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Abstract

Automated patch clamp (APC) technology was first developed at the turn of the millennium. The increased throughput it afforded promised a new paradigm in ion channel recordings: it offered the potential to overcome the time-consuming, low-throughput bottleneck arising from manual patch clamp (MPC) investigations. This has relevance to the fast-paced development of novel therapies for chronic pain. This review highlights the advances in technology, using select examples, that have facilitated APC usage in both industry and academia. It covers both first generation and the latest developments in second-generation platforms. In addition, it also provides an overview of the pain research field and how APC platforms have furthered our understanding of ion channel research and the development of pharmacological tools and therapeutics. APC platforms have much to offer the ion channel research community and this review highlights areas of 'best practice' for both academia and industry. The impact of APC platforms and the prospects for chronic pain ion channel research and improved therapeutics will be evaluated.

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Figures: 2

Tables: 3

Abbreviations: APC: automated patch clamp; CHO: Chinese hamster ovary; CRO: contract research organisation; d.p.: data points; HEK293: human embryonic kidney 293; hERG: human ether-a-go-go-related gene; HTS: high throughput screen; MPC: manual patch clamp; MTS: medium throughput screen; PPC: population patch clamp.

Introduction

The global neuropathic pain market for 2015 was estimated at $5.2Bn, with a projected growth to $8.3Bn by 2024 (Kumar, 2016). A diverse range of ion channels are key molecular targets in combating chronic pain (for reviews see Mathie, 2010; Tibbs et al., 2016; see also other articles in this Themed Issue for further detail). Typically, ion channel research and electrophysiological assays are performed by measuring ionic currents from single, adherent cells via the manual patch clamp (MPC) technique (Hamill et al., 1981; Sakmann and Neher, 1984). While MPC is still held as the benchmark for electrophysiologists, a major drawback is that it requires significant training and technical capability. This is becoming increasingly problematic in academia with a focus on deliverables and feasibility of research programs (Nurse, 2015). Further, MPC is time-consuming and provides very low throughput (~ 20-40 data points, d.p./day; where a d.p. is defined as a current recording under different test conditions e.g. a four-point cumulative drug concentration response would be five d.p., control current, plus four drug modulated currents), hampering the ability to study ion channels and develop drugs targeting ion channels in an efficient and timely manner.

To overcome these technical and low throughput hurdles, several automated patch clamp (APC) technologies were developed in the late 1990s and early 2000s (for reviews on APC technologies see Priest et al., 2004; Dunlop et al., 2008; Milligan et al., 2009; Terstappen et al., 2010). The key development that lowered the technical capability, whilst dramatically increasing throughput, was applying ion channel expressing cells (via heterologous over-expression in e.g. HEK293 cells) in suspension to planar arrays of recording sites (or planar recording chips; see Perkel, 2010). In this way, instead of micro-manipulating a glass electrode pipette onto a single, adherent cell (see Figure 1A), cell suspensions are applied to planar recording chips with multiple recording sites and would settle on recording sites via a combination of gravity and negative pressures (see Figure 1B). Once settled onto the recording aperture, increased negative pressure induces the formation of high electrical resistance seals (100s MΩ – several GΩ) the hallmark of high-quality patch clamp recordings. Lowering the required technical capability democratized the ability to record ion channel currents - though the high costs of APC platforms (typically > £100k) and single-use recording chips has, in general, restricted use of the technology to pharmaceutical industry drug discovery or the best funded academic groups. Though there is evidence of chip re-use this has not been robustly demonstrated for inclusion in either academic or industrial research groups (Kao et al., 2012).

In this review of APC technology examples from academia and industry will be presented, highlighting key developments that have advanced our understanding of pain-related ion channels and how they have been assayed and targeted to develop improved pain therapeutics.

Evolution of APC platforms – first generation

APC platforms have evolved over almost two decades (Figure 2). Briefly, the first APC platforms used one of three different recording formats:

i. automated recording electrode pipette-to-cell recordings, mimicking MPC recordings via robotics (Apatchi-1, Sophion A/S, Denmark; Asmild et al., 2003);
ii. cell suspensions inside inverted pipettes, each forming a seal with a single cell from the suspension e.g. AutoPatch, CeNeS (acquired by Xention), (Mathes, 2003); Flyscreen, Flyion GmbH, Germany; (Lepple-Wienhues et al. 2003);
iii. cell suspensions applied via automated pipettors to planar arrays of recording sites (planar recording chips) e.g. Patchliner, Nanion Technologies GmbH, Germany, (Brüggemann et al., 2006); SyncroPatch96 Nanion Technologies, (Stoelzle et al., 2011); QPatch, Sophion A/S, Denmark, (Asmild et al., 2003; Mathes et al., 2009); PatchXpress, Molecular Devices Corp. (MDC), USA, (Xu et al., 2003; Tao et al., 2004), IonWorks, MDC, USA, (Schroeder et al., 2003; Finkel et al., 2006); CytoPatch, Cytocentrics AG, Germany, (Scheel et al., 2011) and IonFlux Fluxion Biosciences, USA, (Spencer et al., 2012).

It is now generally agreed that the most effective and successful of these recording formats was the application of cell suspensions to multiple recording sites on planar recording chips, and became the standard format that subsequent APC platforms would adopt (see Obergrussberger et al., 2016). In this format, multiple recordings could be achieved simultaneously in a single experimental run.

