The suitability of high throughput automated patch clamp for physiological applications

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Abstract Although automated patch clamp (APC) devices have been around for many years and have become an integral part of many aspects of drug discovery, high throughput instruments with gigaohm seal data quality are relatively new. Experiments where a large number of compounds are screened against ion channels are ideally suited to high throughput APC, particularly when
the amount of compound available is low. Here we evaluate different APC approaches using a variety of ion channels and screening settings. We have performed a screen of 1920 compounds on GluN1/GluN2A NMDA receptors for negative allosteric modulation using both the SyncroPatch 384 and FLIPR. Additionally, we tested the effect of 36 arthropod venoms on NaV1.9 using a single 384-well plate on the SyncroPatch 384. As an example for mutant screening, a range of acid-sensing ion channel variants were tested and the success rate increased through fluorescence-activated cell sorting (FACS) prior to APC experiments. Gigaohm seal data quality makes the 384-format accessible to recording of primary and stem cell-derived cells on the SyncroPatch 384. We show recordings in voltage and current clamp modes of stem cell-derived cardiomyocytes. In addition, the option of intracellular solution exchange enabled investigations into the effects of intracellular Ca2+ and cAMP on TRPC5 and HCN2 currents, respectively. Together, these data highlight the broad applicability and versatility of APC platforms and also outlines some limitations of the approach.

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Abstract figure legend Different cell types including stable cell lines, transfected cells, stem cells and primary cells are used on automated patch clamp to generate high throughput pharmacology data, for mutant investigations and clone selection, and functional analysis of ion channels. Created with BioRender.com.

Key points
- High throughput automated patch clamp (APC) can be used for a variety of applications involving ion channels.
- Lower false positive rates were achieved using automated patch clamp versus a fluorometric imaging plate reader (FLIPR) in a high throughput compound screen against NMDA receptors.
- Genetic variants and mutations can be screened on a single 384-well plate to reduce variability of experimental parameters.
- Intracellular solution can be perfused to investigate effects of ions and second messenger systems without the need for excised patches.
- Primary cells and stem cell-derived cells can be used on high throughput APC with reasonable success rates for cell capture, voltage clamp measurements and action potential recordings in current clamp mode.

Introduction
Automated patch clamp (APC) has been used as an alternative to conventional patch clamp since its development in the late 1990s and early 2000s. The most successful automation of the patch clamp technique involves replacing the patch clamp pipette with a planar substrate (for reviews see Dunlop et al. 2008; Obergrussberger et al. 2015, 2021). The material used for the planar substrate ranges from borosilicate glass to silicon and polymer (Obergrussberger et al. 2015). Cells in suspension are attracted to the patch clamp aperture by the use of suction. The first successful experiments
recording ion channels in artificial bilayers or from cells in the whole cell patch clamp mode using quartz glass chips were shown in 2002 (Fertig et al. 2002). Since this time, a number of APC instruments have come onto the market with varying degrees of throughput ranging from recording from a single cell at a time up to 48 cells with tight, gigohm (GΩ) seals and up to 384 cells simultaneously with loose (MΩ) seals (Dunlop et al. 2008). In 2014, APC instruments were marketed that enabled the recording of 384 wells simultaneously with GΩ seals (Obergrussberger et al. 2016). With this, the emphasis has shifted from fluorescence-based to electrophysiology-based high throughput screening (HTS) strategies. In addition, with this shift in emphasis, the need for high quality, platform-optimized cell lines has become more apparent.

High throughput APC devices can be used in many aspects of physiological research, including hit finding and lead optimization in drug discovery, through cardiac safety testing on hERG and other ion channels (Brinkwirth et al. 2020; Kramer et al. 2020), to biophysical analysis of channelopathies to study loss-of-function (LOF) or gain-of-function (GOF) mutations. This has been done in a number of studies including KCNQ1 (Vanoye et al. 2018), Kv2.1 (Calhoun et al. 2017; Kang et al. 2019), hERG (Kozek et al. 2020; Ng et al. 2020), SCN5A (Glazer et al. 2020), Piezo1 (Rotordam et al. 2019) and NaV and Cav channels (Heyne et al. 2020). In another recent study, 309 variants of acid-sensing ion channel 1a (ASIC1a) were investigated by introducing three non-canonical amino acids (ncAAs) at 103 separate locations (Braun et al. 2021). In addition to being able to compete with fluorescence-based assays in terms of throughput, APC also offers the added benefit of providing additional information about mechanisms of action and kinetics of ion channel activity.

While not all experiments that are performed using conventional patch clamp can be transferred onto a high throughput APC instrument, there are some applications that can be performed on planar APC with much more ease than in conventional patch clamp, for example, exchanging the intracellular solution. Activation and modulation of some ion channels involve intracellular processes such as depletion of calcium from internal stores elevating cytosolic calcium concentration ([Ca²⁺])i, phosphorylation processes and/or the action of second messenger proteins. Although intracellular perfusion of the patch clamp pipette in a conventional patch clamp set-up has been described (Cull-Candy et al. 1981; Lapointe & Szabo, 1987), it is technically challenging (Leech & Holz, 1994).

The aim of this article is to provide an overview of the different applications that are suitable for use on high throughput APC instruments and offer insights into the limitations of the technique.

Methods

Molecular biology for hASIC1a variants

The complementary DNA encoding hASIC1a was kindly provided by Dr Stephan Kellenberger, while plasmids containing AzF-RS, Bpa-RS and tRNA were gifts from Dr Thomas P. Sakmar (Ye et al. 2009). A C-terminal 1D4-tag for purification was added to hASIC1a and two silent mutations were inserted at V10 and L30 to reduce the risk of potential translation reinitiation after proximal TAG codons (Kalstrup & Blunck, 2015). TAG variants were created by site-directed mutagenesis using PfuUltraII Fusion polymerase (Agilent, Denmark) and custom DNA mutagenesis primers (Eurofins Genomics, Germany). Sequences were confirmed by sequencing of the full coding frame (Eurofins Genomics).

Cell culture and harvesting

Standard cell lines

The cell lines used here (hNaV 1.9 stably expressed in HEK 293 cells from Icagen Inc., USA; hNaV 1.5 stably expressed in CHO cells from Charles River, USA; ionotropic glutamate receptors of the variant GluN1/GluN2A stably expressed in HEK293 cells, SB Drug Discovery, UK; α4β2 nicotinic acetylcholine stably expressed in SHSY5Y cells, SB Drug Discovery, UK; GluN1/GluN2A, GluN1/GluN2B, GluN1/GluUN2C and GluN1/GluN2D stably expressed in HEK 293 cells, Charles River, USA; Ionotropic glutamate receptor of the variant GluA2 stably expressed in HEK 293 cells, SB Drug Discovery, UK; hTRPC5 stably expressed in HEK 293 cells, Charles River, USA; hHCN2 stably expressed in HEK 293 cells, Axxam S.p.A., Italy) were cultured as previously described (Brueggemann et al. 2004; Brüggemann et al. 2008; Becker et al. 2013; Obergrussberger et al. 2014). In brief, cells were cultured in T75 culture flasks in the media recommended by the supplier and passaged every 2–3 days when they were 50%–80% confluent. The cells were passaged regularly to ensure that the cells were single when passaged and harvested. The cells were prohibited from reaching 100% confluency so that the cells remained healthy, single and expressed the ion channel of interest when they were harvested into a cell suspension for recordings. For experiments on the SyncroPatch 384, cells were harvested into suspension and suction was used to attract a cell to the patch clamp aperture of each well. Since it was a blind method for capture, cells had to be single with not too many clusters and the cell suspension was free from cell debris, as the presence of cell clusters and debris can decrease the success rate. Cells were typically harvested as described previously (Brueggemann et al. 2004; Brüggemann et al. 2008; Becker et al. 2013;
Obergrussberger et al. 2014) using TrypLE, other suitable enzymes, or even enzyme-free detachment protocols. Cells were then resuspended in extracellular recording solution at a density of 50,000—500,000 cells/ml.

