A GUIDE TO DRUG DISCOVERY

High-throughput electrophysiology: an emerging paradigm for ion-channel screening and physiology

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Abstract | Ion channels represent highly attractive targets for drug discovery and are implicated in a diverse range of disorders, in particular in the central nervous and cardiovascular systems. Moreover, assessment of cardiac ion-channel activity of new chemical entities is now an integral component of drug discovery programmes to assess potential for cardiovascular side effects. Despite their attractiveness as drug discovery targets ion channels remain an under-exploited target class, which is in large part due to the labour-intensive and low-throughput nature of patch-clamp electrophysiology. This Review provides an update on the current state-of-the-art for the various automated electrophysiology platforms that are now available and critically evaluates their impact in terms of ion-channel screening, lead optimization and the assessment of cardiac ion-channel safety liability.

Ion channels are a diverse family of membrane-spanning proteins that lower the free energy required for ions to traverse the plasma membrane. Ionic flux through ion channels provides the foundation for membrane excitability and neurotransmission that is essential for the proper functioning of neurons, cardiac and muscle cells. A precise calculation of the number of ion-channel drug targets and ion-channel targeted drugs varies widely depending on the criteria used for evaluation. However, a recent estimate places ligand-gated and voltage-gated channels as third and fourth (only behind G-protein coupled and nuclear receptors) in the top five gene families that are targeted by currently available drugs, constituting 7.9% and 5.5%, respectively[1]. More specifically, a wide range of ion channels can be explicitly identified as a primary molecular target in the action of many currently used drugs. These include the nicotinic, $GABA_A$ (γ-aminobutyric acid A) and NMDA (*N*-methyl-D-aspartate) ligand-gated channels, the voltage-gated Na^+, K^+ and Ca^{2+} channels, and the ryanodine, inositol 1,4,5-trisphosphate (IP_3) and transient receptor potential Ca^{2+} channel families[2]. Of the 18 new molecular entities approved by the US Food and Drug Administration in 2006 two had their primary mode of action attributed to ion-channel modulation: varenicline (Chantix/Champix; Pfizer), a partial nicotinic receptor agonist for smoking cessation, and lubiprostone (Amitiza; Sucampo/Takeda), a chloride channel activator for constipation[3]. This picture therefore indicates that ion channels are attractive and tractable drug discovery targets.

Despite this apparent validation of ion channels as druggable targets it is accepted in the field that ion-channel-targeted drug discovery has been hampered by the unavailability of high-throughput platforms that utilize electrophysiological techniques for the characterization of compound activity. Although other methods such as measurement of ion flux and use of fluorescent indicator dyes are widely used for ion-channel screening, electrophysiological techniques that control the voltage of the cellular membrane have distinct advantages. Electrophysiological techniques are particularly useful for voltage-gated channels, as the 'ligand' for these channels can then be precisely controlled. Traditionally, electrophysiologists have relied on the gold-standard assay of patch-clamp electrophysiology in mammalian cells (BOX 1) or two-electrode voltage clamp in *Xenopus* oocytes. These approaches are extremely information-rich, but they are also labour intensive, require highly skilled staff and can only support the evaluation of small numbers of compounds. Consequently, higher-throughput methodologies that use cell-based assays with membrane potential or Ca^{2+} sensitive dyes[4–7] or ion-flux measurements[8–11] have become integral components of ion-channel drug discovery programmes. Although these approaches have been useful in supporting efforts to identify and profile compounds,

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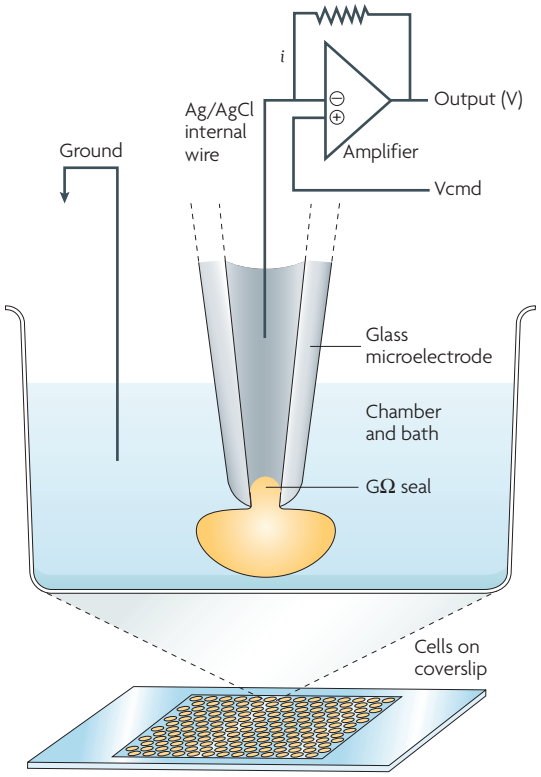
Box 1 | **Manual electrophysiology**

Conventional patch clamping, or manual electrophysiology, refers to the technique using glass microelectrodes patched on to the cell surface with giga-ohm (GΩ) seal resistance to gain electrical access to the cell interior allowing high-fidelity recordings of the ionic currents (*i*) passing through ion channels present in the plasma membrane. A typical electrophysiology rig would encompass a Faraday cage, a vibration isolation table, a microscope for imaging cells, micromanipulators for electrodes, low-noise amplifiers and some kind of drug perfusion device for introducing solutions. In this format, a single cell at a time is patched with a borosilicate glass microelectrode to form a GΩ seal, and subsequently a small patch of plasma membrane under the seal is ruptured by negative pressure to achieve access to the cell interior. This provides excellent dynamic range for voltage control (Vcmd) of the cell membrane, and is considered to be the gold standard for electrophysiological recordings. However, limitations to this approach come both in terms of throughput and relative ease of use; the single-cell format provides for a very low throughput and the methodology itself is technically challenging, requiring the continuous presence of a skilled operator. Conversely, the manual approach allows for a high degree of flexibility in the recording configurations including cell-attached, whole-cell, detached patch, inside-out and outside-out.



they represent indirect measures of ion-channel activity and often generate data that do not correlate well with data from electrophysiological measures. These caveats have resulted in a continued reliance on the gold-standard assay for confirmation of compound activity and efficacy to the point that this has created a significant bottleneck in the drug discovery process.

