Catch and Patch: A Pipette-Based Approach for Automating Patch Clamp That Enables Cell Selection and Fast Compound Application

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ABSTRACT

Manual patch clamp, the gold standard of electrophysiology, represents a powerful and versatile toolbox to stimulate, modulate, and record ion channel activity from membrane fragments and whole cells. The electrophysiological readout can be combined with fluorescent or optogenetic methods and allows for ultrafast solution exchanges using specialized microfluidic tools. A hallmark of manual patch clamp is the intentional selection of individual cells for recording, often an essential prerequisite to generate meaningful data. So far, available automation solutions rely on random cell usage in the closed environment of a chip and thus sacrifice much of this versatility by design. To parallelize and automate the traditional patch clamp technique while perpetuating the full versatility of the method, we developed an approach to automation, which is based on active cell handling and targeted electrode placement rather than on random processes. This is achieved through an automated pipette positioning system, which guides the tips of recording pipettes with micrometer precision to a microfluidic cell handling device. Using a patch pipette array mounted on a conventional micromanipulator, our automated patch clamp process mimics the original manual patch clamp as closely as possible, yet achieving a configuration where recordings are obtained from many patch electrodes in parallel. In addition, our implementation is extensible by design to allow the easy integration of specialized equipment such as ultrafast compound application tools. The resulting system offers fully automated patch clamp on purposely selected cells and combines high-quality gigaseal recordings with solution switching in the millisecond timescale.

INTRODUCTION

Manual patch clamp requires a human operator using a micromanipulator to position the tip of a glass pipette against the membrane surface of a carefully selected cell to achieve the gigaohm seal needed to perform high-quality analysis of ion channel function.[1]

The fact that all steps have to be performed manually, under optical control, and with a single electrode only makes the method tedious and labor-intensive and thus not suitable for higher throughput analysis.[2] On the other hand, this manual mode of operation allows the selection of vital and distinct cells out of a heterogeneous cell preparation, which is a prerequisite for a high experimental success rate and the quality of the results.

Cell selection is routinely based on easily recognizable markers of cell quality, such as size, roundness, and the absence of visual damage or blebs. In addition, if the cell preparation involves a transient gene transfection of low efficiency, cell selection based on fluorescent tagging of successfully transfected cells becomes essential.[3] To date, all commercially available systems for automated patch clamp are based on patch clamp chips. Aiming at high throughput at low cost while maintaining a reasonable data quality, these systems randomly attract cells to a patch hole in a planar chip instead of using active cell handling for cell selection.[4] Thus, as a price for this pragmatic approach to higher throughput, these instruments require absolutely homogeneous cell preparations to achieve meaningful data at high success rates.

Patch clamp pipettes are not only electrophysiological recording tools but also allow for manipulation and translocation of membrane fragments and whole cells. This is a requirement for the integration of high performance tools for ultrafast compound application, a method of high relevance for neuropharmacological research. In chemical synapses, information is transmitted by release and uptake of neurotransmitters at the submillisecond timescale, sensed by

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postsynaptical receptors with equally fast kinetics.[5] Electrophysiological research on these fast-acting ligand-gated ion channels (LGICs) demands repeated delivery and washout of reagents at a millisecond or even submillisecond timescale.[6,7] To achieve state-of-the-art performance in this discipline, it is required to move the recording electrode with the patch-clamped cell away from the bottom of the measurement chamber, where so-called unstirred layer effects slow down the solution exchange, and into the solution stream of a specialized microfluidic device, called a theta tube.[6,8] These devices maintain a laminar flow of two parallel solution streams and use a high performance piezo actuator to switch a cell between the two streams within fractions of a millisecond. Such significant efforts are necessary to study modulators of the complex kinetic properties of neural LGICs, which are promising drug targets for neuronal disorders.[9,10] However, the chip-based design of existent automated patch clamp devices is incompatible with attempts to integrate such specialized microfluidic tools for ultrafast compound application. In addition, achievable exchange rates of compound applications are limited by the unstirred layer effects at the surface of the chip where the patch-clamped cell is located.

In this study, we have overcome these limitations by developing a completely new approach for catching and patching cells out of a suspension that can be run in a standard patch clamp setup by adding our specific hardware and software. It is based on a nonoptical positioning strategy for patch clamp electrodes solely based on electrical potentials. Positioning accuracy is not limited by the optical system, does not require prepositioning of the electrodes in the visual field, and is able to position the electrodes with sub-µm precision in all three dimensions. Using this positioning strategy, we are able to actively make contact to and pick up user-selected cells from a microfluidic cell handling device. Parallelization, and thus higher throughput, is easily achieved by using an array of patch clamp electrodes, yet requiring only a single micromanipulator. Thus, we have designed an automated and parallel process that mimics the manual patch clamp much more closely than any currently available automated system. We demonstrate the power of this approach by integrating a highly advanced method so far restricted to tedious manual patch clamp, the ultrafast compound application using a piezo-driven theta tube device.

MATERIALS AND METHODS

Automated Patch Electrode Positioning

Our method can be implemented as an add-on to a standard patch clamp setup (*Fig. 1*). To enable parallelization as well as automation of the patch clamp technique, two new concepts are introduced. First, the single recording electrode is replaced by an array of three recording pipettes mounted on a single standard micromanipulator. For convenience, standard patch pipettes are inserted into a removable three-channel holder block, which can be easily attached to the micromanipulator by a snap-lock mechanism. Second, an auxiliary pipette, which we call the catch pipette, is mounted on the opposing side. This catch pipette serves several purposes: it is designed for (1) catching a single cell out of a suspension, (2) presenting each caught cell to the field of view of a microscope at a fixed defined location, which is in focus for optical characterization and selection, and (3) stably holding the cell in this place for pickup by any of the recording pipettes. In addition to these cell handling purposes, the catch pipette can (4) send the electrical beacon pulses, which provide the basis of our electrode positioning principle. This active localization of the recording pipette tips is essential for proper patch clamping since imprecision of pipette production and