**Stem cell-derived cardiomyocytes**

The human-induced pluripotent stem cell-derived cardiomyocytes (hiPSCs) were provided as cultured beating monolayers of ventricular cardiomyocytes in T25 culture flasks. Cells were maintained in culture for 2–5 weeks before being used for patch clamp measurements. Cells were harvested as previously described (Stoelzle et al. 2011; Becker et al. 2013).

**Preparation of hASIC1a variants**

HEK 293T ASIC1a-KO cells ( Borg et al. 2020) were cultivated in T175 flasks in supplemented DMEM and passed every 3–4 days using trypsin. Cells were plated into 10 cm dishes (2 million cells/dish) and transfected the next day with pEI ( Polysciences, Germany) and a 1:1:1:1 ratio of DNA encoding hASIC1a TAG variants, AzF- or Bpa-RS, tRNA and eGFP Y40TAG or Y151TAG, respectively. Control cells were transfected with ASIC1a WT and eGFP WT in a 3:1 ratio. nCAAs were added to the TAG variants by exchanging the cell medium for supplemented DMEM containing 0.5 mM AzF ( Chem Impex, IL, USA) or 1 mM Bpa ( Bachem Bio, Switzerland) 6 h after transfection, and cells were subjected to fluorescence-activated cell sorting (FACS) on a FACS Aria I or III ( BD Biosciences, CA, USA) with a 70 μM nozzle to enrich GFP-positive cells before APC experiments.

**Automated patch clamp recordings**

All cells were recorded in the whole cell mode of the patch clamp technique using the SyncroPatch 384PE ( Nanion Technologies, Munich, Germany) or the SyncroPatch 384i ( Nanion Technologies). Electrophysiological protocols were constructed, and data digitized using PatchControl 384 ( Nanion Technologies). Cells were recorded on single- or multi-hole chips of different resistances. Each well of the SyncroPatch 384 has an individual headstage of the amplifier and, therefore, each well is denoted as n = 1. For Z’ calculation (Fig. 1A and B), success rate (Fig. 1C), IC50 values across plates (Fig. 2G) and percentage positive wells for ASIC1a experiments (Fig. 4B), n represents results from 1 NPC-384 chip. With PatchControl 384, parameters such as seal resistance, capacitance and series resistance were determined from each well after application of a test pulse. All parameters are monitored over time and can be recorded for individual experiments.

**Nav1.9-mediated currents** were evoked by applying a voltage step protocol from a holding potential (Vhold) of -120 mV to 40 mV for 60 ms and back to Vhold. Pharmacological experiments were performed by continuous sweeping the single step voltage protocol with a sweep interval of 30 s. Peak current amplitudes were analysed at 40 mV. **Nav1.5-mediated currents** were evoked by applying a voltage double step protocol from a holding potential (Vhold) of -100 mV to -15 mV for 1 s, the voltage was then stepped back to -100 mV for 40 ms and then stepped again to -15 mV for 20 ms before stepping back to Vhold. Pharmacological experiments were performed by continuous sweeping the double step voltage protocol with a sweep interval of 10 s. Peak current amplitudes were analysed at both the first and second step to -15 mV but only IC50 values calculated on the 1st peak were used to construct the box plots shown in Fig. 2G.

** currents obtained from ligand-gated receptors** were recorded at a Vhold of -80 mV except for ASIC1a experiments where the Vhold was 0 mV. Voltage-dependent activation of NMDA receptors was conducted by precisely triggering the ligand addition to voltage steps of 3 s from a holding potential of -100 mV to values between -100 mV and 60 mV in steps of 20 mV. Alternatively, the voltage-dependent relief of magnesium (Mg2+) block of NMDA receptors was also analysed by application of glutamate/glycine precisely timed to a voltage ramp of 500 ms from -100 mV to 60 mV. The potential at which the cell current reversed (ERev) was calculated by using the online analysis function of PatchControl 384.

**hTRPC5 recordings**

A voltage ramp protocol from -100 mV to +100 mV over 100 ms was applied to cells expressing hTRPC5 every 10 s. Intersweep holding potential was -20 mV. Current amplitude at +90 mV and -90 mV was used for analysis.

**hHCN2 recordings**

Currents mediated by HCN (Ih) were evoked by stepping from Vhold of -30 mV in -20 mV increments to -130 mV for 2 s.

**iPSC-derived cardiomyocyte recordings**

Currents were elicited by application of a voltage ramp-step protocol (Voigt et al. 2012) starting from a holding potential of -80 mV, continuing with a ramp to -40 mV over 100 ms and followed by a voltage step from -40 mV in 5 mV increments up to 80 mV. Action potentials were evoked by applying current steps of 5 ms
length starting at 100 pA increasing up to 1.5 nA in 100 pA steps. Recording sweep was 600 ms.

**Recording solutions and compounds**

Standard physiological recording solutions were used for all recordings based on the following recipes: intracellular recording solution contained (in mM) 110 CsF, 10 CsCl, 10 NaCl, 10 EGTA, 10 HEPES; extracellular recording solution contained (in mM) 140 NaCl, 4KCl, 2 CaCl$_2$, 1 MgCl$_2$, 5 d-glucose monohydrate, 10 HEPES. For the GluN2A APC compound screen, MgCl$_2$ was absent from the extracellular recording solution. Compounds were sourced from Sigma or Tocris unless otherwise stated.

Venoms were obtained from spider and scorpions as described in Guo et al. (2018). Spider venoms were collected using small electrical stimulations (see details in Herzig & Hodgson, 2008). Scorpion venoms were collected by a pipette subsequently after aggravating scorpions to sting onto a parafilm membrane. After collection, all venoms were dried by lyophilization or by using a vacuum centrifuge, following which they were reconstituted in water prior to experiments. Recombinant venom peptides were produced using a periplasmic expression system as previously described (Bende et al. 2014; Undheim et al. 2015).

Each compound formulation contained a maximum of 0.3% DMSO. The same DMSO concentration was used as a negative control. For the Na$_V$1.9 experiments full block was achieved with 300 μM tetracaine. Action potential recordings of iPSC-derived cardiomyocytes were performed in intracellular solution containing (in mM) 110 KF, 10 KCl, 10 NaCl, 10 EGTA, 10 HEPES, and extracellular solution as above.