To address this bottleneck, several companies have developed and introduced in the past 5 years automated platforms for performing electrophysiological studies in mammalian cell lines and oocytes. These include both single-channel and multichannel systems for conventional and perforated patch-clamp electrophysiology in mammalian cells, essentially replacing the 'top-down' access to cells via micropipettes in manual recordings with a 'bottom-up' configuration to allow compatibility with plate or chip-based formats (FIG. 1). The implementation of these technologies is now evident in both ion-channel drug discovery and cardiac liability profiling, holding the promise to dramatically impact the delivery of new ion-channel-targeted therapeutics. Finally, systems that are capable of automated brain-slice field recording experiments, as well as those capable of network recordings, are extending the reach of high-capacity electrophysiology to native tissues.

This Review addresses the critical importance of electrophysiological recording methods to ion-channel drug discovery as illustrated by examples of cardiac safety screening, lead optimization and use in higher-order systems to rigorously study physiology.

Faraday cage
An enclosure for blocking out external static electrical fields, made from a conducting material. It is named after the nineteenth-century physicist Michael Faraday.

Automating conventional electrophysiology

The discovery of high-resistance giga-ohm patch-clamp recordings[12], accompanied with advances in micromanipulation and low-noise amplifiers, opened the scientific floodgates to the world of ion channels and bioelectricity. However, conventional (or manual) electrophysiology, utilizing non-planar approaches, as discussed later, is still an intimidating, labour intensive, technically challenging discipline that integrates the art of micromanipulation and giga-ohm seal formation to the science of channel investigation. Attempts to automate this process started in the late 1990s at NeuroSearch with the invention of NeuroPatch that later evolved as Apatchi 1 (reviewed in REF. 13).

Subsequently, a limited number of systems have been reported that fully automate conventional manual patch-clamp recordings with glass microelectrodes, including patch-pipette handling, giga-ohm seal formation, obtaining whole-cell configuration, drug application and data acquisition. These include the Flyscreen[14], AutoPatch and RoboPatch[15,16] for mammalian cells, and the Robocyte[17] and OpusXpress 6000A[18] for *Xenopus* oocytes (TABLE 1).

Validation of automated conventional electrophysiology platforms. Among these automated systems, the RoboPatch, although not commercially available, has been validated for the study of ligand-gated and voltage-gated ion channels. These have appropriate electrophysiological and pharmacological properties when compared with manual patch clamp, as demonstrated for the