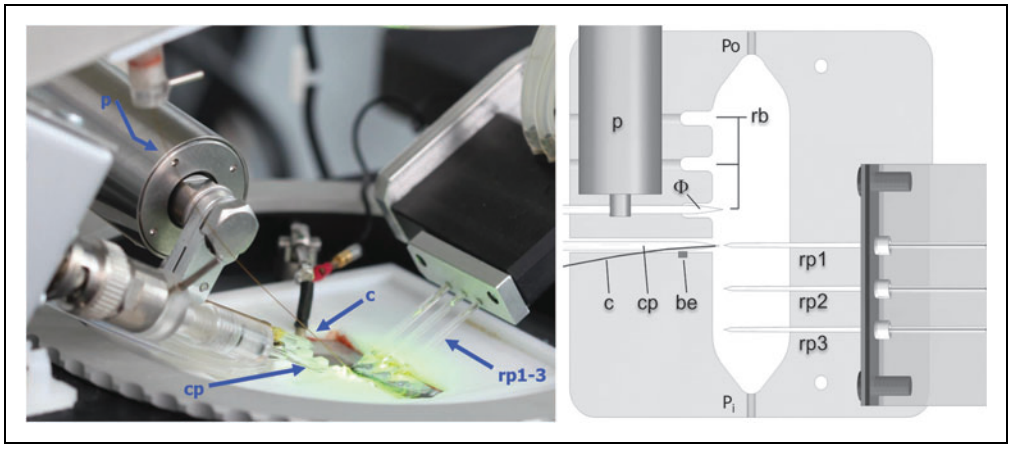

Fig. 1. Catch and patch setup. An array of three recording pipettes (rp1-3) is positioned by a patch clamp micromanipulator so that its tips protrude into the bath chamber, while a single catch pipette (cp) and the bath electrode (be) are mounted to a fixed position. A hair-like capillary (c) transports cells from the cell reservoir (not visible) to the tip of the catch pipette. For conventional (slow) compound application, the array of recording pipettes can be moved into the three recording bays (rb) where they will receive compound application simultaneously. In addition, the chamber can be globally perfused through the perfusion inlet (Pi) and outlet (Po). For ultrafast compound application, a theta tool (Θ) is placed in the first recording bay adjacent to the catch pipette so that it can be reached by each of the recoding pipettes subsequently. A piezo actuator (p) is used to move the theta tool for ultrafast solution switching.

patch pipette holders make it impossible to rely on mechanically predetermined tip positions. To facilitate electrode positioning, the catch pipette is connected to a dedicated amplifier, the catch amp, which is used to inject electrical pulses into the bath chamber. The resulting potentials, which decline with increasing distance from the catch pipette, are sensed by the recoding pipettes through their connected patch clamp amplifiers. Our patented positioning algorithm uses this information to systematically move each recording pipette until it is perfectly positioned right in front of the tip of the catch pipette (*Fig. 2*).[11]

Catch pipettes were manufactured using a standard micropipette puller (DMZ Universal, Zeitz, Germany) to pull pipettes with ~20 µm tip opening diameter. The pipettes were further processed using a microforge to reduce tip openings to ~5 µm and to obtain smooth rounded edges at the tip.

Automated Cell Handling

For automated patch clamp, cells were prepared into acute suspension. The suspended cells are placed into the cell reservoir, a 250-µL container mounted a few centimeters above the measurement chamber. To avoid clumping of the suspended cells, the suspension is agitated by a small pipetting device (a glass tube of 1.2 mm inner diameter protruding into the cell suspension), which is used to aspirate and dispense back a certain amount of cell suspension at user-defined time intervals. A glass capillary with 100 µm ID connected to the reservoir is used to transport the cells slightly above the tip of the catch pipette from where they are released and eventually can be caught and immobilized by gentle suction at the catch pipette (typically 1–2 mbar). Cell delivery through the capillary can be stopped and its rate can be controlled by an adjustable vacuum in the cell reservoir so that cells are released only at times when they are needed. The catch amp uses impedance measurements to immediately detect the presence of a caught cell so that the suction can automatically be reduced to the minimum, which is required to hold the cell at the tip of the catch pipette.

Pressure Controllers

Positive and negative pressures needed at the recording pipettes and the catch pipette to facilitate the automation of