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**Figure 1. Screening of allosteric modulators against NMDA receptor using high-throughput-screening assays FLIPR and SyncroPatch 384i**

In this experiment, 1920 compounds in total were tested for activity. **A**, quality check (QC) results (Z’-factor) for FLIPR plates across the screen, mean Z’ = 0.496 ± 0.075. **B**, QC results (Z’-factor) for NPC-384 chips, mean Z’ = 0.613 ± 0.109. **C**, success rate for completed experimental wells using SyncroPatch 384i. Across the APC campaign 11 negative allosteric modulators (NAMs) were identified and of these, 9 NAMs were verified using a single point assay on the SyncroPatch 384i, but were not identified when tested in the FLIPR platform. Graph shows the mean ± SD percentage inhibition of n = 2–6 wells and compounds with inhibition >25% (as shown by dashed line) were considered as hits. **E**, further verification of NAMs using a concentration response assay in the SyncroPatch 384i. Mean ± SD response and n = 2–6 replicates per concentration are shown. Panels **A–D** adapted with permission from Obergrussberger et al. (2021).
Figure 2. Compound hit finding of venoms acting on Na\textsubscript{v}1.9 expressed in HEK293 cells
A, cartoon of the compound map of 36 arthropod venoms that were added in 6 replicates (P65–P718), two voltage-gated sodium channel specific compounds (tetracaine and TCN-1752) and vehicle are shown mapped on a 384 well plate. Grey wells indicate successful experiments, blue wells indicate failed wells (R\textsubscript{goi} < 100 MΩ).
B and C, current traces from exemplar cells and time plots of average current ± SD for vehicle (B; n = 104) and...
Fast ligand exchange using stacked addition

Ligand-gated receptors (NMDA, ASIC1a and AMPA) were activated by fast application of 5 μl ligand as a stack with 45 μl vehicle at a dispense speed of 60 μl/s in combination with a triggered recording voltage protocol (dispense speed can be increased up to a maximum of 110 μl/s). SHSY5Y-α4β2 nicotinic receptors were activated by a 10 μl ligand stack with 30 μl vehicle at a dispense speed of 40 μl/s. For ‘in-trace normalization’ experiments, solutions were stacked in the solution and pipetted onto the cell in the order: (1) glutamate; (2) modulator; (3) control wash solution. For NMDA receptors, glycine was present for the entire experiment. For pre-incubation experiments, modulator was pre-incubated in the extracellular solution for 2 min before co-application with glutamate.

Mix-and-read compound addition

Extracellular solution was added and removed by a 384-pipette head of a Biomek Liquid Handler, while a voltage protocol was sweeping continuously, e.g. for voltage-gated ion channels. Each well of the 384 well patch clamp plate holds 95 μl. A residual volume of 40 μl always remains in the well to keep cells in solution so that the cells do not become dry. Hence, further added solution is mixed and diluted by the residual volume. The final concentration is calculated by PatchControl 384 (Nanion Technologies).

Intracellular perfusion

The intracellular perfusion system is divided into 12 channels serving 32 wells each. Intracellular solution is supplied by 1 l glass bottles by applying negative pressure underneath the fluidic system. The minimum volume for filling one channel is roughly 2.5 ml (∼30 ml for a full plate). Valve controls facilitate fast switching between different internal solutions.

Quality control parameters

Cells must pass the quality control parameters such as $R_{\text{Seal}} > 100 \, \text{MΩ}$ (ASIC1a experiments) or $200 \, \text{MΩ}$ (all other experiments) for single hole chips, and $R_{\text{Seal}} > 50 \, \text{MΩ}$ for $4 \times$ hole chips to be included in the analysis. Generally wells are also filtered for online analysis parameters such as $I_{\text{peak}} > 100 \, \text{pA}$.

Data analysis

The SyncroPatch 384 platforms have a software package consisting of PatchControl 384 (for data acquisition) and DataControl 384 (for data analysis; both Nanion Technologies).

Concentration-response curves were calculated either using a 4-point cumulative addition of compound on each well, i.e. 4 concentrations of the compound were added to each individual well from lowest to highest and the $IC_{50}$ calculated for each individual well and then averaged, or as single point addition. In this case a single concentration of compound was added to each well and the $IC_{50}$ was calculated across multiple wells. $IC_{50}$ analysis was done with DataControl 384 or Igor Pro (WaveMetrics), concentration-response curves were fitted with a Hill equation: $y = base + \frac{(\text{max} - \text{base})}{1 + (\frac{x}{\text{EC}_{50}})^{n}}$. The data acquisition and analysis software contain a variety of online analysis functions for data generated in voltage clamp or current clamp.

AP analysis (APA) was used for action potential analysis. Action potential amplitude was calculated as the difference between the AP peak voltage (maximum) within cursor region and the baseline (defined by a second cursor pair). Action potential duration (APD) 90 was calculated from the start of the action potential to 90% decay of the peak potential.

The $Z'$ value is a statistical parameter used in HTS (and elsewhere) to evaluate the overall quality of an assay without intervention of test compounds (Zhang et al. 1999; Iversen et al. 2006). $Z$-factor ($Z'$) is calculated using the equation:

$$Z' = 1 - \frac{3 \times SD_{\text{Max}} + 3 \times SD_{\text{Min}}}{AVG_{\text{Max}} - AVG_{\text{Min}}},$$

where AVG is the mean and SD the standard deviation. The subscripts ‘Max’ and ‘Min’ in the formula for $Z'$ refer to the control measurements with maximum current (i.e. response to negative control) and the minimum current (i.e. response to positive control), respectively.

$Z'$ values >0.5 are considered to correspond to an excellent assay for screening (Zhang et al. 1999; Iversen et al. 2006).
Allosteric modulation of ligand-gated ion channels

To investigate ligand binding efficacies, receptor modulation, compound use-dependency and kinetics of NMDA receptor recordings, we chose a normalization method with which the peak current \( (I_{\text{peak}}) \) in the presence of the modulator measured within cursor 2 (C2) was normalized to the steady-state current \( (I_{\text{ss}}) \) elicited by the ligand alone at cursor 1 (C1; for cursor positions see Fig. 5B; so-called 'in-trace normalization'). For pre-incubation experiments, peak current in the presence of modulator \( (I) \) was normalized to the peak current in response to ligand alone \( (I_{\text{control}}) \) in the region of cursor 1 (C1; see Fig. 5C).

Statistical analysis

Data are presented as means ± SD. Differences between groups were tested using the Student's \( t \) test for normally distributed data. \( P \) values are reported in the figures; \( n \) represents the number of wells examined or the number of plates as indicated in the and/or figure legends. For ASIC1a analysis of current with and without FACS, a Mann Whitney test was performed in GraphPad 8, \( P = 0.003 \).