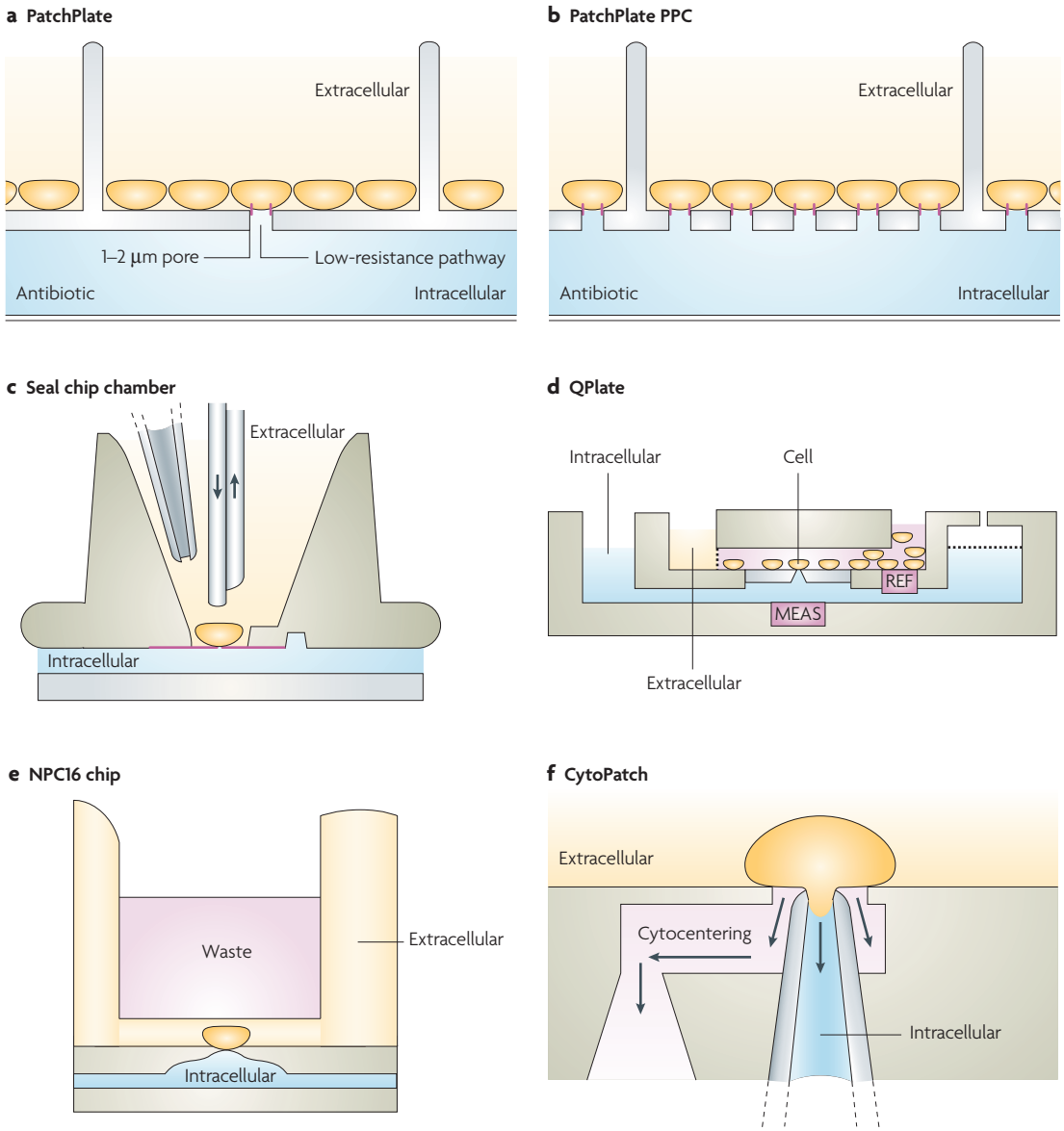


Figure 1 | **Cross-section schematics of chips and flow channels used in automated electrophysiology platforms illustrate the diverse approaches taken. a** | PatchPlate used in IonWorks HT. **b** | PatchPlate population patch-clamp (PPC) used in IonWorks Quattro. **c** | Seal chip used in PatchXpress. **d** | QPlate used in QPatch. **e** | NPC16 chip used in Patchliner NPC16. **f** | CytoPatch chip used in CytoPatch. The QPlate, Cytochip and NPC16 chip have a flow channel built into the chip that enables fluid exchanges. Seal chips are well-based and fluid exchange is achieved using pumps. PatchPlates utilized in IonWorks HT and Quattro have no perfusion capability and only 1–2 additions of compound is possible to each well. The first-generation IonWorks HT uses a single hole in each well of the 384-well PatchPlate, whereas the second-generation Quattro incorporates 64 holes per well, termed the PPC mode. REF and MEAS refer to reference and measurement electrodes, respectively.

voltage-gated hERG (human ether-a-go-go-related gene product) channel and the rapidly desensitizing ligand-gated α7 nicotinic receptor channel[16]. Preliminary validation has been presented for the Flyscreen for the assessment of various cell lines and channels, although extensive pharmacological characterization is currently lacking[14]. All systems discussed are equivalent to manual patch-clamp recordings in terms of data quality, including true giga-ohm seal formation, small access resistance and stability of recordings. A higher than normal throughput for manual patch-clamp recordings in mammalian cells can also be realized by integration of the Dynaflow system from Cellectricon with a conventional manual recording system.

In addition to the systems described above for automating conventional patch clamp in mammalian cells, two approaches to the automation of two-electrode voltage-clamp recordings in the *Xenopus* oocyte expression system have been described. Validation of the Robocyte for higher-throughput electrophysiological screening has been reported[17], whereas the OpusXpress 6000A has been successfully used to rank-order α7 nicotinic receptor

Table 1 | **Comparison of automated conventional electrophysiology platforms**

Method	Configuration	Format	Comments
AutoPatch (Xention)			
Glass pipette	Interface WCPC	1 or 48 channels	• Not commercially available • Overall daily throughput similar to RoboPatch
Flyscreen (Flyion)			
Glass pipette	Cells in a pipette, WCPC	2, 4 channels	• Overall daily throughput similar to RoboPatch
RoboPatch (Wyeth)			
Glass pipette	WCPC or cells in pipette	1 channel	• Not commercially available • Overall daily throughput of 12 to 17 6-point concentration–response curves
Dynaflow (Cellectricon)			
Glass pipette	WCPC	1 channel	• Only solution and voltage is automated • The microfluidic based chip design allows for efficient construction of multiple concentration–response curves with a single cell over a long recording period (several hours) with the added advantage of using minimal volume (µl) of compound solution • Integration of this system can also help overcome perfusion limitations for studying ligand-gated channels with rapid desensitization kinetics
Robocyte (ALA Scientific)			
Two glass pipettes	TEVC, *Xenopus* oocytes	96-well, sequentially	• cDNA injection capable • Script driven software
OpusXpress (Molecular Devices)			
Two glass pipettes	TEVC, *Xenopus* oocytes	8 chambers in parallel	• Closely emulates manual recordings in oocytes

TEVC, two-electrode voltage clamp; WCPC, whole-cell patch clamp.

agonists based on functional potency and relative drug efficacy[18]. Although this provides an option for multiple recordings in parallel towards higher throughput, the field has focused heavily on automated systems for mammalian cells using planar-array based approaches as discussed in the following section.

Planar-array based approaches

A recent explosion in the number and different approaches taken to planar-array patch clamp of mammalian cells has occurred in the past few years, resulting in the commercialization of four planar systems and an additional platform still under development (TABLE 2). Planar-array refers to the use of multi-well configurations either in a plate-based or chip-based format to enable multiple recordings in parallel[19] compared with a single glass patch-pipette in conventional manual patch clamp (BOX 1). Generally, these systems have integrated robotic operation for cell, solution and compound handling. Application has been limited to mammalian stable cell lines or large-scale transiently transfected cells, although progress past the initial HEK-293 and CHO cell lines is now being made.

The IonWorks HT system (Molecular Devices; FIG. 1a), originally invented by Essen Instruments, was the first of the screening systems that became widely available for use[20]. Similar electrophysiological properties of the hERG channel were demonstrated with IonWorks HT compared with conventional electrophysiology, although different outcomes were observed regarding compound inhibitory potencies. For example, consistent rightward shifts in compound IC_{50} values were observed in one study[21], whereas another found that this could be minimized by titrating to low numbers of cells used per well[22]. With respect to the rightward shift in compound potency, it has become well known that lipophilic compounds adsorb nonspecifically to plastic surfaces in the automated systems, although the use of glass has mitigated this issue somewhat.

In our own experience, instrument and assay performance for IonWorks HT has been monitored over a 6-month period, demonstrating frequently right-shifted IC_{50} values for compound screening compared with conventional electrophysiology. The general robustness of the platform is supported by the observed IC_{50} of the reference standard quinidine on hERG channels showing little variability around a mean value of 1 µM. Similarly, negative control wells in the presence of dimethyl sulphoxide performed within 3 standard deviations from the 95% control response, indicating that the assay can be robust in terms of false positives, false negatives and performance over an extended time period.