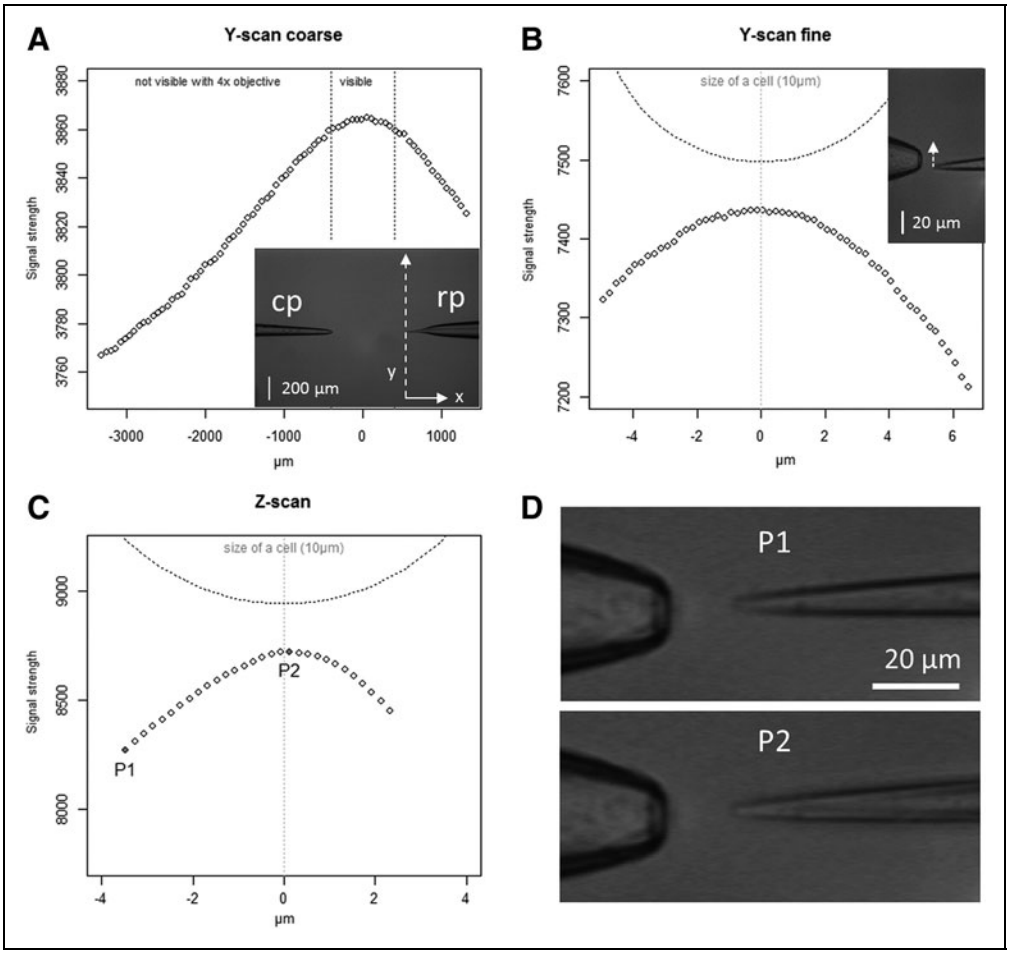

Fig. 2. Automated position alignment of recording pipettes (rp) and catch pipette (cp) in the *y* and *z* axis is achieved by moving along the respective axis while measuring the signal strength of beacon pulses emitted by the catch pipette. In **(A)**, a coarse scan in the *y* direction is shown. Before starting the scan, the recording electrode was placed far outside the field of view of the microscope at 4× magnification. By scanning along the direction of the *white dashed line*, the optimal alignment position for this axis is determined and the electrode is placed at the position shown. In **(B)**, a similar scan is performed in close vicinity of the catch pipette, where sub-µm resolution of positioning is achieved. In **(C)**, a similar scan is performed for the *z* direction. The *dashed circles* in **(B, C)** denote the dimensions of a typical cell of 10 µm diameter. For comparison, **(D)** shows images of the recording electrode corresponding to positions P1 and P2 of the z-scan shown in **(C)**. Please note that in spite of the fact that the electrode moves almost half of a cell's diameter, the corresponding focusing error of the electrode's image is hardly detectable, whereas the electrical signal allows precise determination of the optimal position.

the patch clamp process, as well as variable pressures needed at the cell reservoir to control cell application into the bath chamber, were generated by a pressure controller specifically designed for this project at the NMI, which was controlled by our software. In some experiments, we also used a pressure controller designed by Multi Channel Systems (Reutlingen, Germany).

Cell Lines and Culture

CHO-herg DUO (BSys, Lugano, Switzerland) cells were maintained in HAM's F-12 + Glutamax (31765; Invitrogen, Carlsbad, California) supplemented with 10% fetal bovine serum Gold (PAA, A15-151), 100 µg/mL G418 (PAA, P11-012), and 100 µg/mL hygromycin (PAA, P02-015). TE671/RD cells were maintained in high-glucose Dulbecco's modified Eagle's medium containing 10% horse serum, 5% fetal calf serum, 0.05 mg/mL streptomycin, and 2.5 U/mL penicillin in 7% CO_2. Cells were passaged every 3–5 days at 60%–70% confluence. Before experiments, the cells were detached from the flask with accutase and kept in ringer bath solution.

Patch Clamp Recordings

Recording pipettes for manual patch clamp and automated experiments were pulled with a standard pipette puller (DMZ Universal) to obtain electrode resistance ranging from 2 to 6 megohm. The bath solution contained 145 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM HEPES, and 10 mM glucose; adjust pH to 7.4 with NaOH. The pipette contained 120 mM KCl, 10 mM HEPES, 5 mM $CaCl_2$, 1.7 mM $MgCl_2$, 4 mM K_2ATP, and 10 mM EGTA; adjust pH to 7.2 with KOH.

Manual as well as automated patch clamp recordings were performed using HEKA-EPC10 and EPC triple amplifiers, respectively.

Theta Tube Compound Application

Concentration jumps of agonist were performed as described by Colquhoun *et al.*[8] using an application pipette fabricated from theta glass tubing (Warner Instruments, Hamden, Connecticut; 2.0 mm outer diameter, 0.6 mm wall thickness, 0.2 mm septum). The application pipette was directly mounted on a piezo translator (P-216.40) operated by a piezo power switch (E-481.00; Physik Instrumente, Waldbronn, Germany).

Software Control of Automated Patch Clamp

Control of the hardware and automation of the patch clamp process, including electrode positioning, cell handling, seal formation, break in into whole cell configuration, and conduction of whole cell experiments, were performed by our proprietary control software. The HEKA EPC10 triple patch clamp amplifier was under direct control of the manufacturer's Patchmaster Software, which in turn was remote controlled by our software using Patchmaster's built-in batch control interface.[12]

RESULTS

Automated Patch Clamp Process with Cell Selection

After mounting the pipette array to the micromanipulator (*Fig. 1*), a fully automated patch clamp experiment always starts with the automated alignment of the first recording pipette and the catch pipette (*Fig. 2*). The time needed for this positioning depended on the initial distance of the pipette tip from the final location. If the starting point was only a few 100 µ away from the final position, the positioning process required less than 10 s. On the other hand, if the initial position was very distant, up to 20 s were needed. In any case, positioning was reliable and worked without crashes and converged into a final position where the recording pipette is aligned to the catch pipette with sub-µm precision. This positioning process is repeated for all pipettes of the pipette array. Once the information about all patch pipette tip locations is determined and stored, the catch pipette will be used for cell handling.

After release of a small cloud of cells toward the catch pipette in the recording chamber, a cell was typically caught within 10 s. We found that ~1,000 cells are needed per successful recording, which is similar to Nanion's Patchliner and far below the numbers for most other patch clamp automats, which require between 8,000 and 150,000 cells per successful recording.[13]

The detection of a cell presence at the catch pipette by the catch amp was very reliable and was used to trigger the immediate reduction of the negative pressure. Therefore, the mechanical stress imposed on the cells by the catch pipette was always kept minimal. At this point, the operator was notified by a sound that a new cell is available for visual inspection. Due to the fixed position of the catch pipette, the caught cell was always in focus without further microscope adjustments. After taking a brief look at the cell, the user would simply click yes or no to accept or reject it. Future versions may use simple image analysis algorithms applied to a fixed region of interest to automate this selection process. Rejection of cells and other objects triggered application of a short (1 s) 10 mbar pressure pulse to the catch pipette, thereby discarding the cell and freeing the tip for the next cell to be caught. If needed, cleaning pulses of up to 400 mbar could be applied to the catch pipette so that a single catch pipette could be used for several working days.