This first generation of APC platforms using planar recording chips gave researchers a greater throughput: the ~20-40 d.p./day of MPC was now improved to 10 to 100-fold greater on APC platforms (see Table 1). To achieve the highest throughput of several thousand d.p./day, compromises were made on some of the APC platforms (see Table 1). These compromises may have impacted on the uptake of APC in academia. For instance, the first APC platforms offering 384 simultaneous recordings made use of a 'loose-seal' (typically ~100MΩ) configuration in contrast to GΩ seals routinely obtained in high quality MPC recordings (Hamill et al., 1981). The PatchXpress had lower throughput, with 16 simultaneous recordings, but had the advantage of GΩ seal. On other APC platforms, such as the PatchLiner (Brüggemann et al., 2006) and the SyncroPatch96 (Stoelzle et al., 2011) GΩ seals were achievable, but required the presence of a 'seal enhancer'. This 'seal enchancer' solution contains a high Ca^{2+} concentration (e.g. 40 mM), which is problematic for ion channels that are sensitive and/or modulated by Ca^{2+}. (It should be noted, however, that these seal enhancing solutions are routinely used only at the very start of recording, during seal formation, after which they are washed away using onboard fluidics capabilities that the PatchLiner and SyncroPatch96 possess). Another limitation of the some of the early platforms involved the recording volumes and the ability to wash out test compounds. For example, IonWorks HT (and its later generation sibling IonWorks Quattro) has a fixed recording well volume (~ 20 μl) and is limited to two applications of test solutions to the recording site; consequently, this platform does not have a wash-through capability of test solutions (Schroeder et al., 2003; Finkel et al., 2006), a routine capability in MPC. A pertinent example of a limitation that fixed well-volume causes is the 'lipid-sink effect' reported by (Bridgland-Taylor et al., 2006); excess cells in the

recording well that are not sealed onto a recording site act as 'lipid-sinks' for highly lipophilic compounds (e.g. terfenadine, astemizole). This results in lower potencies of these lipophilic compounds being reported than when using MPC, due to the lowered free concentration of the compounds.

Evolution of APC platforms – second generation

Many of the compromises that allowed for the higher throughput of the first APC platforms have been addressed in the second generation. A summary of the capabilities of these second-generation APC platforms is given in Table 2. For example, the limited fluidics that the first 384-recording capable APC platform IonWorks employed were improved in its later generation IonWorks Barracuda (Gillie et al., 2013; Kuryshev et al., 2014). Similarly, Nanion's SyncroPatch 384 PatchEngine (384PE, Obergrussberger et al., 2016) and Sophion's Qube (Chambers et al., 2016) also employ micro-fluidics channels to allow low-volume, rapid exchange of solutions around the cell recording sites. The second-generation APC platforms maintain high-throughput (384 or 768 simultaneous recordings) whilst achieving the high-quality GΩ seal recordings, though the 384PE does still require the high Ca^{2+} 'seal enhancer'. Through developments in cell dissociation and cell suspension maintenance, the requirement for high Ca^{2+} 'seal enhancer' on the 384PE has been reduced recently, with seals of several hundred MΩ routinely achieved without 'seal enhancer' in many ion channel expressing cell lines (Dr. Alison Obergrussberger, Nanion, personal communication). On both the Qube and 384PE, current-clamp recordings are also possible. Finally, with increased throughput, often accompanied by improved recording success rates with greater knowledge of cell culturing, dissociation and recording solutions, the cost per d.p. has reduced. First generation APCs have an estimated cost per d.p. ranging from €0.31 - €1.50, whilst second generation now range from 3-10-fold less than those costs (€0.10 - €0.16; see Table 2).

Additional APC capabilities advancing ion channel research

Whilst compromises were needed in APC platforms to enable lowering of the technical requirement and increased throughput, the design and engineering of the planar array format of APC technology generated many advantages, providing researchers with several additional capabilities as standard, which would not routinely be standard capabilities or readily achievable in MPC.

The following list summarises the additional capabilities that APC technology allows:

Internal cell solution perfusion – Patchliner, SyncroPatch96, 384PE and Qube all allow exchange of the internal cell solution during recordings. For instance, TREK-1 channel modulation via intracellular pH was demonstrated using the 384PE (Sauter et al., 2016). In another example, activation of TRPC5 channels was shown on the 384PE by perfusing Ca^{2+} containing internal solutions (Brinkwirth et al., 2017). While this is possible in MPC, it is only via internal pipette solution dialysis, meaning control of the timing of dialysis is limited and is a one-time event after achieving whole-cell access to the recording cell.

Temperature control – The flexibility of the temperature control on APC platforms allows for two operational modes: (i) both the recording chamber perfusate and pipette solution to be heated simultaneously, allowing for maintenance of physiological temperatures, or (ii) the intracellular solution can be heated alone, allowing for the study of heat activated channels. This has been demonstrated effectively in TRPV1 and TPRV3 studies with the Patchliner platform (Papakosta et al., 2011; Stoelzle et al., 2011). The biophysical and pharmacological effects of temperature on hERG (Kv11.1) ion channels has also been shown using IonFlux (Golden et al., 2011; Kauthale et al., 2015). Once again, though possible in MPC, it requires specific add-on temperature control units.

Fluidics – The use of micro-fluidic channels (e.g. IonFlux, QPatch, SyncroPatch, PE384, Qube) or low, fixed well-volumes (IonWorks) allows expensive, low quantity compounds and molecules (e.g. peptide toxin fractions see Klint et al., 2015; Shcherbatko et al., 2016b; Deuis et al., 2017) to be tested in limited, small volume applications. Micro-fluidic channels also provide fast external solution exchange rates (complete solution exchange between 10 – 50 ms), sufficiently fast to allow for recordings from fast desensitising ligand gated ion channels (e.g. nicotinic acetylcholine α7 receptors, Dunlop et al., 2007; Friis et al., 2009; Obergrussberger et al., 2014; Hao et al., 2015; Arias et al., 2016; P2X receptors, Shcherbatko et al., 2016a). Once again, though possible in MPC, it requires specific add-on microinjection units.

