

Internal perfusion of Ca^{2+} to activate hTRPC5 on Nanion's SyncroPatch 384PE

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Summary

Transient Receptor Potential Canonical (TRPC) channels are a subfamily of TRP channels. The TRPC family contains at least 7 subunits and are predominantly expressed in neuronal cells where they may play an important role in Ca^{2+} flux¹.

TRPC5 channels are non-selective cation channels expressed in many areas of the brain particularly the hippocampus, amygdala and cerebellum, amongst others². Although the physiological and pathophysiological role of TRPC5 is not fully known, it does appear to be important in neuronal function, in particular during development where it is involved in hippocampal neurite outgrowth and growth cone morphology^{1,3,4}. Knockout mouse studies have also revealed that TRPC5 plays an essential role in innate fear⁵. TRPC5 is expressed in some regions outside of the CNS including the heart² where it contributes to cardiac hypertrophy in heart failure⁶.

TRPC5 is activated by intracellular calcium^{7,8}. Using the SyncroPatch 384PE, TRPC5 expressed in HEK cells could be activated by perfusion of the intracellular solution to contain free- Ca^{2+} . This current was potentiated by the lanthanide, gadolinium (Gd^{3+}), as expected⁹ and blocked by 2-APB with an IC_{50} consistent with that reported in the literature¹⁰.

Results

For the evaluation of the performance of hTRPC5 (HEK293) cells, Seal Resistance (R_{series}) at the start and end of one experiment using single hole chips was determined (Fig. 1). The seal resistance improved over the course of the experiment with 64% of cells with a seal resistance $>0.5 \text{ G}\Omega$ at the start of the experiment increasing to 77% at the end of the experiment which lasted approximately 10 minutes.

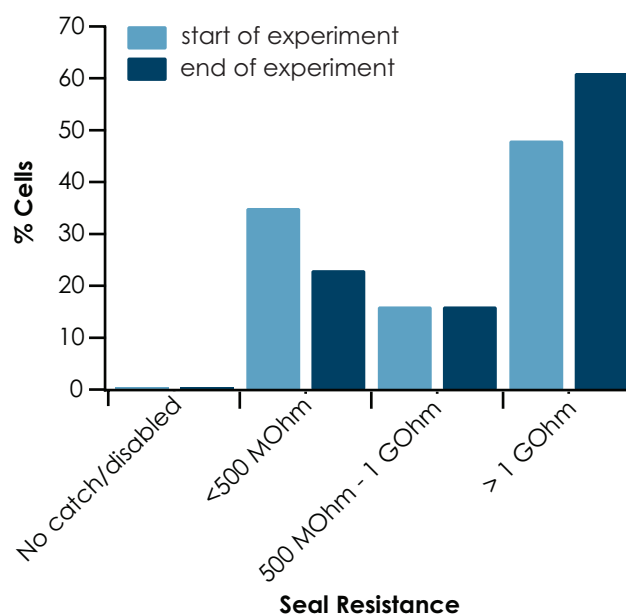


Figure 1: Statistics of TRPC5 expressing HEK293 cells recorded on one NPC-384 chip on the SyncroPatch 384PE. Success rate (seal resistance) of individual HEK cells on the SyncroPatch 384PE. Shown is a bar graph of seal resistances at the start (light blue) and end of the experiment (dark blue).

Application Note

Currents mediated by hTRPC5 were activated by application of 150 nM free- Ca^{2+} in the internal solution. The timecourse of activation of the current following internal perfusion of Ca^{2+} could be monitored online and is shown in Figure 2. There is a gradual increase in current (at 90 mV) over time, peaking approximately 2 min after the start of internal Ca^{2+} perfusion. The TRPC5 current was blocked by application 50 μM 2-APB (full-block concentration). All experiments utilized 150 nM internal free- Ca^{2+} and were performed using multi-hole (4 holes per well) chips in the whole cell configuration.