Results and applications

High throughput pharmacology and hit finding

Fluorescence-based high throughput screening (HTS) instruments, e.g. the Fluorometric Imaging Plate Reader (FLIPR) have typically been used for primary screening of compound libraries due to the throughput (96, 384 or 1536 format), physiological relevance of the assay, and low cost per data point (Xu et al. 2001; Yu et al. 2016). However, the temporal resolution of fluorescence-based methods and voltage-sensing dyes differs from ion channel characteristics recorded using patch clamp (Xu et al. 2001). In addition to this, compound fluorescence and compound-dye interactions can result in high false positive rates when using these kinds of methods. With the introduction of high throughput APC devices, with a throughput and cost per data point almost comparable with a FLIPR assay, compound screening on ion channels can be performed using patch clamp at an earlier stage of the drug discovery process. In data obtained from an HTS screen of 1920 compounds against the GluN1/GluN2A NMDA receptor, comparison of the FLIPR Z’ factor (Fig. 1A) to that obtained in the SyncroPatch 384i (Fig. 1B) assay demonstrated the ability of APC devices to produce high quality HTS data as well as achieve a high success rate per plate (Fig. 1C). The effect of compounds on the fluorescence response or current amplitude in cells stimulated with glutamate EC\(_{20}\) and glycine EC\(_{100}\) were recorded in both platforms. Both 0.2% DMSO and MK-801 (100 \( \mu \)M) controls were included on each test plate as negative and positive controls, respectively. Both the FLIPR and SyncroPatch 384i initial compound screens were completed within 2 days. Initially using the FLIPR assay, 14 negative allosteric modulators (NAMs) were found. However, in follow-up studies, all 14 NAMs were shown to be false positives (data not shown). On the other hand, screening of the same library in the APC assay, 11 NAMs were identified, of which 9 NAMs were verified by displaying an inhibition greater than the 25% threshold, using the SyncroPatch 384i but not the FLIPR platform (Fig. 1D). These NAMs were further characterized in follow-up concentration-response curve studies using APC (Fig. 1E). These data highlight the advantages of high throughput APC over fluorescence-based screening tools in providing more accurate data with fewer false negatives or false positives, and over conventional patch clamp given its vastly higher throughput allowing thousands of compounds to be screened for activity on ion channels in a short space of time.

In another experiment, we screened 36 arthropod venoms (one from an assassin bug, one from centipede, one from scorpion and the remaining 33 from spiders) with replicates of 6 for each venom and ran negative (vehicle; 0.3% DMSO; 128 wells; Fig. 2B) and positive (300 \( \mu \)M tetracaine; Fig. 2C) controls on the plate (for compound map see Fig. 2A). In this screen, compounds were tested on Na\(_V\)1.9 expressed in HEK cells. The recordings were performed in the presence of 1 \( \mu \)M tetrodotoxin (TTX) to block TTX-sensitive background currents present in some of the cells. Figure 2C shows block of Na\(_V\)1.9-mediated currents by tetracaine (0.94 ± 0.4, \( n = 22 \)). Addition of vehicle (0.3% DMSO) did not affect current amplitude (−0.01 ± 1.12, \( n = 104 \)). Time plots of average current ± SD, average current traces for venom samples P185 (\( n = 6 \)) and P483 (\( n = 6 \)) are given in Fig. 2D and E, respectively. P469 (\( n = 5 \)) did not affect peak current amplitude but changes in current kinetics could be observed (Fig. 2F). At the concentration tested (0.05 \( \mu \)g/\( \mu \)l), 18 compounds blocked Na\(_V\)1.9 > 20% compared with control, 1 compound enhanced current >20% compared with control and 17 compounds showed <20% difference compared with control (no effect).

The higher throughput of measuring 36 compounds in one experiment along with low compound requirement per well offer considerable advantages over other, lower throughput, alternatives.

Reproducibility of data across patch clamp chips is also an important factor to consider when performing high throughput screening experiments to ensure that the data is accurate and reliable. We calculated the IC\(_{50}\) values for 3 compounds which block Na\(_V\)1.5: amitriptyline, clozapine and verapamil across 8 different chips and plotted the measured IC\(_{50}\) values as a box plot shown in Fig. 2G.

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was low spread of the data for the 3 compounds, all IC_{50} values were within 2-fold of the mean.

**Clone screening and cell line optimization**

The 384-well capacity of the latest APC systems is ideally suited to the task of functional screening of clones, enabling rapid assessment of large numbers of isolates and delivering vital information on the performance of each one, including current amplitude, seal quality and success rate, as well as confirming homogeneity, ensuring the best clones are selected for downstream research. Figure 3 shows an excerpt of the data generated using such a strategy for the generation of an \( \alpha_4\beta_2 \) nicotinic acetylcholine receptor (nAChR) SHSY5Y stable cell line. Individual clones (24 clones) were first assessed by qPCR to verify the presence of nAChR \( \alpha_4 \) and \( \beta_2 \) mRNA transcripts (data not shown) and positive clones amplified for functional assessment by APC electrophysiology. Using the SyncroPatch 384i, SHSY5Y \( \alpha_4\beta_2 \) nAChR cell line clones were assessed for activity in response to 10 \( \mu \)M of the agonists nicotine and A85380. Figure 3A shows the results for 4 example clones and untransfected SHSY5Y cells, measuring their response to agonist and enabling rapid profiling of the functional activity of each cell line. Utilizing the 384-well capacity of the SyncroPatch 384i, a high number of replicates can be assessed for each clone. Three clones which reached the threshold for current amplitude (clones 43, 114 and 117) also exhibited high success rates for positive wells, whereas clone 34 despite exceeding the threshold for current amplitude, showed a lower success rate for positive wells (Fig. 3B). Therefore, the 384 well format provides insights into success rates for individual isolates (Fig. 3), allowing them to be ranked not only on parameters such as current amplitude, but also on commercially relevant factors, such as assay success rate. A summary of current amplitudes and EC_{50} values for A85380 for clones 43, 114 and 117, a HEK \( \alpha_4\beta_2 \) nAChR cell line and untransfected SHSY5Y are given in Table 1.

**Incorporation of ncAAs and investigations into effects of mutations**

While APC lends itself to mutational screening due to its high throughput, efficiency is limited when using transiently transfected cells, as this often results in low transfection rates, especially when co-transfecting multiple plasmids. In a recent study, this limitation was addressed by adding a fluorescence-activated cell sorting (FACS) step before APC to enrich the population of transfected cells. This enrichment procedure even allowed for the incorporation of ncAAs: HEK293 ASIC1a-KO cells were transiently transfected with 103 different stop codon-containing ASIC1a variants, as well as a tRNA/ncAA-RS system to incorporate ncAAs into the channel using the non-sense suppression approach (Braun et al. 2020). A GFP reporter for monitoring ncAA incorporation was co-transfected and used to enrich the transfected population by FACS before recording currents.
on the SyncroPatch 384PE. ASIC1a-mediated currents were elicited by applying solutions of decreasing pH, after which the activating solution was washed away using control solution at pH 7.4. This protocol can be used to efficiently generate concentration-response curves or to obtain information on pharmacological modulation. Importantly, the success rate (i.e. proportion of wells with a measurable current) was <10% without the FACS step, but increased to about 30% after subjecting the cell population to FACS (Fig. 4; (Braun et al. 2021)).

