensemble currents, its use has been largely confined to whole-cell and perforated patch modes, providing limited insight into individual channel gating. Single-channel measurements in the cell-attached configuration impose strict noise and stability requirements that have historically restricted their adoption in automated systems.

Recent improvements in APC hardware, including substantially reduced amplifier noise, offer the potential to extend automated electrophysiology to single-channel measurements. In this study, we establish a cell-attached single-channel recording assay on the QPatch platform and demonstrate its applicability using recombinantly expressed BK channels in HEK293 cells.

Methods

Electrophysiology recordings were conducted on the QPatch (Sophion Bioscience) using single-hole QPlates. To enable single-channel resolution, an updated low-noise amplifier was used, reducing noise variance by ~10-fold compared to the Polyamp 4.2. Sampling at 10 kHz with 2 kHz cut-off.

Cell Culture: Experiments were performed using HEK293 cells expressing BK channels.

Pharmacology was assessed using 1 µM ionomycin, 10 µM HCTZ, and 10 mM TEA.

Protocol and Acquisition:

Configuration: Cell-attached mode was established using a timed protocol with -50 mbar positioning pressure.

Voltage Protocol: Clamp voltage stepped from -80 mV to +120 mV in 20 mV increments.

Patch Voltage: This corresponds to patch voltages of 140 mV to – 80 mV in 20 mV steps

Results

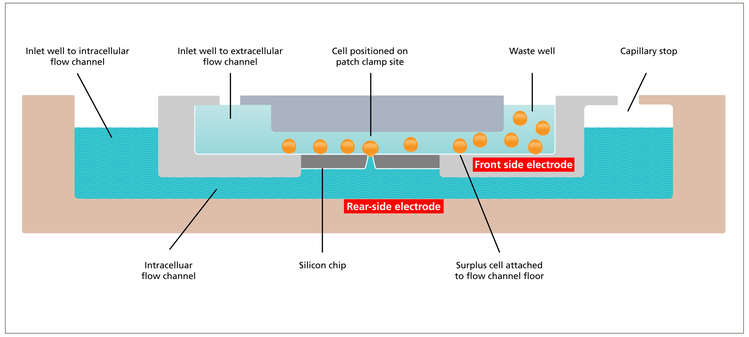

Fig. 1: Schematic of a microfluidic flow channel. Cells are introduced into the external channel, and a single cell is captured at the patch aperture using negative pressure. Each consumable includes built-in Ag/AgCl electrodes for precise and reproducible recording of currents and voltages.

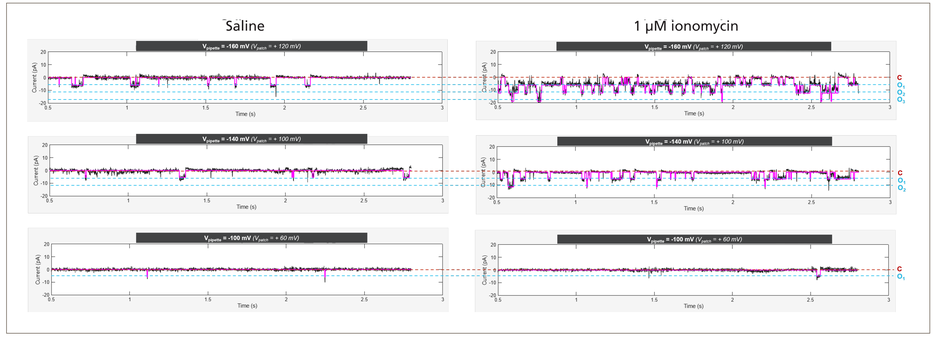

Fig. 3: Single-channel recordings of BK channel activity on QPatch. Representative current traces recorded in cell-attached configuration from HEK293 cells expressing BK channels. Recordings were obtained at pipette voltages ($V_{pipette}$) of -160 mV, -140 mV, and -100 mV, corresponding to patch voltages V_{patch} of +120 mV, +100 mV, and +60 mV. Left panels show basal channel activity in saline. Right panels show the same patch following the addition of 1 µM ionomycin in the external solution, demonstrating a clear increase in open probability (P_o) due to calcium-dependent activation. Red dashed lines indicate the closed state (C), while blue dashed lines indicate discrete channel openings (O_1, O_2, O_3).