A more recent evolution of the IonWorks technology comes in the form of the Quattro, which uses simultaneous recording of 64 cells per well, enabled with 64 holes per well (FIG. 1b), termed the population patch-clamp (PPC) mode[23]. The advantage of the PPC mode compared with the original IonWorks HT single-hole recording has recently been illustrated in a study of calcium-activated K^+ channels (hIK) expressed in CHO cells. This study showed that the multi-well averaging achieved in the PPC mode overcame the considerable well-to-well variability observed with the single-hole approach to allow for the

IC_{50}
The half maximal inhibitory concentration. This represents the concentration of an inhibitor that is required for 50% inhibition of a biological or molecular process.

Z′ values
A measurement that takes into account the dynamic range of the assay (how far apart the positive controls are from the negative controls), as well as data variability (how much variation is seen in the measurements of positive and negative controls).

pharmacological profiling of hIK activators[24]. Moreover, the same study validated the use of the IonWorks Quattro for screening a small compound library with acceptable Z′ values. One important limitation worthy of note for the IonWorks technology stems from the inability to place the recording and pipettor heads simultaneously into the well and thereby maintain continuous voltage clamp. This largely restricts its utility to voltage-gated channels (and perhaps slowly desensitizing ligand-gated channels), but also creates difficulties in measuring use-dependent block, especially for slowly equilibrating compounds at low concentrations. Also, in a typical assay with a single recording of activity before and a second recording after compound addition, the rundown for each well cannot

Table 2 | **Comparison of automated planar-array electrophysiology platforms**

Method	Configuration	Format	Advantages	Shortcomings
QPatch (Sophion Bioscience)				
Planar QPlate with embedded recording and ground contacts	WCPC; giga-ohm seals	16- or 48-wells in parallel; 1 cell/hole per well	• Laminar solution flow • Cumulative/multiple compound additions • Ligand addition during recording • Continuous voltage-clamp and current versus time experiment flow • Cell preparation unit stirs suspension and prepares cells before use • Built-in elevators for QPlates and compound plates with a bar-code reader allows for unattended automation • Option of one or two pipetting robot arms each having four channels, enabling a greater ease of liquid handling	• Estimates of compound potency influenced by propensity of certain compounds to adhere to the plate surfaces • Synchronized channels without manual override • No simultaneous visualization of channels • No intracellular perfusion
PatchXpress (Molecular Devices)				
Planar SealChip with electrodes separate from chip	WCPC; giga-ohm seals	16-wells in parallel; 1 cell/hole per well	• Desynchronized control of chambers • Continuous voltage-clamp versus time plots • Cumulative/multiple compound additions • Ligand addition during recording • Simultaneous visualization of all 16 channels • Disposable pipette tips	• Estimates of compound potency influenced by propensity of certain compounds to adhere to the plate surfaces • No cell maintenence or preparation unit • No stackers available to enable hands-free operation • Single pipetting robot with one channel • No intracellular perfusion
IonWorks HT and Quattro (Molecular Devices)				
Planar 384-well PatchPlate	Perforated WCPC; ~100 mega-ohm seals on IonWorks HT and 30–50 mega-ohm on Quattro	384-well; 48-channels sequentially; 1 (IonWorks HT) or 64 (Quattro) cells/holes per well	• Highest throughput unit • Allows for collection of 384 data points with ~60% success with IonWorks HT and > 95% success with Quattro	• Esitmates of compound potency negatively influenced by propensity for lipophilic compounds to adhere to the plate • No high-resistance membrane seal • No voltage-clamp between reads • No simultaneous ligand addition and recording • Single pipetting robot with 12 or 48 channels for 96- or 384-well compound plates • No intracellular perfusion
Patchliner (Nanion Technologies)				
Planar NPC-16 chip	WCPC; giga-ohm seals	2, 4 or 8-wells in parallel; 1 cell/hole per well	• Borosilicate chip surface • Use of primary cells possible with single-well version • Perfusion of external and internal solutions • Unlimited ligand perfusion volume • Continuous voltage versus time plots • Cumulative/multiple compound additions • Ligand addition during recording	• System performance information limited • Single pipetting robot with one channel
CytoPatch (Cytocentrics)				
Planar, electrode tip shape surrounded by aperture in borosilicate glass surface	WCPC	1 cell/hole per well	• Constant laminar flow • Positive pressure on electrode is independent of suction in surrounding aperture, mimicking manual patch-clamp	• Still in development, no current validation data

TEVC, two-electrode voltage clamp; WCPC, whole-cell patch clamp.

Fluorescence-activated cell sorter
(FACS). A machine that can rapidly separate cells in suspension on the basis of size and the colour of their fluorescence.

be assessed. However, recording parallel wells with vehicle alone has proved useful in this regard, and a repetitive scan mode is available in the IonWorks version 2 software that addresses this limitation.

PatchXpress (Molecular Devices) was the first of the planar-array-based systems to enable giga-ohm seal patch-clamp electrophysiology on a chip[25,26] (FIG. 1c; TABLE 2). A related system — and similar in many aspects to PatchXpress — the QPatch (Sophion; FIG. 1d) was the first planar system to enable microfluidics flow capability into their design[13,27]. As with the IonWorks technology, both PatchXpress and QPatch have been used successfully for recording currents from hERG channels expressed in stable cell lines. They have demonstrated similar electrophysiological responses compared with conventional patch-clamp[25,28–30], and revealed a potential for differences in pharmacology for the planar systems (right-shifted IC_{50} values), which is mediated, at least in part, by the ability of certain compounds to adhere to the plate surfaces.

Patchliner (Nanion Technologies; FIG. 1e) has expanded the microfluidics capability into the arena of both extracellular and intracellular fluid exchange on a glass chip[31]. A single channel, manual version of the technology with no liquid handling is available as a Port-A-Patch unit[32,33], an offering that can either act as an assay development device for the larger unit, and/or as a simple, benchtop unit for patch-clamp recording. Finally, CytoPatch (Cytocentrics) is one of the newer robotic systems still in development, although chip design (FIG. 1f) and development prototypes do exist in the company laboratories[34].