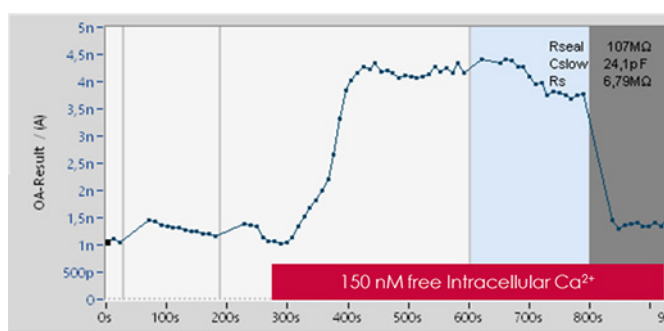


Figure 2: Activation of TRPC5 by internal perfusion of 150 nM free- Ca^{2+} . Online analysis (current amplitude at 90 mV) is plotted versus time and application of internal Ca^{2+} is indicated by the red bar. At the end of the experiment, a full-block concentration of 2-APB (50 μM) was applied (grey region). The current increased upon application of internal Ca^{2+} reaching a peak approximately 2 min after the start of perfusion. The current was stable and then blocked by external 2-APB.

TRPC5 activated by elevated internal Ca^{2+} was blocked by external 2-APB in a concentration-dependent manner. In these experiments, the cells were activated by internal perfusion of 150 nM internal free- Ca^{2+} following which the cell was exposed to a single concentration of 2-APB applied externally and the concentration response curve calculated across the whole plate. A second (full block) concentration of 2-APB (50 μM) was also applied across the whole plate as a positive control. The average concentration response curve for 330 cells is shown in Figure 3. The calculated IC_{50} was 11 μM ($n = 330$) which is similar to that reported in the literature (19 μM)¹⁰. In this experiment, 330 cells were used for the concentration response curve giving a success rate of 86% for completed experiments.

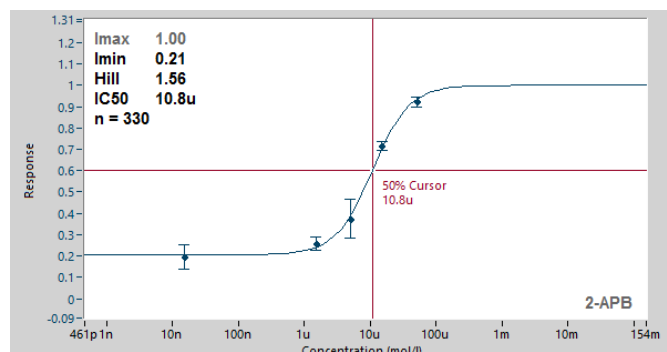


Figure 3: Average concentration response curve for 2-APB. The concentration response curve was constructed across the whole plate. Only cells which returned to baseline after the full block concentration of 2-APB were used for analysis giving an $\text{IC}_{50} = 10.8 \mu\text{M}$ ($n = 330$) and a success rate of 86% for completed experiments.

TRPC5 is modulated by the lanthanides⁹, La^{3+} and Gd^{3+} . Gd^{3+} (100 μM) potentiated the TRPC5 current activated by internal Ca^{2+} , particularly the inward current at -90 mV as previously reported⁹.

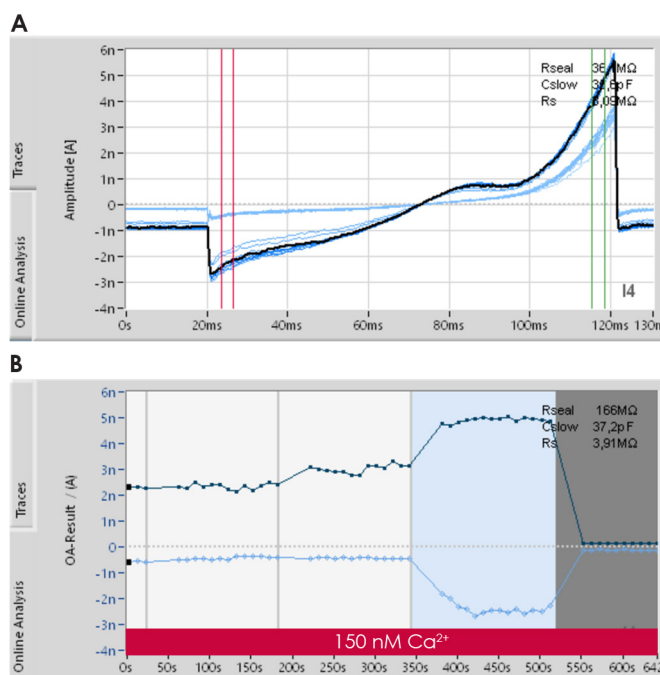


Figure 4: TRPC5 is potentiated by Gd^{3+} . **A** Raw traces from an exemplar cell in the presence of internal Ca^{2+} and potentiation of the response following application of external Gd^{3+} (see black trace). **B** Corresponding online analysis showing current amplitude at 90 mV (dark blue squares) and -90 mV (light blue circles) in the presence of internal Ca^{2+} (white), 100 μM external Gd^{3+} (blue) and full block by 50 μM 2-APB (grey).