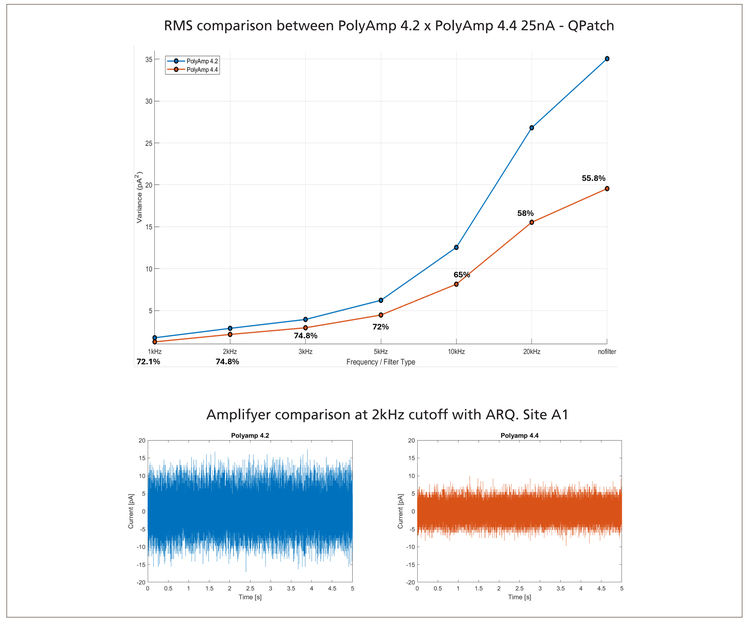

Fig. 2: Noise optimization and performance of the updated QPatch amplifier (Q Amp) for single-channel recordings. (Top) RMS noise comparison between the legacy Q Amp (Polyamp 4.2) and the updated Q Amp (Polyamp 4.4) amplifiers. (Bottom) Comparison of current noise in the cell-attached configuration at $V_{pipette}$ = 0 mV. The legacy Q Amp (red) exhibits a filtered noise floor of σ = 3.4 pA (5 kHz sampling, 1 kHz Bessel filter). In contrast, the updated Q Amp (blue) achieves a 38% reduction in filtered noise (σ = 2.1 pA) and an approximately 45% reduction in unfiltered noise (50 kHz sampling). This improved signal-to-noise ratio enables reliable resolution of single-channel current steps as small as 2 pA on an automated platform.

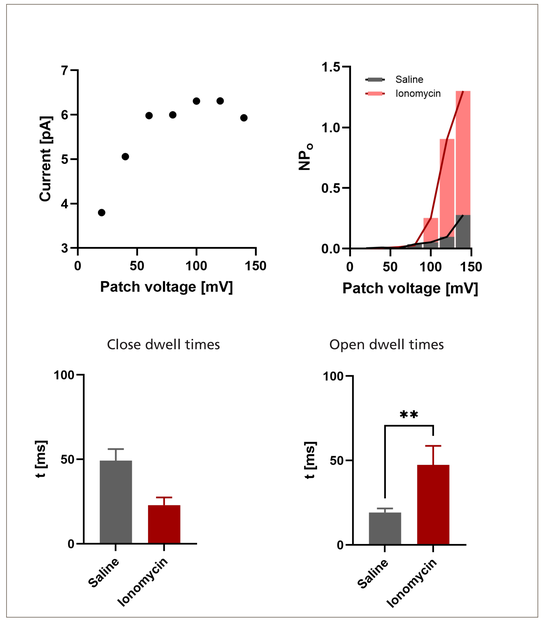

Fig. 4: Quantitative analysis of BK single-channel gating and ionomycin activation of a representative cell. (Top Left) Single-channel current amplitude (pA) plotted against patch voltage (mV), showing the unitary conductance of the BK channel. (Top Right) Open probability NP_o as a function of patch voltage in the absence (Saline, black) and presence of 1 µM ionomycin (red). Ionomycin significantly increases NP_o across depolarized potentials. (Bottom) Dwell time analysis at V_{patch} = +120 mV. Ionomycin treatment decreases the mean closed dwell time (Bottom Left) and significantly increases the mean open dwell time (Bottom Right, **p < 0.01), consistent with calcium-dependent stabilization of the open state. Data represent mean ± SEM.

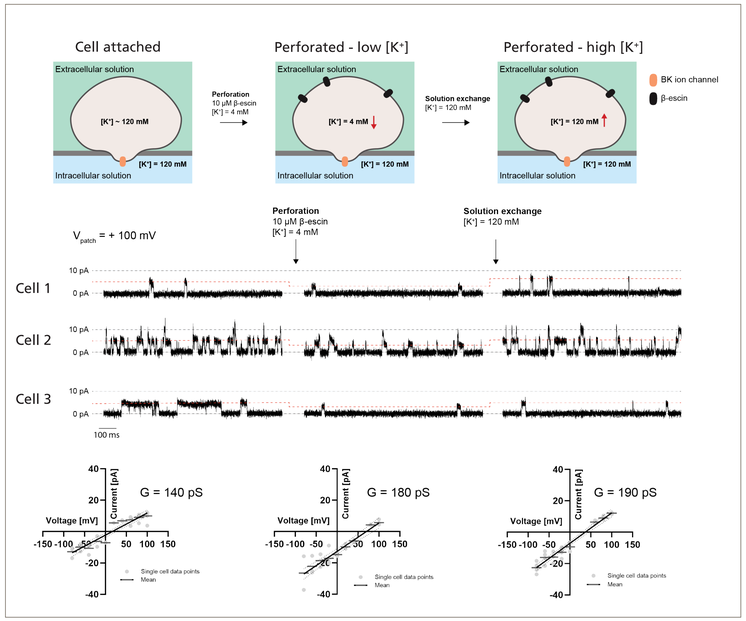

Fig. 5: Validation of single-channel BK conductance in pseudo-inside out configuration. To activate the BK channel, 10 µM HCTZ was present in the pipette solution. Recordings were initiated in the on-cell configuration with an estimated symmetrical K⁺ gradient (estimated cytosolic [K⁺] ≈ 120 mM), yielding a reversal potential close to the calculated Nernst potential for K⁺ ($E_{rev}(K^+)$ = 0 mV). Addition of 10 µM β-escin to the external flow channel permeabilized the membrane, allowing entry of 4 mM K⁺ from the external solution and resulting in a positive shift of the reversal potential ($E_{rev}(K^+)$ = +87 mV). Finally, exchanging the external solution to 120 mM K⁺ restored a symmetrical gradient and shifted the reversal potential to a more negative potential ($E_{rev}(K^+)$ = 0 mV).The top panel shows a schematic representation of the different stages of the experiment, the middle panel shows the related raw traces and the corresponding IV curve is shown in the bottom panel.

Presented @BPS2026