Practical considerations with automated electrophysiology. On a practical side, all the multi-channel automated systems require an initial costly capital investment and use expensive plates or chips (although small economies of scale are possible), which are proprietary designs (for the foreseeable future) for the specific instrument and one-time use consumables. The capacity of each platform for compound screening (BOX 2) is clearly an important question to be considered. The software and analysis is generally complex, requiring significant expertise in electrophysiology. Automation in giga-ohm seal recordings has resulted in acquisition of enormous amounts of high-quality data that lend themselves to various analyses. This can be cumbersome and can become a bottleneck post-acquisition if the process is not automated. Both QPatch and PatchXpress have adopted a database-based approach to address the issue of data acquisition, archival and analysis. Although the initial versions of these databases and analysis modules were rudimentary with limited capability, the newer releases have fixed many of the shortcomings and made the analysis automated and more user-friendly.

The cell-culture challenge. Despite the sophisticated technologies that are now available, automating patch-clamp electrophysiology often presents underestimated challenges regarding the reproducibility with the cells being used. One requirement of all the current systems is the need for large numbers of dissociated cells, something that is more applicable to cell lines rather than to primary cells isolated from animals. This adds a significant need for cell culture (or development of the assay from frozen cell stocks[35]), with high cell quality and robustness being crucial factors for the success of these platforms, something that is not always appreciated or optimized by traditional ion-channel laboratories.

Most systems are designed to use stably expressing cell lines with well-behaved patch-clamp characteristics (membrane seal and stability properties). Limited success has been reported using an alternative approach of transiently transfecting cells, selecting expressors based on the co-expressed green-fluorescent-protein marker using a fluorescence-activated cell sorter, followed by measuring in an automated patchclamp[35]. Use of transiently expressing cells is more likely to be broadly feasible if a high expression rate is obtained (for example, those using a viral expression such as BacMam), but this approach is still in development. The initial starting point for working with these systems is usually with an existing cell line that works well for manual patch clamp. An immediate challenge, however, can occur if only a small subset of cells express currents of sufficient amplitude for testing. This might not be appreciated from manual patch-clamp experiments, as its visually guided nature often results in an experimenter imposing cell choice based on multiple characteristics. Automated patch-clamp systems, on the other hand, record from cells blindly. One standard approach to overcome this problem is to enhance current expression in the cell line using techniques for optimizing expression.

In a typical scenario, a cell line already exists and is being used for other assays, so a common approach is to generate subclones of the original cell line, using standard techniques, and then screen the subclones on the automated platform. Selecting the 'best' cell line(s) is often done empirically on the instrument, searching for the best balance of compatibility on the instrument, current expression, seal characteristics and current stability, as in the case reported for large-conductance calcium-activated potassium channels (BK_{Ca})[35]. Methods to improve the initial quality of the subclones by starting

Box 2 | **The question of compound throughput**

In comparing the planar-array based systems, IonWorks Quattro (Molecular Devices) is the system that wins the throughput race, but it comes with the price of several compromises. Such compromises reflect this system's unique emphasis on a screening approach to electrophysiology, and its consequent test-add-test experimental workflow. IonWorks is the only system incapable of giga-ohm seals, although surprisingly the pharmacological correlations with manual patch-clamp do not reflect a large error in the measurements. Similarly, only IonWorks is unable to perform ligand application with simultaneous voltage clamping, a significant limitation for all but the most slowly desensitizing ligand-gated channels. All the other systems take a more traditional electrophysiology approach, but perform in a parallel multi-recording manner. A major advantage to these latter systems is in the giga-ohm seals that provide comparable quality of recordings to manual patch clamp and allow precise voltage control. The capabilities of PatchXpress (Molecular Devices) and QPatch (Sophion) for experimental design, flexible voltage protocols, cumulative concentration responses with flexible application times, washout of compounds, constant voltage clamp, and fast ligand application onto cells is quite valuable and of particular relevance to rapidly desensitizing ligand-gated channels. Among these systems, the 48-channel QPatch HT has become the system of choice if throughput is the major consideration.

Box 3 | **Automated electrophysiology: fundamental principles**

Even when a well-behaved cell line has been identified and validated on an automated platform, good and healthy cells are the key to success in automated electrophysiology. Most recombinant ion-channels are expressed either in HEK-293 or in CHO cells. The efficiency of sealing and the success is higher with CHO cells. However, irrespective of the background expression system a few other factors are also crucial for success. Optimal well-regimented cell-culture conditions are critical for success. Cells should be plated and maintained under constant culture conditions, at an optimized plating density for a fixed duration before the experiments. Over-confluent cells that work well in manual electrophysiology might fare poorly in automated systems. Cell viability and success in experiments can be enhanced by using milder and gentler dissociation reagents such as detachin and accutase rather than the traditionally used reagent trypsin. Detachin also helps prevent the clumping of cells post-dissociation. Trafficking of ion-channels to the cell surface and expression of channels can be significantly enhanced by treating the cells with sodium butyrate, or by incubating them at reduced temperatures (25–30°C) for several days before the experiment. Finally, the passage number of cells needs to be monitored carefully, as some channel types lose expression after several passages.

The composition of intra- and extracellular solutions and osmolarity difference can influence success. Unlike manual electrophysiology where solutions can be prepared and stored for weeks under refrigerated conditions, preparing solutions fresh improves the rate of success in automated systems. All the solutions should be filtered before use in experiments. Substituting Cl^- with either F^- or gluconate ions either partially or completely in the intracellular buffer can enhance giga-ohm seal formation on automated systems. However, such interventions can affect junction potentials and the biophysical properties and pharmacology of ion channels, and care should be taken to optimize them. Similarly an enhanced extracellular Ca^{2+} concentration also helps improve the seal formation. For enhanced success in platforms utilizing perforated-patch technique, it is essential to use fresh solutions of pore forming antibiotics, such as amphotericin B. A low concentration of antibiotic will prevent precipitation.

Although some of these considerations seem simple, and perhaps obvious, overlooking any of them can have a profound impact on the success of experiments on automated electrophysiology platforms.

only with those cells containing the visual characteristics similar to those used in manual patch clamp has been successful at improving the odds of identifying an appropriate cell line in one case[36], combined with empirical testing as the final selection method.