To demonstrate the usefulness of cell selection *Figure 3* shows a collection of cells caught out of a typical preparation of stem cell-derived cardiomyocytes. A number of intact cells of different sizes are accompanied by cells with clearly visible deformations such as membrane damage and blebs. Not shown are a range of objects such as debris and cell fragments that have been rather aspirated than caught by the catch pipette. With common, chip-based, automated patch clamp systems, which randomly attract any object in the suspension to their patch holes, this preparation would have yielded a very low success rate due to debris or damaged cells blocking the chip holes. In contrast, our system allows focusing on actively selected healthy cells of a favored size, resulting in significantly improved success rate and data quality.

After acceptance of a cell, our algorithm advances to initiate the patch clamp process. Using the previously stored positioning information, the recording pipette is moved to its starting position right in front of the catch pipette, and then moves forward until it makes contact with the caught cell, which is presented there for pickup (*Fig. 4*). At this point, the process continues mimicking best practice of manual patch clamp, meaning that the recording pipette is kept slightly pressurized while it approaches the cell surface, which helps keep the pipette tip clean.[1] The pressure of the recording pipette is not released until contact with the cell surface is detected electrically by a small increase of pipette resistance. Thus, the inner pipette tip is not contaminated by bath fluid or debris, which guarantees high-quality seals. Again, this is in contrast to other automated systems, which need to use suction to attract cells to the recording sites, inevitably also attracting extracellular fluid and debris. After establishing the first contact of the recording pipette with the cell membrane, the software monitors the increase in seal resistance while automatically adjusting the negative pressure at the recording pipette until a gigaseal is achieved. Finally, pressure ramps or short negative pressure pulses of up to −400 mbar were used to rupture the cell membrane inside the recording pipette, resulting in the whole cell recording configuration.

To automatically establish recording configurations on all pipettes of the array, one pipette tip after the other picked up its cell from the catch pipette to establish a gigaseal. For each recording pipette, withdrawal of the cell from the catch pipette was initiated right after the sealing process had started. Subsequent improvement of seal resistance could be performed on all three pipettes in parallel. Finally, break-in to the whole cell recording configuration was performed for all pipettes of the array, and recordings of ion channel currents, including drug applications, can be performed for all recording pipettes in parallel.

To compare success rates, gigaseal rates, and whole cell rates to manual patch clamp, CHO cells expressing the hERG potassium channel were prepared and patched either manually (56 pipettes) or in automated mode using a pipette array (137

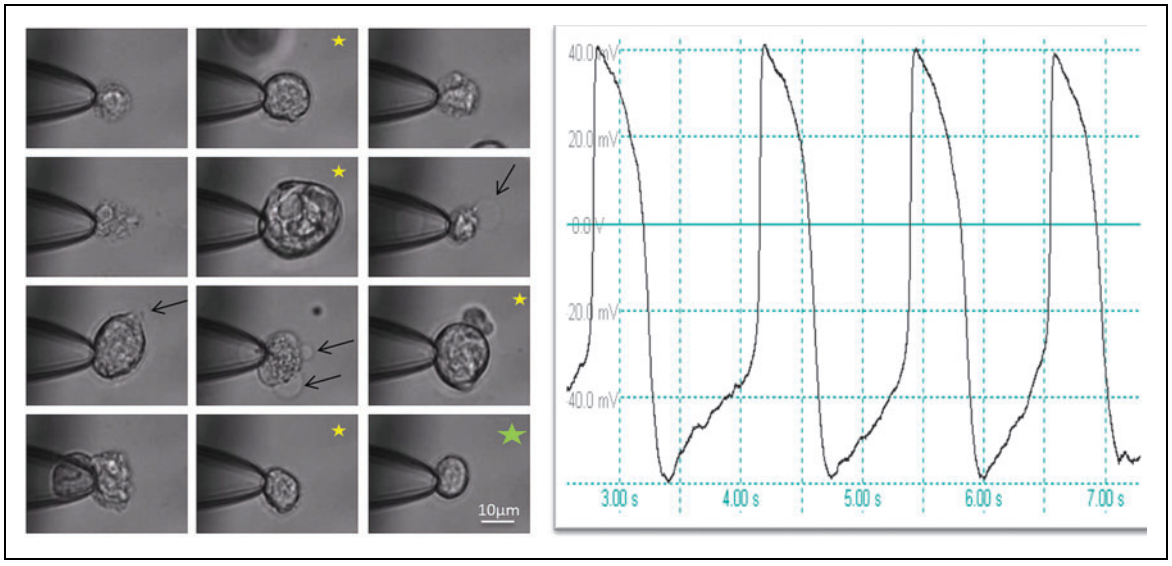

Fig. 3. Cherry-picking from a preparation of stem cell-derived cardiomyocytes. *Left*, collection of 12 Cor.4U cardiomyocytes, which were subsequently caught within 60 s. Cells that show blebs or sites of damaged membrane (indicated by *arrows*), or lack a clear round border, have a low probability to generate meaningful results and can easily be identified and excluded from patch clamp analysis to obtain a higher success rate. Presumably good cells are marked by *yellow stars*. *Right*, spontaneous cardiac action potentials recorded from the cell that was finally chosen (*green star*) for automated patch clamping.