Application Note

384 color coded depictions of data traces eases judgement of success rate

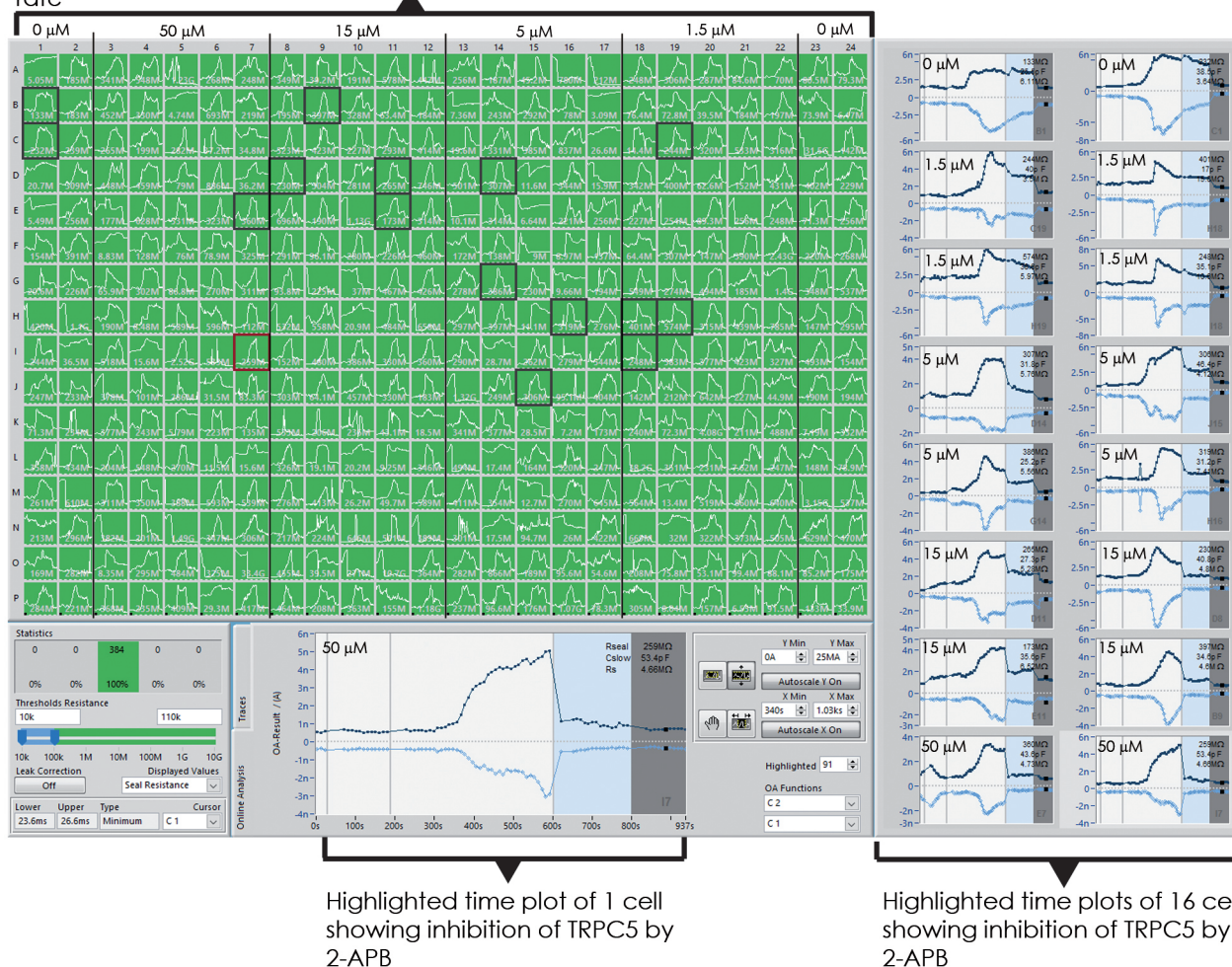


Figure 5: Graphical user interface of the screening and data analysis software used on the SyncroPatch 384PE. Screenshot of depiction of online analysis data of hTRPC5 expressing HEK293 cells as recorded on one NPC-384 (multi-hole, 4 holes per well) patch clamp chip. Three hundred and eighty-four small color-coded pictures are seen in the upper left part displaying 384 recordings. One highlighted experiment is displayed at the bottom, 16 selected experiments are seen in the right part. Graphs show current amplitudes of internal Ca^{2+} (150 nM) activation (white region), block by 2-APB (blue region) at the indicated concentrations and full block (grey region) using 50 μM 2-APB. Five minutes of baseline current was recorded prior to application of internal Ca^{2+} . Only cells which returned to baseline after the full block concentration of 2-APB were used for analysis.