Alternatively, starting from scratch, generating a new cell line for use on an automated platform provides the most versatility, but also the most time and effort. Choice of the initial cell background can be crucial for expression, as auxiliary subunits required for membrane expression are found endogenously in some cell lines. One example of this is the α7 nicotinic receptor, which requires expression of the chaperone protein ric-3 for its function. GH4C1 cells were found to express α7 receptors well, and only later was ric-3 found endogenously in the cell line[37,38]. This finding has allowed expression of α7/ric-3 in other cell types such as HEK-293 cells[38]. Peripherally expressed tetrodotoxin-resistant Na^+ channels present similar challenges, being best expressed in just a few cell types; the identification of a required associated subunit that allows widespread expression has so far been elusive. Finally, other factors must also be appraised in the choice of starting cell-line, such as its patch-clamp seal characteristics, growth rate and any contaminating endogenous conductances, as overlooking even the simplest of considerations can have a profound effect on the assay performance using automated systems (BOX 3).

Automation in cardiac liability screening

Toxicological findings continue to be one of the factors contributing to failure of drug candidates[39,40]. Drug-induced inhibition of I_{Kr} (cardiac hERG potassium channel) in a concentration-dependent manner, a consequent prolongation of QTc interval on cardiac electrocardiogram and accompanying potentially fatal polymorphic ventricular arrhythmia, Torsades-de-pointes (TDP) has been a cause for either the withdrawal from the market (in the case of astemizole, cisapride, terfenadine, sertindole, grepafloxacin and terodiline) or the restriction of the use of a number of non-anti-arrhythmic drugs in the past decade[41]. Most drugs that produce TDP in humans prolong QTc interval and inhibit I_{Kr}, hence hERG has become the major, but not the only culprit in safety pharmacology[42,43]. Regulatory agencies from the US (Food and Drug Administration), Europe (European Medicines Agency) and Japan have issued guidance[44] on pre-clinical strategies for testing the pro-arrhythmic potential of human pharmaceuticals that strongly recommend *in vitro* I_{Kr} assays, including assessment of compound effects on the clonal hERG channel, and *in vivo* QT assessment[45,46].

Pharmaceutical companies have implemented the same strategies — adopted for higher-throughput ion-channel screening in general — to assess the potential for drug candidates to inhibit cardiac ion-channels, particularly hERG. These include *in silico* assessments[47–49], radioligand binding assays[50], optical assays using fluorometric imaging plate reader[4] or fluorescence-polarization measurements[51]. In addition, ion-flux assays using the ion-channel reader[21,22] are typically used early on in the assessment of potential for hERG interaction, and the lower-throughput gold-standard electrophysiological assays are traditionally done as the compounds progress towards development. Although the higher-throughput assays have a reasonable predictive potential, they have several limitations that might be technically or chemically limiting. This has resulted in the increased use of electrophysiological assays early on to assess ion-channel liabilities and has been one of the key drivers for implementation of automated electrophysiology.

Several electrophysiology platforms, including IonWorks HT, IonWorks Quattro, PatchXpress, QPatch and Patchliner, have been evaluated and subsequently validated for use in safety pharmacology studies. Using CHO cells that stably express hERG channels, IonWorks allowed for assessment of approximately 200 data points per PatchPlate and about 80 concentration–response curves per day[21,22,52]. The seal success rate and stability were > 75% and a good pharmacological correlation — that is, rank order of compound activity compared with conventional electrophysiology data — were observed, even in the presence of rightward shifted concentration–response curves (within 3–5-fold). PatchXpress, QPatch and Patchliner allow stable recording of high-quality hERG currents from giga-ohm seals for 20–30 minutes, allow the complete concentration–response experiment to be performed on a single cell, and exhibit good pharmacological data correlation with conventional

Table 3 | **Automation in higher-order systems**

Biological substrate	Recording chamber	Stimulation and recording	Channel number	Automation software	Advantages	Shortcomings
Slice Master (Scientifica)						
Brain slices	Conventional design; individual baths	Conventional electrodes; motorized manipulators	4 or 8	Requires third-party add-on	• Remote control and monitoring system • Innovative, miniaturized recording chambers • DC-powered stimulus isolators	• Lack of bundled automation software • Recording chamber allows submerged slices only • High cost
SynchroSlice (Lohmann Research Equipment)						
Brain slices	Space-saving multi-bath design	Conventional electrodes; manual manipulators	4 or 8	Bundled	• Simple experimental setup with bundled software • Online data analysis • Recording chamber can be submerged or interface	• Rigid experimental designs • Limited built-in LTP/LTD induction protocols
Synaptic Explorer (Wyeth Neuroscience)						
Brain slices	Conventional design; individual baths	Conventional electrodes; manual manipulators	4	Integrated	• Graphical interface allows easy and flexible experimental design • Online data analysis • Swappable recording chambers	• Limited to four concurrent recordings • Not commercially available
MED64 (Alpha MED Sciences)						
Tissue slices; cell cultures	The MED-64 probe; various designs	Multichannel electrode array	64	Bundled	• Software permits ready execution of various standard slice experiments	• Temperature control of preparation is lacking • Wash-in of drug solutions variable
MEA System (Multi Channel Systems)						
Tissue slices; cell cultures	The MEA biosensor; various designs	Multichannel electrode array	60	Bundled	• Wider selection of custom biosensors • Integrated , accurate temperature control	• Wash-in of drug solutions variable

DC, direct current; LTD, long-term depression; LTP, long-term potentiation; MEA, multi-electrode array; MED, multi-electrode device.

experiments[25,28–30]. Automated electrophysiology platforms allow the direct assessment of potency of compounds on hERG channels and give reliable information to medicinal chemists to enable structure–activity optimization. However, lack of perfusion, excess of cells per well and high surface area are some caveats that can produce potency shifts with hydrophobic compounds in these automated systems[22,29,52].