Planar chips – The development of a planar recording chip reduces the potential for mechanical noise that long, glass electrode pipettes used in MPC recordings may cause. Consequently, greater success rates for longer recordings (> 30 minutes) are possible on APC platforms (Milligan et al., 2009).

Population (ensemble or multi-hole) patch clamp – The ability to record the average current from a population or ensemble of multiple cell currents recorded from multiple recording sites in a single recording well. In this way, recording multiple cells gives improved recording success rates and averages variable ion channel expression across several cells (Finkel et al., 2006; Dale et al., 2007).

These advancements were guided by MPC capabilities, but have often surpassed existing MPC technologies (e.g. internal perfusion systems). APC technology now provides a robust recording capability to deliver a higher throughput.

Using APC platforms in ion channel research – the academic perspective

2006 saw the introduction of APC platforms with a Patchliner (Nanion) and the first QPatch (Sophion) taking up residence in academic laboratories. These first steps into automated electrophysiology were primarily development partnerships between APC manufacturers and academics, with academia employing full-time staff to run the platforms. This then led to the first characterization of APC (Patchliner, Nanion) versus MPC, to provide protocols and troubleshooting guidance for APC adopters (Milligan et al., 2009). This study highlighted the potential for HTS to become part of academic

laboratory research portfolios. However, it did also point to some drawbacks with regards to cell specific experiments and the need for raised concentrations of Ca^{2+} (e.g. Nanion's 'seal enhancer' solution) or F^- in the intracellular solutions. This has led to further publications with more focus on specific cell and channel subtypes to gauge where the APC can provide benefit in the academic environment when compared to current practices (Estacion et al., 2010; Becker et al., 2013; Haraguchi et al., 2015).

From a pain therapeutics perspective, the APC platforms have been used to good effect by various groups (for example, Xu et al., 2008; Miller and Aricescu, 2014; Klint et al., 2015; Deuis et al., 2017). These studies have examined a host of different molecular targets, ranging from transient receptor channels (TRPC5) to voltage gated sodium channels (Nav1.7). The Nav1.7 story that has developed over the last decade has been supported by APC platforms in both academia and industry (Klint et al., 2015; Alexandrou et al., 2016; Deuis et al., 2017). The data generated have provided a wealth of both structural and biophysical data. For instance, Klint et al. (2015) describe Nav1.7 channel inhibition by spider venom peptides on the QPatch system (Sophion): by employing APC to generate cumulative concentration response data, using heterologous expression systems (e.g. CHO cells), the researchers made the most of APC functionality, i.e. rapid, low volume exchange of extracellular solution; this solution exchange maximizes use of limited volumes of venom peptide.

Another molecular pain target that has been examined using APC platforms is the glycine receptor (Gilbert et al., 2009). Again, here it has been used for HTS of novel compounds that can modulate receptor function. They employed a twin strategy: the primary screen was based on fluorescent assays, with 'hits' confirmed by APC. Here an 80% success rate of recordings was reported in line with other published data (Milligan et al., 2009); additionally, 50 % of these recordings lasted for 10 minutes or more. These studies again display the efficiency of the APC, primarily employing heterologous expression systems. More recently, data has been published on primary cells and induced pluripotent stem cells (Haythornthwaite et al., 2012; Cao et al., 2016). Regarding pain therapeutics, Cao et al., 2016 used induced pluripotent stem cell-derived sensory neurons from patients with erythromelalgia, which opens up much needed relevant human models to investigation via APC. With these heterogeneous cell populations, the success rate varies, often reduced and similar with what is achievable by an experienced MPC electrophysiologist. It is of note these data were generated for sole industrial publications or industrial and academic partnerships.

GABA receptors have become relevant targets in pain therapeutics following a number of studies (Enna and McCarson, 2006; Knabl et al., 2008; Ji and Neugebauer, 2011; Kahle et al., 2016). To this end they have also received attention for recording on APC-based technology in academic labs. Most notably Miller and Aricescu (2014) provided the first 3D structure of a GABA receptor. In parallel with structural studies, functional data collected on the Port-a-Patch platform (Nanion) provided pharmacological concentration response data to a variety of modulators (Miller and Aricescu, 2014). Here the Port-a Patch was used not to facilitate HTS, but to provide access to functional data for labs that do not have the electrophysiological capabilities

and training required to perform MPC experiments. The Port-a-Patch is well suited to academic labs looking to complement existing structural or biochemical data with important functional read outs. Though technically not an APC platform, the Port-a-Patch uses the same planar technology developed on APC platforms (i.e. a static recording site, onto which cell suspensions are applied), providing a more accessible ion channel recording format for first time users (Fertig et al., 2009). Further, this ease of use makes it ideally suited as an educational tool to open the field of electrophysiology to undergraduate students. Here, low cost experiments could be set up to engage with students to reinforce taught physiological content, where previously MPC usage would only be considered for PhD students or postdoctoral researchers where training time is feasible.

While APC usage has been used and developed in academia, often it is in partnership with pharmaceutical companies. However, while this facilitated the early adoption of APC in academia it is something that has not become widely implemented. On the academic front, APC runs into difficulties: for greatest efficiency and research output, an APC would need to be used and maintained by a full-time researcher and would need to be performing numerous experiments several times per week (e.g. testing different modulating molecules, different ion channels, etc.). Most academic labs have neither the research support nor the throughput requirements to efficiently use an APC. However, one usage model that has been adopted by academia is that of a shared, core facility where several research groups club together to fund and generate the volume of experimental testing required – much like the usage model previously adopted for earlier, expensive technology platforms (e.g. confocal microscopy, DNA sequencing).