Therefore, APC is a suitable approach for investigating mutant channels and ncAA-containing channel variants, provided the cells are either stably transfected, the transfection rate is high, or a sorting method (such as FACS) is used as an intermediate step between transfection and APC recordings.

### Allosteric modulation of ligand-gated ion channels

The double-stack solution addition approach adopted by APC platforms allows brief ligand application and fast solution exchange rates. To this end, wash solution is first aspirated into the pipette followed by ligand so that the ligand is rapidly washed away after application to minimize exposure time and desensitization. Here, we show the activation of cells expressing different NMDA receptor subunit compositions (GluN1/2A, GluN1/2B, GluN1/2C, or GluN1/2D) by application of 10 μM glutamate/10 μM glycine or 100 μM NMDA/10 μM glycine (Fig. 5A). Under these conditions, NMDA receptors show the typical slow gating kinetics, with a sustained current after activation, and relatively weak (GluN1/2B) or no desensitization (GluN1/2C and GluN2D) (for reviews see Monyer et al. 1992; Vicini et al. 1998; Wyllie et al. 1998; Traynelis et al. 2010). For comparison, we show recombinant AMPA receptor subtype GluA2 (Fig. 5A, right), which shows fast activation and rapid and pronounced desensitization (for reviews see Mosbacher et al. 1994; Edmonds, 1995; Erreger et al. 2004; Traynelis et al. 2010). The mean current amplitudes for different ionotropic glutamate receptors are summarized in Table 2.

Using a planar chip approach has the advantage that different subunit combinations can be recorded on the same plate, on the same day and under the same conditions (as shown in Fig. 5A and B; Table 2), allowing direct comparisons about peak amplitude, desensitization properties and compound potency to be made. In this way, any subunit specific compounds that are identified are automatically validated by testing them on multiple subunit combinations under the same conditions. Although testing compounds for subunit specificity can be conducted using conventional patch clamp, this can only be performed on one cell at a time. Additionally, it is unlikely that all experiments will be conducted on the same day, giving rise to potential differences in solutions and temperature, which could affect compound potency of allosteric modulators (Sitzia et al. 2011; Milligan & Möller, 2013).

Different assay designs can be utilized for investigating allosteric modulation of ligand-gated ion channels. The first involves activation of the receptor by ligand and this is followed by co-application with the modulator so that both control and modulator effects are measured in the same trace (Fig. 5B). Using this approach, we observed potentiation of NMDA GluN1/GluN2B receptor-mediated responses by the polyamine spermine by two distinct mechanisms – increase of peak current and slowing NMDA channel desensitization. The concentration-response curve was calculated across the whole plate and revealed an EC50 of 186.6 ± 1.7 μM (n = 307 cells). Further, the tetrahydroisoquinoline CIQ has been shown to enhance NMDA GluN1/2C and 2D receptor responses 2-fold with an EC50 of 2.7 μM (GluN1/2C) and 2.8 μM (GluN1/2D) by increasing channel opening frequency (Mullaaseril et al. 2010). Our data obtained from the SyncroPatch 384i APC platform matched these values (EC50GluN2C = 4.5 ± 0.9 μM (n = 155 cells); EC50GluN2D = 5.3 ± 1.6 μM (n = 170 cells); see Fig. 5B and Table 2). This approach is suitable for ligand-gated ion channels that desensitize slowly or not at all, but for fast desensitizing channels such as AMPA receptors shown in Fig. 5A (right), a classical pre-incubation with the modulator and co-application of modulator and ligand is recommended. This is shown in Fig. 5C where LY404187 was pre-incubated for 2 min before co-application with glutamate. When pre-incubated, LY404187 enhances peak amplitude of GluA2-mediated responses by approximately 16 times compared with control and completely abolishes desensitization. The mechanism by which LY404187 modulates AMPA receptors has been reviewed by Baumbarger et al. (2001). LY404187 stabilizes the open

| Cell line | A83580 EC50 (μM) | Max. current amplitude (nA) | Number of cells |
|-----------|------------------|-----------------------------|-----------------|
| Clone 117 | 1.17             | −3.9 ± 1.1                  | 7               |
| Clone 114 | 1.56             | −3.8 ± 1.4                  | 7               |
| Clone 43  | 1.12             | −3.9 ± 1.0                  | 8               |
| HEK-α4β2  | 0.30             | −4.4 ± 1.4                  | 6               |
| SHSY5Y UT | 4.83             | −1.1 ± 0.9                  | 8               |

The maximum peak amplitude, given as mean ± SD for the number of cells indicated, was larger in the clones compared with the SHSY5Y-untransfected cell line.

Table 1. Summary of EC50 values for A83580 and maximum current amplitude for clones 117, 114, 43 and HEK-α4β2 (stable cell line) and untransfected SHSY5Y cells.

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Table 2. Summary of mean current amplitudes of different ionotropic glutamate receptor subtypes and EC$_{50}$ values for positive allosteric modulators acting on the NMDA receptors GluN2B, GluN2C, GluN2D and the AMPA receptor GluA2

| Glutamate receptor | Mean current amplitudes ± SD in nA (n) | Compound | Mean EC$_{50}$ ± SD (μM) | Number of cells |
|--------------------|----------------------------------------|----------|--------------------------|----------------|
| GluN2A             | $-8.3 \pm 4.1$ (86)                    | —        | —                        | —              |
| GluN2B             | $-4.2 \pm 2.4$ (90)                    | Spermine | $186.6 \pm 1.7$          | 307            |
| GluN2C             | $-0.15 \pm 0.06$ (81)                  | CIQ      | $4.5 \pm 0.9$            | 155            |
| GluN2D             | $-0.28 \pm 0.09$ (83)                  | CIQ      | $5.3 \pm 1.6$            | 170            |
| GluA2              | $-7.8 \pm 5.2$ (365)                   | LY404187 | $0.4 \pm 0.6$            | 365            |

state of AMPA receptors, while it allows desensitized AMPA receptors to make a transition to an open state either directly or through intermediate desensitized and/or closed states (Baumbarger et al. 2001).

The pre-incubation and co-application approach is standard procedure for investigating ligand-gated ion channels using either conventional or automated patch clamp. The in-trace normalization approach has been used to record GABA$_A$ receptor mutants using conventional patch clamp (Scheller & Forman, 2002); however, a specialized perfusion system including a piezo-driven multi-chamber pipette is required. The fast switching from one solution, e.g. glutamate/glycine followed by co-application of glutamate/glycine with the modulators spermine or CIQ (as shown in Fig. 5) on an automated patch clamp system is achieved by simply