Repolarization assays, although not mandated by the ICH regulations, are recommended as follow-up assays for assessing pro-arrhythmic risk[46]. These organotypic assays are technically demanding and lower in throughput. A semi-automated Purkinje fibre recording station with automated liquid handling capabilities that can run three assays at a time and conserves the operator time and animal usage has been developed by Scientifica. Planar 96-well plate-based, automated, high-throughput QT screens using multi-electrode arrays have been developed by Multi Channel Systems and by Alpha MED Sciences. A limited validation data set for the potential use of these systems in safety pharmacology studies using avian and mammalian myocytes and embryonic stem cells is available from the vendors, but the routine use of these systems in the pharmaceutical industry is yet to be documented[53,54].

Automation in higher-order systems

Compound screening against molecularly isolated, heterologously expressed ion channels, although intrinsic to the modern drug discovery process, will often identify drug candidates whose higher-order impact on networked neuronal systems are not necessarily inferable from their effects on individual conductances. Raising the throughput of pharmacological evaluation in such higher-order systems presents a distinct set of challenges. Of particular relevance to neuronally targeted therapies are their effects on various standard measures of synaptic physiology, most notably synaptic plasticity, which includes long-term potentiation (LTP)[55–58] and long-term depression (LTD)[59], both widely regarded as the physiological correlates underlying learning and memory[57,60]. Deficits in synaptic plasticity have been identified in animal models of neurodegenerative disorders such as Alzheimer's disease[61,62], Parkinson's disease[63,64] and schizophrenia[65,66].

For these reasons, neuroscience drug discovery efforts are seeking to scale-up the assessment of synaptic function in brain preparations from animal models of disease, along with the subsequent reversal of synaptic deficits following treatment with novel pharmacological therapies. Of the many neuronal preparations available, *in vitro* brain-slice extracellular field-potential recordings

ICH regulations
International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). The ICH works to bring together government regulators and drug industry representatives from the United States, the European Union and Japan to make the international drug regulatory process more efficient and uniform.

Long-term potentiation
(LTP). The prolonged strengthening of synaptic communication, which is induced by high-frequency patterned input and is thought to be involved in learning and memory formation.

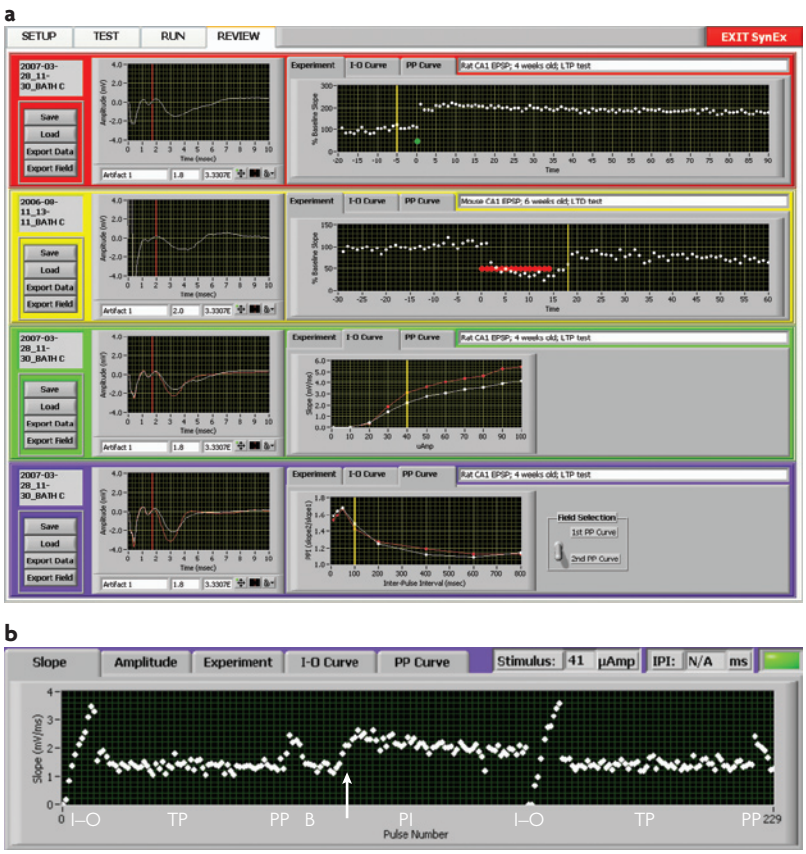


Figure 2 | **Illustration of the various types of data obtained using hippocampal-slice field recordings. a** | The top, red quadrant shows a long-term potentiation (LTP) experiment, in which potentiation was induced by a triple theta burst, indicated by the green dot on the chart. The quadrant below (yellow) illustrates a long-term depression (LTD) experiment, with the red bar representing 15 minutes of 1 Hz paired-pulse stimulation. The bottom half of the screen shows additional synaptic measures collected during the LTP experiment shown. The input–output (I–O) curve (green quadrant) is a measure of basal synaptic transmission, and is determined both at the beginning of the experiment (white line), from which to calculate the proper baseline stimulation, and again at the conclusion (red line), to judge general slice viability. The paired-pulse (PP) curve (purple quadrant) is used to measure presynaptic facilitation. In this example, as the pre- and post-LTP PP curves overlay, the potentiation can be deemed to have a postsynaptic locus. In all quadrants, the field excitatory postsynaptic potential (fEPSPs) shown in the graphs to the left correspond to the data points under the yellow cursors on the corresponding charts to the right. **b** |Cumulative record of field potential analyses calculated on-line during the course of a typical synaptic physiology experiment. Each point represents the slope (mV ms^{-1}) derived from an individual fEPSP. Various segments of this data set are later automatically parsed into the various summary charts shown in part **a**. Typically, four such recordings are performed in parallel across individual slices subjected to particular treatment conditions. The various synaptic tests are listed below the relevant portion of the chart as follows: I–O curve; TP, test pulse determination; PP facilitation curve; B and PI refer to the baseline and post-induction phases of the LTP segment of the recording, with the induction by high-frequency stimulation indicated by the arrow.

are particularly amenable towards this end. A large body of literature, spanning several decades[60,67–70], attests to the unique usefulness of this preparation. While allowing many of the advantages of an *in vitro* preparation, brain-slice field recordings, in most instances, remain indistinguishable from their *in vivo* counterparts. Unlike intracellular recordings, field potentials typically remain stable for many hours. Finally, brain-slice field recording is, relative to most other electrophysiological methods, a relatively simple process, allowing a competent investigator to perform multiple recordings in parallel.