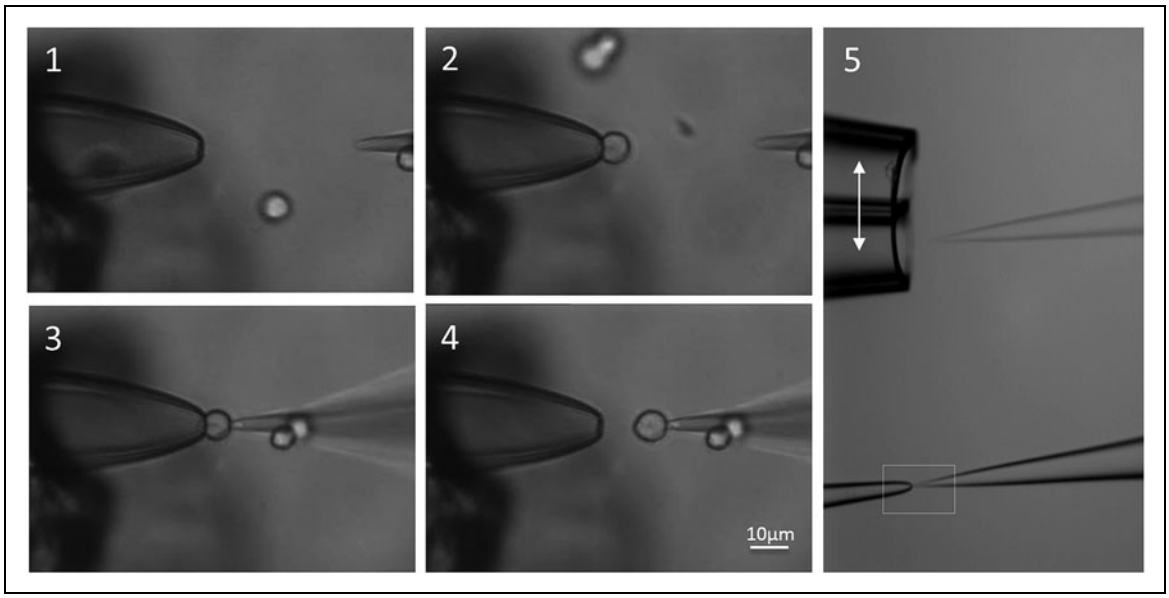

Fig. 4. Automated patch clamp experiment. **(1)** Catch pipette and recording pipette are aligned; **(2)** cell is caught; **(3)** cell has been contacted by the recording pipette and sealing has started; **(4)** patch clamped cell is withdrawn from catch pipette; and **(5)** recording pipette is moved to the theta tool for compound application (superimposed images).

pipettes). Automated mode yielded equally high gigaseal and whole cell rates per pipette (*Fig. 5*). Thus, the use of many recording pipettes in parallel significantly enhances efficiency in comparison with manual patch clamp, where only one pipette at a time can be used.

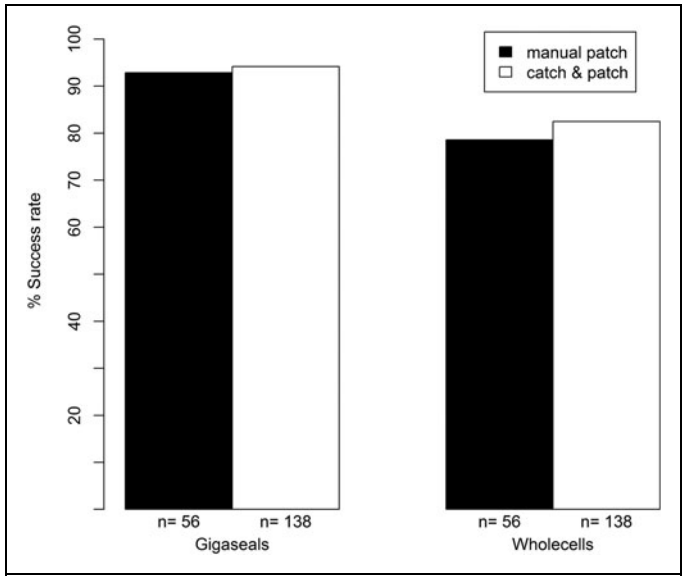

Fig. 5. Success rate evaluation. Using the same cell preparation of CHO cells expressing the hERG potassium channel, 56 single recording pipettes and 46 pipette arrays containing a total of 138 pipettes were consumed to evaluate the success rates of manual patch clamp (*black bars*) and automated operation (*open bars*), respectively. The gigaseal rate was 92.9% and 94.2% of all pipettes, and whole cell rate was 78.6% and 82.5% of all pipettes, respectively.

As a result of this automated patch clamp process, a number of cells are now available in whole cell recording configuration for simultaneous drug application. The cells are levitated by the recording pipettes and not attached to any surface. This configuration is resistant to vibrations of the setup and avoids problems with drug binding to nearby surfaces. Most importantly, it is the essential prerequisite for ultrafast compound applications. In manual patch clamp, this requires additional efforts to strip the cell from the glass bottom of the chamber.

Since the requirements for an ideal drug application system strongly depend on the type of experiment, we implemented two different drug perfusion systems, each optimized for conventional and ultrafast compound applications, respectively.

Conventional Compound Application

While drug testing on voltage-gated ion channels does not require that test compound applications are completed within milliseconds, it is still important that a steady-state drug concentration is reliably achieved within a few seconds. At the same time, many assays require the continuous flow of drug-containing solution over 10 min or more. Since test compounds may be expensive or limited in quantity, low fluid consumption is an additional requirement for a good drug perfusion system. In a typical setup for manual patch clamp, perfusing the complete measurement chamber may yield acceptable performance only if the chamber is kept small. In the case of our system with its array of recording pipettes, however, the measurement chamber is rather big (11 × 40 mm). We

found that global perfusion of the chamber can require unexpectedly long times of more than 60 s before a steady-state drug concentration is reached.

We therefore designed a measurement chamber with laterally positioned separate inlet channels for each recording pipette (*Fig. 1*). These inlets are connected to a standard perfusion controller. After the whole cell recording configuration is achieved, the patch clamp micromanipulator is used to automatically reposition the recording pipettes to a user-definable position inside of these recording bays. This also ensures that different compounds can be used with each recording bay without cross-contamination. The recording bays have dimensions of 2×5 mm and a fluid volume of ~ 30 µL. With a flow rate of 1 mL/s, a complete solution exchange inside the recording bays is reliably achieved within 2–3 s, while a continuous drug perfusion of 15-min duration could be maintained from a standard 15-mL reservoir. In addition to the recording bays, there is a global perfusion inlet to facilitate fast washing of the complete chamber (*Fig. 1*). A common perfusion outlet is used to drain the chamber. By maintaining a constant slow flow from the perfusion inlet to the outlet, upstream flow from recording bays toward the catch pipette can be efficiently avoided.

To validate the performance of this chamber layout, we executed dose–response experiments for three hERG active reference compounds (verapamil, haloperidol, and quinidine) with a three-channel setup (*Fig. 6*). In these experiments, the recording pipettes used in parallel received the same compound through the recording bays so that each run contributed up to three recordings to the average IC_{50} value. The resulting IC_{50} values of 330, 13.7, and 549 nM, respectively, are in good agreement with established literature values of 239, 27, and 686 nM, respectively.[14]

Ultrafast Compound Application

Due to the pipette-based design of our system, integration of a theta tube system for ultrafast compound application was straightforward. A piezo actuator holding a theta tube was mounted on the base plate so that the compound application capillary resided at a fixed location in the first recording bay adjacent to the catch pipette (*Fig. 1*). This allowed access to the theta tool by all recording pipettes.

A common test to validate overall performance and correct positioning of a theta tube device in a cell-free experiment uses a brief application of pure water to produce a liquid junction potential shift at the recording pipette.[6] In such cell-free tests, our system was able to reproducibly achieve solution exchange rates in the range of a few 100 µs (*Fig. 7*).