Using APC platforms to drive ion channel drug-discovery – the industry perspective

With the vastly increased drug testing throughput afforded by APC technology, pain drug discovery programmes in pharmaceutical and biotechnology labs were perfectly suited to adopt APC technology, driving medicinal chemistry in the iterative generation of improved drugs (i.e. improved in efficacy, target specificity, selectivity and safety profiles). Not surprisingly, the labs involved in ion channel drug discovery, or providing these screening capability services to the drug discovery industry (e.g. contract research organisations, CROs), were early adopters of this new electrophysiological recording technology.

In particular, given their role in pain pathways, the voltage-gated sodium channels (Nav1.7-1.9) have received significant R&D time, money and attention on a range of APC platforms (Castle et al., 2009). Thus, shortly after commercial availability, IonWorks HT started to be used for the first high throughput screens: for instance, a 21k screen of compounds selected for ion channel-like pharmacophores from a 700k compound library of small molecules was performed, searching for selective blockers of the neuropathic pain target Nav1.7 (Southan et al., 2008). A few years later the same group, targeting the same ion channel, performed a far larger screen of 130k compounds (Dr. Gary Clark, Charles River Labs, 2011, personal communication). The PatchExpress platform has been used in studies examining the pharmacology of

Nav1.8 channels. A combination of MPC and APC (PatchExpress) was used to demonstrate a novel pharmacological tool in pain research, through blockade of the Nav1.8 channel (Payne et al., 2015). The PatchExpress platform was also used by Lin et al. 2016, to generate data surrounding the biophysical identity of HEK293-Nav1.9 cells with an interest in tetracaine modulation (Lin et al., 2016). This study has offered valuable insight into the Nav1.9 mediated currents and will benefit both industrial and academic pain related research endeavors.

Equally important in the early adoption of APC technology for pain drug discovery was drug safety testing, and to this end the cardiac safety of drug libraries for hERG (Kv11.1) ion channel liability were also being screened on IonWorks HT (Sorota et al., 2005). For a comprehensive review of APC and identifying hERG liability, readers should see Danker and Möller (2014) and recent work towards a standardized hERG liability definition (Windley et al., 2017)

To researchers routinely performing high-throughput screens (HTS), these ion channel examples of 10 - 100k compound screens fall short of the usual compound library screens, with true HTS often running to millions of compounds screened. Consequently, APC technology has been used in many drug discovery programmes as a secondary screen to ensure that high-throughput compound 'hits' are modulating the ion channel target and not an artefactual response observed in the primary screen (e.g. compound auto-fluorescence in a fluorescent based assay). For instance, a fluorescent screen can be used to perform the larger, first (primary) HTS screen, followed by a secondary, smaller and more focused medium throughput screen (MTS) to verify the primary HTS compound 'hits' (e.g. Gilbert et al., 2009); for a review of this screening cascade see Terstappen et al., 2010. In this way pain-related ion channels have been targeted in drug discovery programmes with APC technology typically performing this secondary screen (i.e. compound activity verification) in the drug discovery cascade: e.g. HCN channels (Vasilyev et al., 2009); Nav channels (Trivedi et al., 2008; Klement et al., 2012; Bagal et al., 2014; Kornecook et al., 2017), Cav3 (T-type) calcium channels (Xie et al., 2007) and Cav2 (N-type) calcium channels (Swensen et al., 2012).

After the first generation paved the way, driving ion channel drug discovery via MTS, the second generation of APC platforms have been quickly harnessed in the search for improved analgesics. For instance, the Qube, whilst still in beta-testing, was used by Chambers et al. (2016) to perform a 158k compound screen to find compounds modulating Nav1.7 to find new small molecules to treat neuropathic pain.

Conclusions

With the advent of APC technology, data throughput has risen dramatically, whilst the technical ability needed to make ion channel recordings has been lowered. In both academia and industry this has led to significant advances in pain-related ion channel research.

For instance, all the pharmaceutical drug discovery programmes behind the drugs targeting ion channels in chronic pain states listed in Table 3 used APC screens and testing through their medicinal chemistry evolution. (For reviews of APC technology used in advancing pain ion channels targeted in drug discovery see Bagal et al., 2014 and Chambard et al., 2014). The drugs in Table 3 are a selection of the first of the post-APC generation of chronic pain therapeutics entering clinical trials, with the potential of attaining improved analgesia in chronic pain states, whilst avoiding the side effects associated with existing chronic pain therapies (e.g. opioids – side effects include addiction, constipation, over-dose; for a review see Stannard, 2011). These drugs (Table 3) may produce such a pain medicine, or at least provide the research foundations that can be built on. The improved recording capabilities that the second generation of APC technology are now providing will be critical to further accelerate the pace of future pain drug discovery programmes (Chambers et al., 2016; Obergrussberger et al., 2016).

At this early stage of the second generation of APCs, combined with publications from industry often lagging behind their latest research, it's hard to define the true potential that these platforms may bring to pain drug discovery. However, early adoption and data suggests that the higher throughput and cheaper cost per data point that they provide is already having a positive impact on drug discovery programmes. For example, the APC-based HTS that Chambers et al., 2016, performed would previously have been a two-step screen: an HT primary screen (e.g. via a fluorescent read-out), followed by an APC secondary screen, to rule-out artefactual screening 'hits'.