Figure 4. FACS prior to APC increases success rate of recordings from HEK293T ASIC-KO cells transiently transfected with acid-sensing ion channel 1a (ASIC1a) variants
A, currents were recorded in <10% of wells when transiently transfected cells were used without fluorescence-activated cell sorting (FACS) (highlighted by black boxes). B, the proportion of cells with a measurable current increased to ca 30% when combined with FACS (highlighted by black boxes). One row of WT and one row of untransfected HEK293T ASIC-KO cells were also used on the plate as positive and negative controls, respectively (data source: Braun et al. 2021). The data of the 384 well plate representation are colour-coded for easy assessment of data. Depending on the seal resistance (number in bottom right corner of each well), each square is colour-coded: green: $R_{\text{Memb}} > 100 \ \Omega \cdot \text{cm}^2$; blue: $R_{\text{Memb}} = 30–100 \ \Omega \cdot \text{cm}^2$, light blue or grey: $R_{\text{Memb}} < 30 \ \Omega \cdot \text{cm}^2$ or cells disabled). C, bar graph with percentage of wells with current for cells with and without FACS. Values shown as mean ± SD, n = 8 for each condition. Mann Whitney test, $P = 0.003$. © 2021 The Authors. *The Journal of Physiology* published by John Wiley & Sons Ltd on behalf of The Physiological Society.
Figure 5. NMDA and AMPA receptor activation and pharmacology

A, different subunit combinations of NMDA were recorded on a single plate. Shown are subunit combinations GluN1/GluN2A, GluN1/GluN2B, GluN1/GluN2C and GluN1/GluN2D expressed in HEK293 cells which were recorded in different wells on a single patch clamp chip. The different current amplitudes and kinetics of activation, deactivation and desensitization between the different subtypes are clearly visible. For comparison, GluA2 (expressed in HEK293) current trace from an example cell is shown in response to a maximal concentration of glutamate. A fast peak current is followed by a sustained current until washout of glutamate.

B, raw data traces of GluN1/GluN2B subunit containing channels after activation by glutamate/glycine (red cursor), followed by co-application with spermine (green cursor) at the concentrations indicated. Spermine was stacked in the pipette with glutamate/glycine and quickly applied to individual wells at varying concentrations (including control wells) across the plate. Multi-hole chips were used where 4 holes were present per well. GluN2B, 2C or 2D currents in the presence of PAM were normalized to the response generated with glutamate/glycine and quickly applied to individual wells at varying concentrations (including control wells) across the plate. Multi-hole chips were used where 4 holes were present per well. GluN2B, 2C or 2D currents in the presence of PAM were normalized to the response generated with glutamate/glycine and the concentration response curve for spermine or CIQ calculated across the whole plate. The curves were fitted with Hill’s equation and the EC50 calculated to be 186.6 ± 1.7 μM (n = 307) for spermine, 4.5 ± 0.9 μM (n = 155) for CIQ block of GluN2C and 5.3 ± 1.6 μM (n = 170) for CIQ block of GluN2D. Data represent mean ± SD. C, AMPA GluA2 receptors expressed in HEK293 cells were recorded and the positive allosteric modulator LY404187 was applied by pre-incubation and then co-application with glutamate. EC50 = 0.4 ± 0.6 μM (n = 365). D, the current-voltage plot of average NMDA (GluN2A) receptor-mediated peak...
stacking the different solutions inside the pipette. Upon application, the solutions are pipetted onto the cells in one pipetting action, without any added time to switch between the solutions or dead volume. This is the case for any robotic APC device which uses a standard pipetting robot for exchanging external solution.

**Voltage dependence of ligand-gated ion channels**

Some ligand-gated receptors, as well as being activated by ligands or other external stimuli, also exhibit a voltage-dependent gating mechanism (Voets et al. 2004; Nilius et al. 2005; Clarke & Johnson, 2008). Under resting conditions NMDA receptors are blocked by external Mg$^{2+}$. Depolarization relieves the Mg$^{2+}$, unblocking the pore and leading to cation influx when ligands are present. To determine the voltage-dependent relief of Mg$^{2+}$, we observed activation of canonical NMDA receptors, obtained with conventional patch clamp recording from one cell at a time. To determine the voltage-dependent relief of Mg$^{2+}$, we observed activation of canonical NMDA receptors, obtained with conventional patch clamp recording from one cell at a time. Activated by internal Ca$^{2+}$, mediated by TRPC5 were blocked by external 2-APB in a concentration-dependent manner (Fig. 6C). The calculated IC$_{50}$ was 7.5 ± 2.2 μM (n = 330) which is similar to that reported in the literature (19 μM) (Xu et al. 2005). Furthermore, we observed modulation of currents mediated by hyperpolarization-activated cyclic nucleotide-gated (HCN) channels through internal application of cAMP. Figure 6D shows current responses to decreasing voltage steps for an exemplar HEK cell expressing HCN2 and the corresponding current-voltage plot for an average of 346 cells (Fig. 6E). Intracellular perfusion of cAMP did not further increase the current amplitude but modulated the current kinetics by decreasing the time constant (tau) to reach the peak current from 224 ± 45 in the absence of cAMP to 156 ± 33 in the presence of 2 mM cAMP for an average for 230 cells (Fig. 6F), a difference that was statistically significantly different (P < 0.0001, paired Student’s t test). These results are in good agreement with the literature which showed an acceleration of tau in the presence of cAMP for HCN2 and HCN4 (Ludwig et al. 1999).

**Primary cell recordings and stem cells**

Applications involving stably expressing cell lines and, to a certain degree, transiently transfected cells as described above, will yield excellent success rates and a vast amount of data giving insights into mutant channels and drug discovery. Primary cell cultures are also suitable for use on APC, although in some cases this can be associated with a lower success rate compared with cell lines. For example, primary rat cortical cells have been recorded on the SyncroPatch 384PE with a success rate for $R_{\text{Seal}}$ > 200 MΩ of about 40% (Toh et al. 2020) whereas primary T-cells from rat could be used on the SyncroPatch 768PE with a success rate for $R_{\text{Seal}}$ > 500 MΩ of approximately 12% (Li et al. 2017). Note that this success rate may be improved by reducing the size of the chip hole. Even with a comparatively low success rate of 40% or 50%, this results in 100—150 cells in one recording which may take 30–40 min and is therefore a notable increase in throughput compared with conventional patch clamp recording from one cell at a time.
Primary cells such as red blood cells (RBCs) are also suitable for use on HTS APC (Rotordam et al. 2019). These cells can be captured to the patch clamp aperture of the SyncroPatch to record currents, for example Piezo1-mediated currents recorded from RBCs on the SyncroPatch 384PE after activation with Yoda1 (Rotordam et al. 2019). Patient-derived red blood cells have also been recorded on the SyncroPatch 384 (Rotordam et al. 2019) and Piezo1-mediated currents compared between hereditary xerocytosis patients and healthy controls. In this way, automated patch clamp may be useful in interpreting RBC-related channelopathies, as well as providing potential diagnostic tools (Kaestner & Bianchi, 2020).