Quality biological samples for such recordings require significant manual labour, both in terms of time and technical skill. Thus, sample preparation represents a fundamental bottleneck in any implementation of a high-capacity brain-slice field-recording system. Likewise, the selection of usable stimulus-evoked field potentials is itself a highly nuanced process, requiring slice-to-slice comparison, followed by individualized testing of each slice ultimately judged worthy of prolonged recording. Nonetheless, once these manual aspects of the experimental procedure are accomplished, subsequent recordings lasting several hours, encompassing various assessments of synaptic physiology, including responses to investigational compounds, are fully amenable to automation.

The electronics required for successful field recordings are relatively modest. At the most basic level, all brain-slice extracellular field experiments amount to the continuous monitoring of physiological voltage-change responses to stimulation sequences, which are precisely defined, both in terms of their temporal patterns and the current intensity of the individual pulses within those patterns. Within that basic framework, the possible variations in experimental protocol are virtually limitless. Thus, the challenge in developing an automated multi-slice field recording system does not depend on hardware as much as on the available software. That is, the challenge lies in the virtual organization and physical implementation of the large parameter sets that are necessary for the flexible definition and precise execution of any conceivable number of synaptic tests to be performed in parallel across multiple individual slices.

Two multi-slice field-recording systems have become commercially available in recent years. SliceMaster[71], developed by Scientifica in conjunction with Merck and NPI Electronic, is available in three scaled versions, allowing concurrent and independent field recordings from up to a maximum of eight brain slices. A competing commercial system is Synchroslice, developed by Lohmann Research Equipment. Each system comes with its own pros and cons and generally the software does not allow for a lot of flexibility in experimental design (TABLE 3).

In response to such limitations we have developed a third, custom system, Synaptic Explorer (SynEx), internally at Wyeth Neuroscience. SynEx enables a variety of experimental designs, including a wide diversity of plasticity-induction protocols to proceed unattended following the initial slice setup. The various types of data that are routinely derived from slice field recordings using SynEx are illustrated in FIG. 2. In practice, this system has allowed us to reduce the time for a two-arm study (for example, control versus transgenic) from several weeks to 4–5 days.

A different approach to the design of high-capacity field recordings is the multi-channel electrode array (MCEA), of which two competing commercial systems are available: the MEA System, from Multi Channel Systems, and the MED64 System from Alpha MED Sciences (a subsidiary of Panasonic). Neither system falls strictly into the automation category but both provide for high-capacity recordings (TABLE 3). Initially, MCEAs were applied to

Long-term depression (LTD). An enduring weakening of synaptic strength that is thought to interact with long-term potentiation (LTP) in the cellular mechanisms of learning and memory in structures such as the hippocampus and cerebellum. Unlike LTP, which is produced by brief high-frequency stimulation, LTD can be produced by long-term, low-frequency stimulation.

traditional brain-slice field recordings[72–76] and although readily amenable to such, the cost of the arrays make MCEAs more expensive than traditional approaches. Despite this expense, MCEAs prove to be largely reusable and, with care, can be long lived. All in all, however, MCEAs have proved to be an inferior choice for traditional brain-slice field-potential recordings, at least in our hands. In practical terms, slice-to-slice differences being the greatest source of variability in brain-slice field experiments, single pathway recordings from multiple slices generally prove far more meaningful than multiple pathway recordings from a single slice.

While MCEAs are at best adequate for traditional brain-slice field recordings, these devices have been subject to evolving research into novel applications that capitalize on their unique properties. Apart from housing the electrode grids, MCEA recording chambers are amenable to the culture of brain slices[75,77], as well as dissociated cells[78–80], albeit with significant cell attachment difficulties. The network properties of neurons[81–83], retinas[84,85] and cardiomyocytes[86,87], among others, are all under active investigation by the MCEA research community. At the same time, as the collection of enormous amounts of network data becomes possible, its organization and analysis becomes a new strain on the investigative process. Given the limitations of the software provided with both the MEA and MED64 systems, investigators have developed various individualized analysis tools using third-party software, such as Neuroexplorer and MATLAB. Even so, MCEA data management is extremely labour-intensive and poorly amenable to drug screening. Last, it remains speculative as to which parameters of network activity will prove predictive of which particular diseases. Nonetheless, MCEA technology offers potential for future insight into diseases involving pathological hyperactivity, including epilepsy and neuropathic pain.

Future directions and expectations

As few as 6 years ago the availability of automated platforms for ion-channel electrophysiology was limited to prototype systems that had limited impact on the drug discovery workflow. This is in sharp contrast to the current availability of a number of high-throughput and validated platforms that have been fully integrated into drug discovery programmes spanning hit identification, lead optimization, cardiac liability screening and neuronal or slice physiology. That being said, although the benefits of automation seem obvious at face value, in practical experience it is seldom the case that a well-behaved manual patch-clamp assay with mammalian cells transitions easily to an automated platform. More typically, significant effort and accompanied resources are required to optimize conditions for both cell performance and assay reproducibility. In this regard, a close collaboration with instrument vendors can be extremely beneficial to expediting success. Once success is achieved, the true benefits of automation can be realized, as exemplified in our own experience in which one of these platforms is now frequently over-subscribed. In the case of the automation for higher-order systems such as slices, this is even more in its infancy and the same types of challenges exist as described above. Nevertheless, the availability of these technologies has re-energized ion-channel targeted drug discovery by allowing the development of screening paradigms that were not feasible in the pre-automation era. In our opinion this holds much promise for the discovery and development of innovative new ion-channel targeted drugs. Tractability of ion channels as drug targets coupled with future advances in technology platforms and decreased cost of consumables are expected to support an even wider implementation of these automated systems.

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Competing interests statement
The authors declare competing financial interests: see web version for details.

FURTHER INFORMATION
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