Without the consistent, higher throughput that drug discovery requires, academia APC adoption has not matched that of industry. However, in recent years' academia has developed a shared core facility approach to generate the volume of throughput needed to make APC use efficient. In this shared model, by forming consortia, APC platforms used as a core facility allows costs of purchase (of platform and consumables), maintenance and day-to-day running of the facility by full-time, trained users to be spread over several groups. Each individual academic group then has access to APC to advance their own individual or broader multi-group research programs. In addition, APC ion channel training workshops (e.g. Sophion and Nanion both host several APC user meetings across the globe) that bring together ion channel physiologists from academia and industry increase the potential for future partnerships using APC platforms to further our understanding of pain pathways and therapeutics.

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Competing interests

The authors declare no competing interests.

Figure Legends

Figure 1. Manual and planar patch clamp methodologies. *(**A**., top panel)* Manual patch clamp (MPC) involves the use of a glass pipette that is manoeuvred to an adherent cell ('Approach'). Contact is then made with the cell and through application of negative pressure an electrical seal forms, in the order of GΩ magnitude ('Attach'). Two recording formats ('Record') may then be employed: (i) whole cell format, achieved by further negative pressure, rupturing the membrane, causing the pipette solution (shaded blue) and cytosol to become one compartment that is electrically and chemically contiguous. (ii) perforated patch format uses membrane perforating agents (e.g. amphotericin-B, β-escin) to achieve electrical continuity with the cell membrane and pipette solution, and allows limited dialysis (based on perforation diameter: e.g. monovalent ions, but not larger molecules like sugars, ATP or peptides) of the pipette recording solution into the cytosol (denoted by mixed blue/beige shading). (**B**., *bottom panel*) Automated patch clamp (APC) uses cells in suspension that are applied to planar arrays of recording sites on the recording chip (for clarity only a single recording site is shown; 'Approach'). Under gravity and negative pressure an electrical seal forms between cell membrane and a micropore on the recording chip ('Attach'). Like MPC, APCs have the capability to record in both perforated patch (via perforating agents applied beneath the recording chamber; with dialysis limited by the size of the perforations created, denoted by the mixed red/beige shading) and whole cell format (via negative pressure applied to the recording site to give the whole cell configuration), whereby the electrode and recording solution (shaded red) situated beneath the recording site are made electrically and chemically contiguous with the cytosol ('Record'). (IonWorks, IonWorks Quattro and IonWorks Barracuda are limited to perforated patch format, without the ability to apply negative pressures to perform whole cell recordings).

Figure 2. Evolution of automated patch clamp platforms. A timeline showing the development of automated patch clamp (APC) platforms. Abbreviation: PPC – population patch clamp; platforms following IonWorks Quattro (on the market in 2005), the first PPC capable, have similar multi-hole (or ensemble) current recording capabilities.

Tables

Table 1. Key features of 1st generation APC platforms. A comparison of key features of the first generation of APC platforms. (Abbreviations: d.p./day – data points per day; PDMS – poly-dimethyl-siloxane).

Table 2. Key features of 2nd generation APC platforms. A comparison of key features of the second generation of APC platforms. Abbreviation: d.p./day – data points per day. Estimated cost/d.p. was provided by manufacturers (for Barracuda and 384PE) or estimated by the authors (for Qube); estimates are based on four-point cumulative

concentration responses and only account for recording consumable costs (i.e. no platform, service and maintenance costs were included).

Table 3. APC platforms in pain research. A selection of drugs targeting pain-related ion channels developed using APC platforms and their respective stage of development.

References

Alexandrou, A.J., Brown, A.R., Chapman, M.L., Estacion, M., Turner, J., Mis, M.A., et al. (2016). Subtype-selective small molecule inhibitors reveal a fundamental role for Nav1.7 in nociceptor electrogenesis, axonal conduction and presynaptic release. PLoS One *11*: e0152405.

Arias, H.R., Ravazzini, F., Targowska-Duda, K.M., Kaczor, A.A., Feuerbach, D., Boffi, J.C., et al. (2016). Positive allosteric modulators of α7 nicotinic acetylcholine receptors affect neither the function of other ligand- and voltage-gated ion channels and acetylcholinesterase, nor β-amyloid content. Int. J. Biochem. Cell Biol. *76*: 19–30.

Asmild, M., Oswald, N., Krzywkowski, K.M., Friis, S., Jacobsen, R.B., Reuter, D., et al. (2003). Upscaling and automation of electrophysiology: toward high throughput screening in ion channel drug discovery. Receptors Channels *9*: 49–58.

Bagal, S.K., Chapman, M.L., Marron, B.E., Prime, R., Storer, R.I., and Swain, N.A. (2014). Recent progress in sodium channel modulators for pain. Bioorg. Med. Chem. Lett. *24*: 3690–3699.

Becker, N., Stoelzle, S., Göpel, S., Guinot, D., Mumm, P., Haarmann, C., et al. (2013). Minimized cell usage for stem cell-derived and primary cells on an automated patch clamp system. J. Pharmacol. Toxicol. Methods *68*: 82–87.

Bridgland-Taylor, M.H., Hargreaves, A.C., Easter, A., Orme, A., Henthorn, D.C., Ding, M., et al. (2006). Optimisation and validation of a medium-throughput electrophysiology-based hERG assay using IonWorks^{TM} HT. J. Pharmacol. Toxicol. Methods *54*: 189–199.

Brinkwirth, N., Friis, S., Goetze, T., Rapedius, M., Costantin, J., Brüggemann, A., et al. (2017). Investigation of the Ion Channels TMEM16A and TRPC5 and their Modulation by Intracellular Calcium. Biophys. J. *112*: 413a.

Brüggemann, A., Stoelzle, S., George, M., Behrends, J.C., and Fertig, N. (2006). Microchip technology for automated and parallel patch-clamp recording. Small *2*: 840–846.