In accordance with the literature, exchange rates depended critically on the optimal positioning of the recording electrode

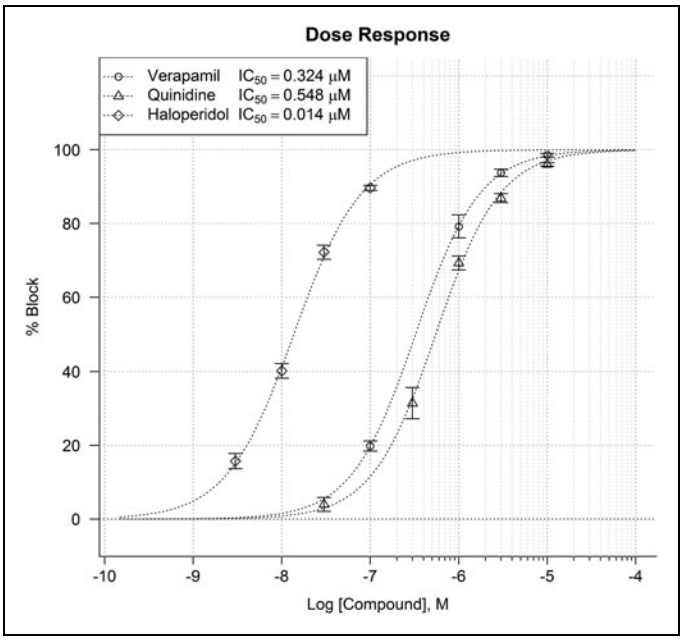

Fig. 6. Dose–response curves for three hERG active tool compounds. A three-channel catch and patch setup was used to evaluate dose–response relationships of three standard hERG tool compounds, verapamil (○, IC50 = 0.324 µM, $n = 5$), quinidine (Δ, IC50 = 0.548 µM, $n = 7$), and haloperidol (◇, IC50 = 0.0137 µM, $n = 7$). IC50 values were obtained by fitting the data to the hill equation y = ch/(IC50h+ch) with c and h representing the drug concentration and hill coefficient, respectively. The error bars represent mean ± standard error of the mean (SEM).

in front of the theta tube for every recorded cell.[15] In manual patch clamp, finding and validating this position for every recording are tedious and critical tasks.

With our approach, we could automate this process by reusing the positioning information already obtained for the catch pipette for exact and reproducible positioning of the recording electrodes in front of the theta tube. In addition to the convenience that the complete experiment can be run unattended this way, this had the advantage that the relative position of the cell in front of the theta tube, and hence the resulting exchange rate, was more reliable and standardized compared with manual positioning.

For a validation study of automated patch clamp with ultrafast compound application, we used TE671 cells as a test system. These cells have been described to natively express $\alpha 1\beta 1\gamma\delta$ nicotinic acetylcholine receptors (AChRs).[16] Application of brief 1 mM acetylcholine (Ach) pulses resulted in currents showing a fast onset with a 10%–90% rise time of 1.1 ms (*Fig. 9B*). For lower ACh concentrations of 100 µM, the current rise time was expectedly slower (4 ms, *Fig. 8*).

The theta tool allowed for the application of very brief agonist exposures. This allowed for separate determination of

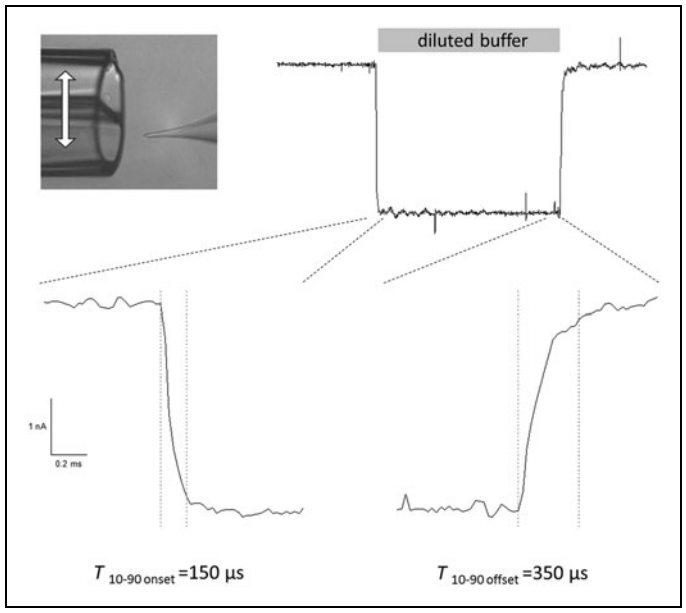

Fig. 7. Performance of the piezo-driven compound application cell-free *open tip* recording to test the performance of a rapid switch between two solutions. Different ionic strength of the solution was used to shift the baseline current of a voltage-clamped patch pipette. The time course of the current change reflects the true time course of the solution exchange. Washin was achieved in 150 µs, and washout was completed in 350 µs.

the rates for desensitization during agonist exposure and inactivation after agonist removal. In one set of experiments, we applied short (5 ms) and long (>200 ms) pulses of 100 µM ACh to the cells. This resulted in deactivation and desensitization time constants of 8.6 ± 1 ($n = 3$) and 21.6 ± 1.3 ($n = 3$) ms, respectively (*Fig. 8*). This is in good agreement with Elenes *et al.*[5] who reported 4.6 and 31 ms for this receptor subtype.

When applying 1 mM Ach with intermediate pulse duration of 20 ms, the agonist is removed before the receptor is fully desensitized. The performance of the theta tube compound application allowed to clearly distinguish between the two phases of receptor desensitization and deactivation (*Fig. 9A*).

A particularly impressive demonstration of the capabilities of the theta tube application system is double-drug application with short, yet precisely timed, interpulse intervals of varying duration to investigate the kinetics of recovery from receptor inactivation. For example, the partially desensitizing 20-ms pulses of *Figure 9* showed recovery at a rate constant of 338 ± 55 ms ($n = 4$) (*Fig. 9C*). For comparison, full desensitization was induced by applying 1 mM ACh pulses of 200-ms duration. In this study, we observed a similar recovery time constant of 375 ± 129 ms ($n = 3$) (*Fig. 10*). For validation, we repeated this experiment using pure manual patch clamp and found a very similar recovery rate of 352 ± 24 ms ($n = 10$). These findings are also in good agreement with Elenes *et al.*[5] who reported a recovery rate of 290 ms for this receptor under similar conditions in manual patch clamp experiments. Finally, if the receptor is further exposed to Ach for a prolonged period after its full desensitization is reached, the recovery rate is known to increase.[17] Accordingly, when we applied longer pulses of 500-ms duration, the recovery time increased to 794 ± 165, $n = 4$.