Stem cell-derived cells, e.g. human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes or neurons, are also suitable for use on high throughput APC instruments. Stem cell-derived cardiomyocytes have been used on high throughput APC (Obergrussberger et al. 2016, 2018; McKeithan et al. 2020; Potet et al. 2020) devices to record cardiac voltage-gated ion channels and action potentials, offering the advantage over acutely isolated cardiac myocytes that they are of human origin. They also have the added advantage that they can be modified to carry mutations from patient-derived cells. Figure 7 shows voltage and current clamp recordings of iPSC-derived ventricular cardiomyocytes: 102 cells exhibit currents with characteristic voltage dependence of voltage-gated calcium channels (VGCCs; Fig. 7A) and voltage-gated sodium channels. Currents mediated by VGCCs could be fully blocked by 300 nM nifedipine. Further, we observed partial block with 333 μM tetracaine (data not shown). In current clamp recordings (Fig. 7C), we elicited action potentials in 117 cells with an average APD90 of

Figure 6. Activation and modulation of ion channels by internal perfusion of calcium and second messenger proteins
A, representative current-voltage traces of TRPC5 (expressed in HEK 293)-mediated currents activated by intracellular perfusion of 150 nM free calcium (blue) and blocked by extracellular addition of 15 μM 2-APB (dark blue), black shows control current. B, time plot of average peak current at +70 mV for 28 cells. Grey background indicates ± SD. Arrow indicates start of internal perfusion of 150 nM [Ca²⁺]. C, concentration response curve for 2-APB calculated from concentrations distributed across the plate. Data were fitted with a Hill equation and IC₅₀ calculated to be 7.5 ± 2.2 μM (n = 298). Data represent mean ± SD. D, raw traces from an exemplar HEK 293 cell expressing hHCN2, activated by hyperpolarizing voltage steps. Shown are current responses to decreasing voltage steps from −30 to −130 mV. E, current-voltage plot for an average of 346 cells. Shown are mean of steady-state current ± SD. V₁/₂ = −93.2 ± 2.8 mV (n = 346). F, box plot showing tau activation before (0 mM cAMP) and after application of internal cAMP (2 mM), P < 0.0001, paired Student’s t test. Raw current traces from an example cell showing hHCN2-mediated current activation by stepping the voltage from −30 mV to −130 mV (black trace) and current modulation by additional intracellular application of 2 mM cAMP (blue trace). The inset displays a higher resolution of current kinetics.
Variable ion channel expression in these cells could be an explanation for the high variability of the action potential duration observed in the cells, examples of 3 different action potential shapes are shown in Fig. 7C.

**Discussion**

**Advantages**

- Up to 384 experiments (patch clamp) are performed simultaneously vastly increasing throughput of data compared with manual patch clamp.
- Low false positives and false negatives compared with other methods, e.g. fluorescence-based techniques.
- Detailed kinetic analysis of biophysical properties of ion channels can be obtained as well as pharmacological analysis.
- Significantly lower compound consumption compared with manual patch clamp.
- Internal solution can be perfused routinely and is easy to do.
- Mutant channels or subunit combinations can be recorded on the same chip, on the same day and under the same conditions, minimizing influence of experimental parameters, e.g. temperature.
- The use of multi-hole chips increases chances of successful experiments.
- Unbiased method for choosing cell to be recorded from.
- Experiments are easy to perform and do not require skilled electrophysiologists to perform the experiments.

**Limitations**

- Tissue slices cannot be used on automated patch clamp devices.
- Single cells must be in suspension for use in the devices.
- Cell to be recorded cannot be visually selected.
- Patches cannot be pulled for inside-out or outside-out patch clamp experiments (although experiments can be performed in cell-attached mode and internal solution exchanged so this may negate the need to pull patches).

**Figure 7. Voltage and current clamp recordings of iPSC-derived cardiomyocytes**

A, example I-V curve showing typical voltage dependence for a current mediated by voltage-gated calcium channels and corresponding raw current traces. B, bar chart representing percentage of qualified wells in voltage clamp (VC) and current clamp (CC) mode at different time points in the recording. Data was filtered at start of VC-recording for seal resistance ($R_{\text{seal}}$) > 250 MΩ that qualified 70% (270 cells) for analysis of $I_{\text{Ca}}$ from which 38% (102 cells) displayed a current larger than $-100 \text{ pA}$ at $-5 \text{ mV}$. After switching to CC mode 117 of 270 cells (43%) elicited an action potential (AP) selected upon a passive response during voltage-current protocol using the AP-Search function. C, action potentials elicited at different current injections showed a variety of different shapes in different cells. Three examples are shown here in Ca–c. The action potentials shown in Ca are from the same cell as shown in A.
High throughput APC has been implemented in electrophysiology laboratories worldwide for drug discovery, safety pharmacology and basic research into channelopathies (Calhoun et al. 2017; Vanoye et al. 2018; Kang et al. 2019; Glazer et al. 2020; Heyne et al. 2020; Ng et al. 2020) and precision or personalized medicine (Kang et al. 2019; Heyne et al. 2020; McKeithan et al. 2020). One of the major advantages of APC is undoubtedly the throughput compared with manual patch clamp where only one cell is recorded at a time. Large numbers of cells and/or compounds can be tested on a single plate, up to 384 at one time, reducing the time required for experiments enormously. To test 36 compounds with 6 replicates can take as little as 30 min. Using conventional patch clamp, this would have taken weeks, if not months. High throughput APC can now compete with other methods traditionally adopted for HTS, for example FLIPR, in terms of throughput and cost per data point, with the added advantages of lower false positive rates and information about channel kinetics, which cannot be obtained from fluorescence or calcium imaging experiments. Therefore, APC can be used at earlier stages of drug discovery and allow for screening of large compound libraries to identify hit compounds that can be further characterized in follow-up studies. Another advantage of the 384 well-plate format of high throughput APC is the low compound volumes required for the experiments. Each well of the 384-well plate holds a maximum of 95 μl and the volume used to exchange the solution in each well is just 40 μl. This is considerably less than the several millilitres of solution required on a manual patch clamp rig. This becomes particularly important when analysing compounds that are only available in small amounts, for example peptide toxins (Liu et al. 2019). In this study we used venoms and tested small volumes (40 μl per well) of each venom in replicates of 6 on NaV1.9 currents. Other laboratories have used high throughput APC to test spider peptides on NaV1.7 (Nicolas et al. 2019; Xu et al. 2019), αC-conotoxin PrXA on acetylcholine-mediated responses (Taiwe et al. 2019) and scorpion toxin block of Kᵥ4 channels (Zoukimian et al. 2019), among others.

In addition to this, APC can be used for clone selection and cell line optimization. While fluorescence-based clone screening still has its place, selecting clones using electrophysiology provides important information about performance statistics and allows for the identification of the optimal cell line for downstream electrophysiology applications. It is not uncommon to find a low correlation between clones selected using fluorescence-based platforms and their performance in more sophisticated electrophysiology assays, resulting in sub-optimal screens with reduced throughput and data reliability. For this reason it is important to ensure identification of platform-optimal cell clones when embarking on APC HTS campaigns. As such, electrophysiological assessment of different clones should rank high on the list of clone screening strategies, ensuring that potentially useful clones are not filtered out due to low performance correlation between assay formats. This results in shorter assay development times, more reliable data generation and ultimately acceleration of research. This strategy can also be applied to the investigation of mutant ion channel constructs, enabling large numbers of individual point mutations to be functionally characterized simultaneously with the added benefit of multi-hole recording options to overcome potential challenges with transfection efficiency.