Cao, L., McDonnell, A., Nitzsche, A., Alexandrou, A., Saintot, P.-P.P.-P., Loucif, A.J.C.C., et al. (2016). Pharmacological reversal of a pain phenotype in iPSC-derived sensory neurons and patients with inherited erythromelalgia. Sci. Transl. Med. *8*: 335ra56-335ra56.

Castle, N., Printzenhoff, D., Zellmer, S., Antonio, B., Wickenden, A., and Silvia, C. (2009). Sodium channel inhibitor drug discovery using automated high throughput electrophysiology platforms. Comb. Chem. High Throughput Screen. *12*: 107–22.

Chambard, J.-M., Tagat, E., Boudeau, P., and Partiseti, M. (2014). Transforming TRP channel drug discovery using medium-throughput electrophysiological assays. J. Biomol. Screen. *19*: 468–77.

Chambers, C., Witton, I., Adams, C., Marrington, L., and Kammonen, J. (2016). High-Throughput Screening of Na $_{V}$ 1.7 Modulators Using a Giga-Seal Automated Patch Clamp Instrument. Assay Drug Dev. Technol. *14*: 93–108.
Dale, T.J., Townsend, C., Hollands, E.C., and Trezise, D.J. (2007). Population patch clamp electrophysiology: a breakthrough technology for ion channel screening. Mol. Biosyst. *3*: 714.
Danker, T., and Möller, C. (2014). Early identification of hERG liability in drug discovery programs by automated patch clamp. Front. Pharmacol. *5*: 203.
Deuis, J.R., Dekan, Z., Wingerd, J.S., Smith, J.J., Munasinghe, N.R., Bhola, R.F., et al. (2017). Pharmacological characterisation of the highly NaV1.7 selective spider venom peptide Pn3a. Sci. Rep. *7*: 40883.
Dunlop, J., Bowlby, M., Peri, R., Vasilyev, D., and Arias, R. (2008). High-throughput electrophysiology: an emerging paradigm for ion-channel screening and physiology. Nat. Rev. Drug Discov. *7*: 358–368.
Dunlop, J., Roncarati, R., Jow, B., Bothmann, H., Lock, T., Kowal, D., et al. (2007). In vitro screening strategies for nicotinic receptor ligands. Biochem. Pharmacol. *74*: 1172–1181.
Enna, S.J., and McCarson, K.E. (2006). The Role of GABA in the Mediation and Perception of Pain. Adv. Pharmacol. *54*: 1–27.
Estacion, M., Choi, J.S., Eastman, E.M., Lin, Z., Li, Y., Tyrrell, L., et al. (2010). Can robots patch-clamp as well as humans? Characterization of a novel sodium channel mutation. J. Physiol. *588*: 1915–1927.
Fertig, N., Bruggemann, A., Kreir, M., George, M., Stoelzle, S., Haarmann, C., et al. (2009). Port-a-Patch and Patchliner: High Fidelity Electrophysiology for Secondary Screening and Safety Pharmacology. Comb. Chem. High Throughput Screen. *12*: 24–37.
Finkel, A., Wittel, A., Yang, N., Handran, S., Hughes, J., and Costantin, J. (2006). Population Patch Clamp Improves Data Consistency and Success Rates in the Measurement of Ionic Currents. J. Biomol. Screen. *11*: 488–496.
Friis, S., Mathes, C., Sunesen, M., Bowlby, M.R., and Dunlop, J. (2009). Characterization of compounds on nicotinic acetylcholine receptor alpha7 channels using higher throughput electrophysiology. J. Neurosci. Methods *177*: 142–8.
Gilbert, D.F., Islam, R., Lynagh, T., Lynch, J.W., and Webb, T.I. (2009). High Throughput Techniques for Discovering New Glycine Receptor Modulators and their Binding Sites. Front. Mol. Neurosci. *2*: 17.
Gillie, D.J., Novick, S.J., Donovan, B.T., Payne, L.A., and Townsend, C. (2013). Development of a high-throughput electrophysiological assay for the human ether-à-go-go related potassium channel hERG. J. Pharmacol. Toxicol. Methods *67*: 33–44.
Golden, A.P., Li, N., Chen, Q., Lee, T., Nevill, T., Cao, X., et al. (2011). IonFlux: a microfluidic patch clamp system evaluated with human Ether-à-go-go related gene channel physiology and pharmacology. Assay Drug Dev. Technol. *9*: 608–19.
Hamill, O.P., Marty, A., Neher, E., Sakmann, B., and Sigworth, F.J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch. - Eur. J. Physiol. *391*: 85–100.
Hao, Y., Tang, J., and Wang, K. (2015). Development of Automated Patch Clamp Assay for Evaluation of α7 Nicotinic Acetylcholine Receptor Agonists in Automated QPatch-16. Assay Drug Dev. Technol. *13*: 174–184.