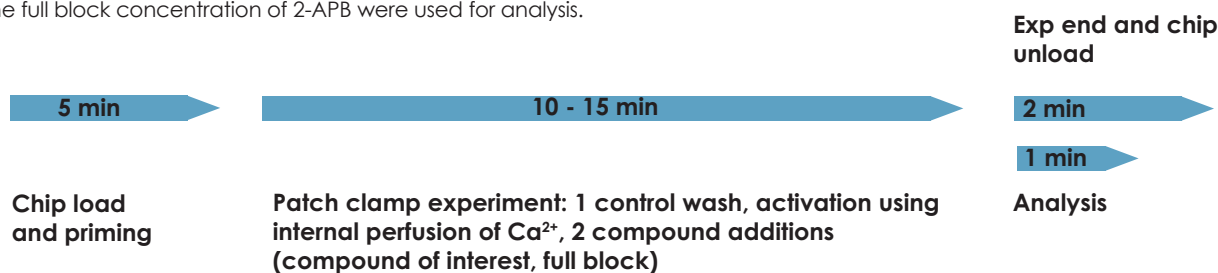


Figure 6: The completion of 1 experiment on the SyncroPatch 384 patch clamp chip (384 wells) for a single point concentration response curve plus activation of TRPC5 with internal Ca^{2+} and full block at the end of the experiment took approximately 17-22 min.

Application Note

Figure 5 shows a screenshot of the SyncroPatch 384 software during an experiment. The user can choose whether to visualize raw traces or online analysis. In this case, online analysis is chosen and the graphs represent current amplitude plotted against time. An individual well can be highlighted to monitor the progression of the experiment. In the Online Analysis view, the time points at which solution additions have been made are indicated by vertical lines, as well as different background colors. In this case, white shows activation of TRPC5 by internal Ca^{2+} , blue shows inhibition by the indicated concentration of 2-APB and grey shows full block by 50 μM 2-APB.

In conclusion, hTRPC5 expressed in HEK293 cells can be recorded on the SyncroPatch 384PE with an excellent success rate for completed experiments. The internal perfusion feature of the SyncroPatch 384PE was used to activate TRPC5 and increase in the current could be monitored online. This continuous monitoring of current amplitude is important for distinguishing compound effect from increase in leak or loss of seal and can be a benefit for assay development. The timeline of each experiment

was about 17-22 minutes (start – end) and included wash, activation with internal Ca^{2+} , time to reach a stable current, single compound concentration application and full block, all performed on each individual well, drastically reducing the consumable cost per data point to < \$0.60.

The activation properties of hTRPC5 recorded on the SyncroPatch 384PE are in excellent agreement with those reported in the literature^{8,9,10}. As expected, hTRPC5 could be activated by internal application of calcium^{7,8}, potentiated by gadolinium⁹ and inhibited by 2-APB in a concentration-dependent manner with an IC_{50} similar to that reported in the literature¹⁰.

The SyncroPatch 384PE is a high throughput and highly reliable automated patch clamp device for recording hTRPC5 currents. User-friendly software, excellent success rates, single additions or multiple additions of compound to each cell, perfusion of the internal solution and easy analysis result in reliable high quality data at an increased throughput with an economical cost per data point.

References

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Methods

Cells

hTRPC5 expressing HEK293 cells were supplied by Charles River.

Cell culture

Cells were cultured according to the manufacturer's recommendations and harvested according to Nanion's standard protocol using detachin.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the SyncroPatch 384PE using either single- or multi-hole (4 holes per well) chips. A voltage ramp protocol from -100 mV to +100 mV over 100 ms was applied to the cells every 10 s. Intersweep holding potential was -20 mV. Current amplitude at +90 mV and -90 mV was used for analysis. Compounds were applied via the internal and external solutions.