Indeed, APC has been adopted in several academic laboratories studying channelopathies and genetic variants of a variety of different ion channels. Generally, cell lines that have been transfected with different genetic variants are used. Stable cell lines have been generated to investigate genetic variants of hERG which are trafficking-deficient variants (Kozek et al. 2020) or to distinguish between loss-of-function and benign variants (Ng et al. 2020); reclassifying variants of unknown significance of SCN5A in Brugada Syndrome (Glazer et al. 2020); and classifying Naᵥ and Caᵥ pathogenic variants (Heyne et al. 2020). Alternatively, transiently transfected cells can be generated, for example by electroporation, which yields a high transfection rate and these can be used directly on APC devices. This has been achieved to classify variants of unknown significance of KCNQ1 (Vanoye et al. 2018) as well as Kᵥ2.1 (Calhoun et al. 2017; Kang et al. 2019). When transiently transfected cells with low transfection rates are used, success rate, i.e. number of usable wells in APC, can be increased by pre-sorting cells using, e.g. FACS. This has been shown recently with 309 variants of the ASIC1a channel (Braun et al. 2021).

In conventional patch clamp experiments, the cell for recording is chosen by the experimenter, often based on fluorescence due to a fluorescent tag co-expressed with the protein of interest. In APC experiments the cell cannot be chosen. This has added the benefit of a more unbiased cell selection process in a mixed population of cells. In addition to a prior FACS step, success rate in APC measurements can be further increased by using multi-hole chips with 4 or 8 holes per well. The current amplitude of the sum of all the wells is then recorded, thus increasing the total measured current if current amplitude in all the cells is small, whilst also increasing success rate if not all the cells express the ion channel of interest (data not shown).

In addition to the use of cell lines, stem cell–derived cells derived from patients harbouring a particular mutation can be studied using APC. This has been validated recently with hiPSC-CMs derived from a patient carrying a mutation in the SCN5A gene, which causes long QT syndrome 3 (McKeithan et al. 2020) and highlights the power of combining stem cell technology with APC. We
have further demonstrated that VGCC-mediated currents can be recorded in hiPSC ventricular cardiomyocytes and that action potentials are generated in approximately 40% of wells (117/270) captured to the NPC-384 chip. This illustrates the suitability of high throughput APC for applications involving hiPSC cardiomyocytes.

Fast external ligand exchange and minimal exposure time to an activating ligand is achieved on APC instruments by stacking the solutions inside the pipette of the robotic device. Using this method, ligand-gated ion channels can be investigated where the switching between activating and non-activating solution occurs without any lengthy aspiration steps (e.g. aspiration of control solution), time to switch between different solutions, or dead volume. Exposure time can be further adjusted by introducing a waiting time between the ligand application and the washout. In addition to classical ligand application at a constant holding potential, voltage protocols can be run at the same time as ligand application in order to investigate voltage dependence of LGICs (Fig. 5). In addition to exchanging the external solution, the internal solution (equivalent to the pipette solution in conventional patch clamp) can also be exchanged easily on high throughput APC devices. This allows for investigations into intracellular second messenger systems or with different intracellular solutions. Using the conventional patch clamp approach, this can be achieved using the inside-out configuration. This involves excising a patch of membrane from the rest of the cell (Hamill et al. 1981), which can be technically challenging and is inherently low in throughput. In contrast to this, and as a result of the open design of the automated patch clamp chip, exchange of intracellular solution can be performed with relative ease in the whole cell configuration. This keeps the cell, including its compartments, intact (Brüggemann et al. 2006; Farre et al. 2007; Sauter et al. 2016; Brinkwirth et al. 2017; Obergrussberger et al. 2018) and obviates the need to pull inside-out patches. Importantly, intracellular signalling pathways are likely to remain intact and data can be obtained from the same cell before and after application of, for example, intracellular Ca2+ or cAMP, thereby reducing the number of cells needed to obtain statistically significant data.

There are, naturally, some limitations when assessing the APC-based approach. For use on APC devices cells must be isolated and in a cell suspension with preferably few clusters. This is to ensure that a single cell can be attracted to the patch clamp aperture and a tight seal formed between the glass of the patch clamp chip and the cell membrane. There are, therefore, some applications which are unsuitable and, in fact, impossible to perform on planar patch clamp devices. Tissue slice recordings, e.g. whole cell patch clamp measurements from brain slices, measurements of pre- or postsynaptic currents and paired electrode recordings are all examples of applications that cannot be used on planar patch clamp devices. In addition to this, acutely isolated adult cardiomyocytes are largely unsuitable for use on APC devices due to a very low success rate, although in some cases the cells can be attracted to the patch clamp aperture of the HTS APC chips. For experiments involving cardiomyocytes, either freshly dissociated neonatal cardiomyocytes or stem cell-derived cardiomyocytes will yield superior success rates compared with adult cardiomyocytes (Rajamohan et al. 2016; Li et al. 2019). As with most techniques, some assay development may be required to find the optimal recording solutions, voltage parameters, cell culture protocols and, in the case of ligand-gated ion channels, ligand application speed and exposure time. The 32-well mode of the SyncroPatch 384, which allows part of the plate to be used at a time, facilitates such assay development phases.

Ion channels remain an important target for drug discovery (McManus, 2014; García & Kaczorowski, 2016) and patch clamp is the gold standard to investigate ion channel activity. Here, we have highlighted several examples of research applications within ion channel function and pharmacology that are suitable for use on high throughput APC devices. The effects of ion channel variants or compounds can be investigated on a single 384-well plate in a single experiment with identical conditions. This means that functional differences can be identified within a short time frame. Although not suitable to all electrophysiological settings (e.g. brain slice recordings), the vast throughput achieved with devices recording 384 cells simultaneously, coupled with the information gained on ion channel kinetics and compound action, make high throughput APC an excellent complementary technique to fluorescence and conventional patch clamp assays. As more core facility and drug discovery units in academic laboratories, along with contract research organizations and the pharma industry, adopt high throughput automated patch clamp in their repertoire of equipment, more researchers will have access to these instruments to facilitate their research into ion channel physiology, pathophysiology and drug discovery.

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Additional information

Data availability statement

All data are included in the figures, statistical analysis is included in the manuscript and uploaded in the statistical summary table. Raw data files used to construct the figures are available upon request from the corresponding author.

Competing interests

A. Obergrussberger, I. Rinke-Weiß, T. A. Goete, M. Rapedius, N. Brinkwirth, M. G. Rotardam, S. Friis & N. Fertig are all employees of Nanion Technologies, the manufacturers of the high throughput automated patch clamp devices described in this manuscript.

Author contributions

Experiments were performed at Nanion Technologies GmbH, Munich, Germany, University of Copenhagen, Denmark and SB Drug Discovery, Glasgow, UK. A.O., I. R.-W., N. Becker and S.A.P contributed to conception or design of the work, acquisition, analysis or interpretation of data for the work and drafting the work or revising it critically for important intellectual content. N.F. & D.D. contributed to conception or design of the work and drafting the work or revising it critically for important intellectual content. T.A.G., M.R., N. Brinkwirth, M.G.R., L.H., P.M., D.P., S.F. and N. Braun contributed to...
acquisition, analysis or interpretation of data for the work and drafting the work or revising it critically for important intellectual content. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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**Supporting information**

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

- Peer Review History
- Statistical Summary Document