These experiments demonstrate that reliable and accurate recovery from desensitization experiments can be performed with our approach. With our current setup, this advanced experiment could only be performed using one whole cell recording at a time. However, this combination of high-quality gigaseals with ultrafast solution exchanges has not been demonstrated on any other automated patch clamp platform so far, thus opening up a new route of applications in ion channel analysis by using our system.

DISCUSSION

We have developed a process for patch clamp automation that mimics the manual method as close as possible, yet running fully automated and performing on multiple recording channels in parallel.

Historically, some now discontinued attempts on patch clamp automation based on patch pipettes have been made,[18–21] However, just like their chip-based competitors, they randomly attracted cells through suction, and no attempts on integrating fast compound application were made.

Other approaches of pipette-based automation systems have been based on image processing. Electrode positioning by image processing is problematic for several reasons. It requires that the electrode is sufficiently prepositioned to be visible in the microscopic image. While high precision in the two dimensions of the visual plane is usually achievable, positioning in the third dimension has to completely rely on the analysis of focusing errors, which has limited precision and is thus error-prone. A commercial system for drug screening offered only a single recording channel and was discontinued soon after its market release.[22] Concurrent image-based systems focus on brain slice research rather than drug screening and restrict themselves to offer computer assistance for electrode positioning rather than full automation.[23] If these systems feature more than one recording electrode, this requires separate micromanipulators for each, resulting in very costly and complex equipment.[23]

With our system, a single catch pipette can be used to serve many pipettes of a recording pipette array, which will subsequently be used serially or in parallel, depending on the desired assay format.

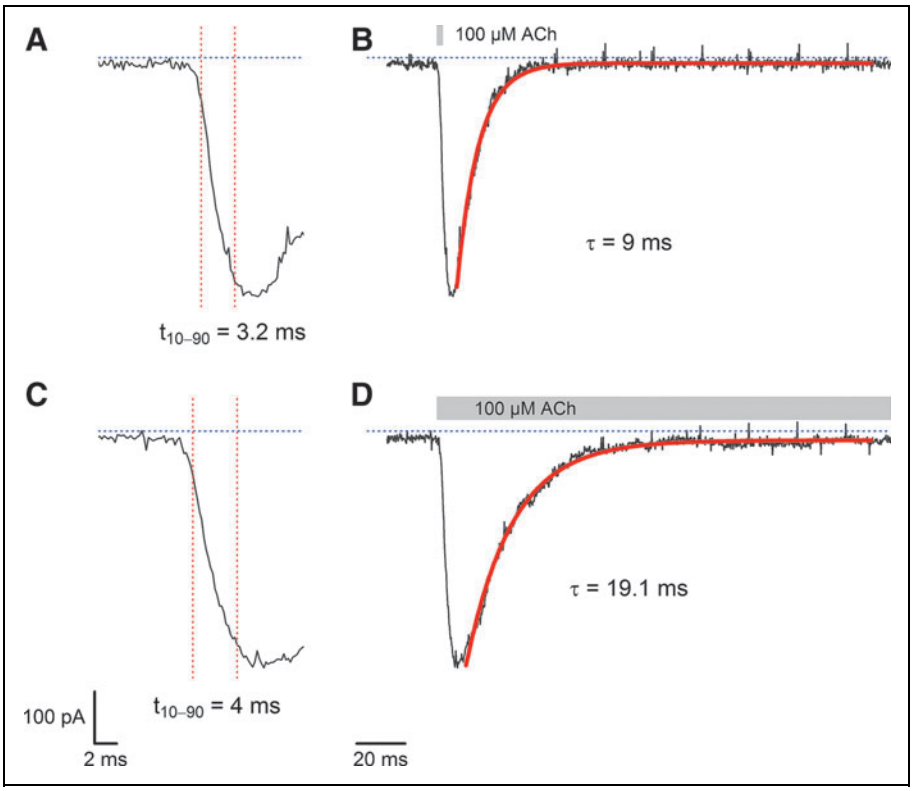

Fig. 8. Deactivation versus desensitization. The response of a TE671 cell to a short (2 ms; **A, B**) and a long (1 s; **C, D**) exposure of 100 µM ACh is shown. The deactivation time constant after short agonist application is 9 ms **(B)**. During longer exposure, a desensitization time constant of 19.1 ms is observed **(D)**. Ach, acetylcholine.

The system employs automated positioning of conventional patch clamp pipettes to combine gigaseal recordings of highest quality with key features formerly restricted to manual patch clamp.

This specific way to use pipettes for automated patch clamp recordings bears some inherent advantages when compared with patch clamp chips (*Table 1*).

Throughput and Costs Per Datapoint

Patch clamp recording pipettes can be manufactured on demand with standard laboratory equipment at low costs of less than 30 cents. This makes them very cheap disposables compared with patch clamp chips, which typically have costs of 6–8 € per patch clamp site. For typical drug testing based on conventional perfusion, doing parallel recordings is a straightforward approach to significantly enhance throughput with our method. In a first phase, the recording configuration is established one by one for the pipettes, followed by the measurement phase, where the system will record from all pipettes in parallel. During this measuring phase, the patch-clamped cells typically are exposed to various compounds for drug testing. The overall process of automatically establishing the recording configuration typically takes some minutes, while the measurement period tends to be much longer. Typically, protocols for dose–response analysis and drug testing have durations from 15 to 45 min, during which several concentrations or compounds can be tested. The measuring phase is conducted for all recording pipettes of a pipette array in parallel, leading to a much higher throughput of the system compared with single-pipette manual work. For ultrafast compound applications, the recording pipettes of a pipette array would have to share a common theta tube. Even with an installation of our system using only a single recording electrode, the high degree of automation offered by our system is very helpful when conducting these otherwise tedious experiments. With recording electrode arrays, an obvious mode of operation for theta tube equipped systems will serially use the recording electrodes one by one. However, in many typical assays, drug applications and recording of the responses usually take less than a second and are repeated at rather long intervals of tens of seconds. This would allow for circular use patterns where the recording pipettes take turns to be moved in front of the theta tube and receive their shot.