Haraguchi, Y., Ohtsuki, A., Oka, T., Shimizu, T., Kola, I., Landis, J., et al. (2015). Electrophysiological analysis of mammalian cells expressing hERG using automated 384-well-patch-clamp. BMC Pharmacol. Toxicol. *16*: 39.
Haythornthwaite, A., Stoelzle, S., Hasler, A., Kiss, A., Mosbacher, J., George, M., et al. (2012). Characterizing human ion channels in induced pluripotent stem cell-derived neurons. J. Biomol. Screen. *17*: 1264–72.
Ji, G., and Neugebauer, V. (2011). Pain-related deactivation of medial prefrontal cortical neurons involves mGluR1 and GABA(A) receptors. J. Neurophysiol. *106*: 2642–52.
Kahle, K.T., Schmouth, J., Lavastre, V., Latremoliere, A., Zhang, J., Andrews, N., et al. (2016). Inhibition of the kinase WNK1/HSN2 ameliorates neuropathic pain by restoring GABA inhibition. Sci. Signal. *9*: ra32.
Kao, L., Abuladze, N., Shao, X.M., McKeegan, K., and Kurtz, I. (2012). A new technique for multiple re-use of planar patch clamp chips. J. Neurosci. Methods *208*: 205–210.
Kauthale, R.R., Dadarkar, S.S., Husain, R., Karande, V. V, and Gatne, M.M. (2015). Assessment of temperature-induced hERG channel blockade variation by drugs. J. Appl. Toxicol. *35*: 799–805.
Klement, G., Babich, O., Larsson, O., Lund, P.-E., Malmberg, A., Sandberg, L., et al. (2012). Identification of novel NaV1.7 antagonists using high throughput screening platforms. Comb. Chem. High Throughput Screen. *15*: 713–20.
Klint, J.K., Chin, Y.K.-Y., and Mobli, M. (2015). Rational Engineering Defines a Molecular Switch That Is Essential for Activity of Spider-Venom Peptides against the Analgesics Target NaV1.7. Mol. Pharmacol. *88*: 1002–1010.
Knabl, J., Witschi, R., Hösl, K., Reinold, H., Zeilhofer, U.B., Ahmadi, S., et al. (2008). Reversal of pathological pain through specific spinal GABAA receptor subtypes. Nature *451*: 330–334.
Kornecook, T.J., Yin, R., Altmann, S., Be, X., Berry, V., Ilch, C.P., et al. (2017). Pharmacologic Characterization of AMG8379, a Potent and Selective Small Molecule Sulfonamide Antagonist of the Voltage-Gated Sodium Channel NaV1.7. J. Pharmacol. Exp. Ther.
Kumar, R. (2016). Global Market Study on Neuropathic Pain: Anticonvulsants Drug Class Segment Projected to Witness the Highest Growth Through 2024. Persistence Mark. Res. 1–145.
Kuryshev, Y.A., Brown, A.M., Duzic, E., and Kirsch, G.E. (2014). Evaluating State Dependence and Subtype Selectivity of Calcium Channel Modulators in Automated Electrophysiology Assays. Assay Drug Dev. Technol. *12*: 110–119.
Lepple-Wienhues, A., Ferlinz, K., Seeger, A., and Schäfer, A. (2003). Flip the tip: an automated, high quality, cost-effective patch clamp screen. Receptors Channels *9*: 13–17.
Lin, Z., Santos, S., Padilla, K., Printzenhoff, D., Castle, N.A., and West, C. (2016). Biophysical and Pharmacological Characterization of Nav1.9 Voltage Dependent Sodium Channels Stably Expressed in HEK-293 Cells. PLoS One *11*: e0161450.
Mathes, C. (2003). Ion channels in drug discovery and development. Drug Discov. Today *8*: 1022–1024.
Mathes, C., Friis, S., Finley, M., and Liu, Y. (2009). QPatch: the missing link between HTS and ion channel drug discovery. Comb. Chem. High Throughput Screen. *12*: 78–

95.
Mathie, A. (2010). Ion channels as novel therapeutic targets in the treatment of pain. J. Pharm. Pharmacol. *62*: 1089–95.
Miller, P.S., and Aricescu, A.R. (2014). Crystal structure of a human GABAA receptor. Nature *512*: 270–275.
Milligan, C.J., Li, J., Sukumar, P., Majeed, Y., Dallas, M.L., English, A., et al. (2009). Robotic multiwell planar patch-clamp for native and primary mammalian cells. Nat. Protoc. *4*: 244–255.
Nurse, P. (2015). Ensuring a successful UK research endeavour - A Review of the UK Research Councils.
Obergrussberger, A., Bru ggemann, A., Goetze, T.A., Rapedius, M., Haarmann, C., Rinke, I., et al. (2016). Automated Patch Clamp Meets High-Throughput Screening: 384 Cells Recorded in Parallel on a Planar Patch Clamp Module. J. Lab. Autom. *21*: 779–793.
Obergrussberger, A., Brüggemann, A., Goetze, T.A., Rapedius, M., Haarmann, C., Rinke, I., et al. (2015). Automated Patch Clamp Meets High-Throughput Screening: 384 Cells Recorded in Parallel on a Planar Patch Clamp Module. J. Lab. Autom. EPub ahead of Print.
Obergrussberger, A., Haarmann, C., Rinke, I., Becker, N., Guinot, D., Brueggemann, A., et al. (2014). Automated patch clamp analysis of nAChα7 and Nav1.7 channels. Curr. Protoc. Pharmacol. *65*: 11.13.1-48.
Papakosta, M., Dalle, C., Haythornthwaite, A., Cao, L., Stevens, E.B., Burgess, G., et al. (2011). The Chimeric Approach Reveals That Differences in the TRPV1 Pore Domain Determine Species-specific Sensitivity to Block of Heat Activation. J. Biol. Chem. *286*: 39663–39672.
Payne, C.E., Brown, A.R., Theile, J.W., Loucif, A.J.C., Alexandrou, A.J., Fuller, M.D., et al. (2015). A novel selective and orally bioavailable Na $_{v}$ 1.8 channel blocker, PF-01247324, attenuates nociception and sensory neuron excitability. Br. J. Pharmacol. *172*: 2654–2670.
Perkel, J.M. (2010). High-throughput ion channel screening: A 'patch'-work solution. Biotechniques *48*: 25–29.
Priest, B.T., Cerne, R., Krambis, M.J., Schmalhofer, W.A., Wakulchik, M., Wilenkin, B., et al. (2004). Automated Electrophysiology Assays (Eli Lilly & Company and the National Center for Advancing Translational Sciences).
Sakmann, B., and Neher, E. (1984). Patch clamp techniques for studying ionic channels in excitable membranes. Annu. Rev. Physiol. *46*: 455–472.
Sauter, D.R.P., Sørensen, C.E., Rapedius, M., Brüggemann, A., and Novak, I. (2016). pH-sensitive K(+) channel TREK-1 is a novel target in pancreatic cancer. Biochim. Biophys. Acta *1862*: 1994–2003.
Scheel, O., Himmel, H., Rascher-Eggstein, G., and Knott, T. (2011). Introduction of a modular automated voltage-clamp platform and its correlation with manual human Ether-à-go-go related gene voltage-clamp data. Assay Drug Dev. Technol. *9*: 600–7.
Schroeder, K., Neagle, B., Trezise, D.J., and Worley, J. (2003). IonWorks (TM) HT: A new high-throughput electrophysiology measurement platform. J. Biomol. Screen. *8*: 50–64.
Shcherbatko, A., Foletti, D., Poulsen, K., Strop, P., Zhu, G., Hasa-Moreno, A., et al. (2016a). Modulation of P2X3 and P2X2/3 Receptors by Monoclonal Antibodies. J.

Biol. Chem. *291*: 12254–12270.
Shcherbatko, A., Rossi, A., Foletti, D., Zhu, G., Bogin, O., Galindo Casas, M., et al. (2016b). Engineering Highly Potent and Selective Microproteins against Nav1.7 Sodium Channel for Treatment of Pain. J. Biol. Chem. *291*: 13974–13986.
Sorota, S., Zhang, X.-S., Margulis, M., Tucker, K., and Priestley, T. (2005). Characterization of a hERG Screen Using the IonWorks HT: Comparison to a hERG Rubidium Efflux Screen. Assay Drug Dev. Technol. *3*: 47–57.
Southan, A., Clark, G., Maidment, S., Easthope, E., Ashcroft, K., Slater, M., et al. (2008). Voltage-gated Na Channels. Biophys. J. *94*: 1032.
Spencer, C., Li, N., Chen, Q., and Johnson, J. (2012). Ion channel pharmacology under flow: automation via well-plate microfluidics. Assay Drug Dev. Technol. *10*: 313–324.
Stannard, C.F. (2011). Opioids for chronic pain: promise and pitfalls. Curr. Opin. Support. Palliat. Care *5*: 150–157.
Stoelzle, S., Obergrussberger, A., Brüggemann, A., Haarmann, C., George, M., Kettenhofen, R., et al. (2011). State-of-the-Art Automated Patch Clamp Devices: Heat Activation, Action Potentials, and High Throughput in Ion Channel Screening. Front. Pharmacol. *2*: 76.
Swensen, A.M., Herrington, J., Bugianesi, R.M., Dai, G., Haedo, R.J., Ratliff, K.S., et al. (2012). Characterization of the Substituted N-Triazole Oxindole TROX-1, a Small-Molecule, State-Dependent Inhibitor of Cav2 Calcium Channels. Mol. Pharmacol. *81*:.
Tao, H., Santa Ana, D., Guia, A., Huang, M., Ligutti, J., Walker, G., et al. (2004). Automated tight seal electrophysiology for assessing the potential hERG liability of pharmaceutical compounds. Assay Drug Dev. Technol. *2*: 497–506.
Terstappen, G.C., Roncarati, R., Dunlop, J., and Peri, R. (2010). Screening technologies for ion channel drug discovery. Future Med. Chem. *2*: 715–730.
Tibbs, G.R., Posson, D.J., and Goldstein, P.A. (2016). Voltage-Gated Ion Channels in the PNS: Novel Therapies for Neuropathic Pain? Trends Pharmacol. Sci. *37*: 522–542.
Trivedi, S., Dekermendjian, K., Julien, R., Huang, J., Lund, P.-E., Krupp, J., et al. (2008). Cellular HTS assays for pharmacological characterization of Na(V)1.7 modulators. Assay Drug Dev. Technol. *6*: 167–79.
Vasilyev, D. V., Shan, Q.J., Lee, Y.T., Soloveva, V., Nawoschik, S.P., Kaftan, E.J., et al. (2009). A Novel High-Throughput Screening Assay for HCN Channel Blocker Using Membrane Potential-Sensitive Dye and FLIPR. J. Biomol. Screen. *14*: 1119–1128.
Windley, M.J., Abi-Gerges, N., Fermini, B., Hancox, J.C., Vandenberg, J.I., and Hill, A.P. (2017). Measuring kinetics and potency of hERG block for CiPA. J. Pharmacol. Toxicol. Methods.
Xie, X., Deusen, A.L. Van, Vitko, I., Babu, D.A., Davies, L.A., Huynh, N., et al. (2007). Validation of High Throughput Screening Assays Against Three Subtypes of Ca_v3 T-Type Channels Using Molecular and Pharmacologic Approaches. Assay Drug Dev. Technol. *5*: 191–204.
Xu, J., Guia, A., Rothwarf, D., Huang, M., Sithiphong, K., Ouang, J., et al. (2003). A Benchmark Study with Seal Chip ™ Planar. Assay Drug Dev. Technol. *1*: 675–684.
Xu, S.-Z., Sukumar, P., Zeng, F., Li, J., Jairaman, A., English, A., et al. (2008). TRPC channel activation by extracellular thioredoxin. Nature *451*: 69–72.