Low Cell Consumption

The vast amount of cells needed by most automated patch clamp systems to achieve a single high-quality recording can be an insurmountable obstacle to use such a system for certain research projects. In many cases, the required number of cells is simply not available or too expensive. Typical examples are stem cell research and primary cultures.

In such cases, manual patch clamp can be economically superior to its automated variant. In the extreme case, visual identification of a single suitable cell can result in successful recording. Our system is similar to other automated patch clamp systems, in that it requires a cell suspension; however, it is much more economical with cell consumption than most other systems. In its current development state, our system typically needs less than 1,000 cells per experiment, and this number is likely to be further improved in the future. Only a few chip-based systems, such as the Nanion Patchliner, are able to operate

at a similar low cell consumption.[13] Typically, chip-based systems require from 25,000 to 150,000 cells/well.[13,24]

Cell Selection

The ability to actively select a suitable cell for recording out of a mixed population is a natural yet important issue for a researcher doing manual patch clamp. Even a huge portion of dead cells and debris can be tolerated due to cherry-picking of the most promising candidates for recording, which can usually easily be identified by their size and visual properties (*Fig. 3*). In addition, manual patch clampers frequently use fluorescent or other tags to identify the subpopulation of interest. A common example is the fluorescent tagging of exactly those cells that express the ion channel of interest after transient transfection of the ion channel gene fused to a fluorescent protein.

In contrast, chip- based automated patch clamp devices are not capable of cell selection at all, and the decision of which one of all cell-like objects will land on the patch clamp hole of the chip is purely random. As a consequence, homogeneous high-quality cell preparations are an absolute requirement for chip-based automated patch clamp devices to achieve meaningful data at high success rates. While this can be made available for many standard assays in the form of specially optimized cell lines and cell preparation protocols, it is usually difficult or impossible to achieve with primary cultures or stem cell cultures.

With our current system, the cell selection is done by the operator, who attends the automated cell catching process and is notified by a sound as soon as a cell has been caught. Since the catch pipette is at a fixed location, the caught cell is always in focus without further adjustment. The operator simply takes a brief look at the cell and clicks yes or no. If a caught cell is rejected by the operator, a new one is usually caught within a few seconds. It is obvious that these cell selection capabilities can easily be extended by using image processing for further automation.

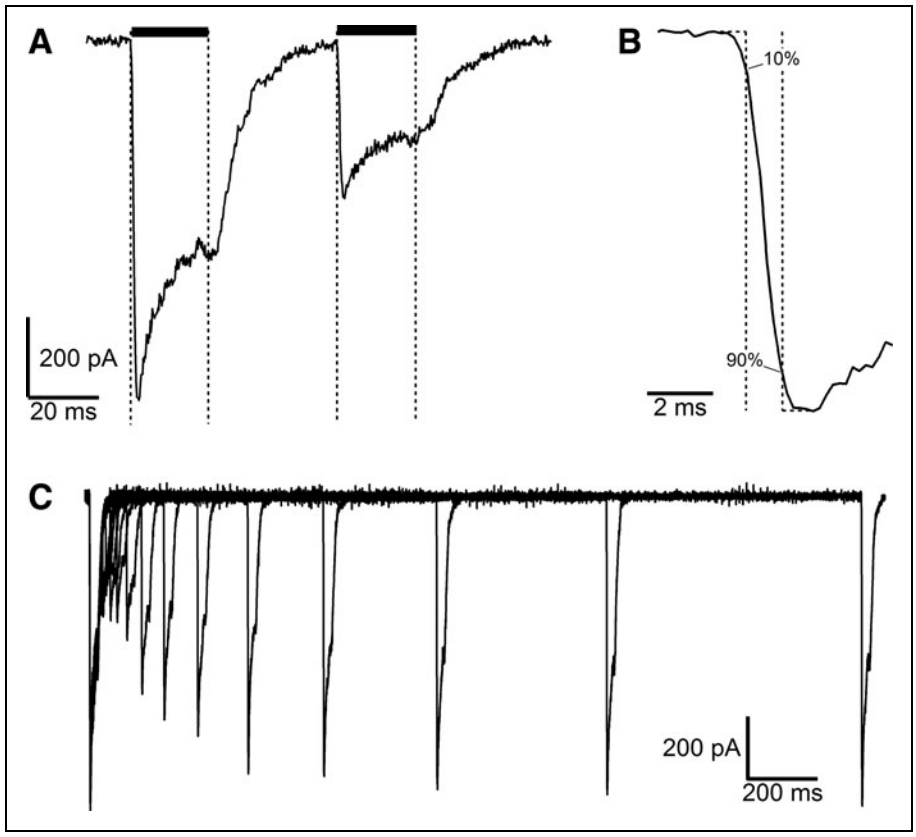

Fig. 9. Application of ACh to TE671 cells using the theta tool. **(A)** Double pulse agonist application of 1 mM ACh. Each application has a duration of 20 ms, and the interpulse interval is 40 ms. Note that the different kinetics of desensitization and deactivation are clearly resolved. **(B)** Close-up of the onset of nACh-induced current from **(A)**. The 10%–90% rise time of the current onset is 1.1 ms. **(C)** Series of superimposed double applications with varying interpulse intervals showing recovery from desensitization.

Ultrafast Perfusion Using a Theta tube

Many chip-based automated patch clamp systems have shown to be capable of eliciting LGIC currents that rise within a few milliseconds.[25–27] However, this does not necessarily mean that the performance of the perfusion system is equally fast. Notably, a fast rise time of the ion channel current does not guarantee that the stimulating agonist application has been applied with an equally fast exchange speed: at agonist doses much higher than the EC_{90}, it is obvious that the current rise time will be much faster than that of the agonist concentration. The systematic errors that arise from this situation for the generation of agonist dose–response curves have been discussed extensively.[28] A workaround suggested by these authors is to use the area under the curve (AUC) as a surrogate measure for EC_{50} determinations. Indeed, when using a conventional chip-based system to analyze responses of the alpha7 nAChR to its agonist ACh, the peak response-based EC_{50}, but not the AUC-based EC_{50}, was right shifted up to 15-fold compared with manual patch clamp literature, indicating that the speed of compound application was much slower than the activation kinetics of the channel.[26]

In addition to the performance of the washin, a fast and efficient washout of compounds is equally important. Since most

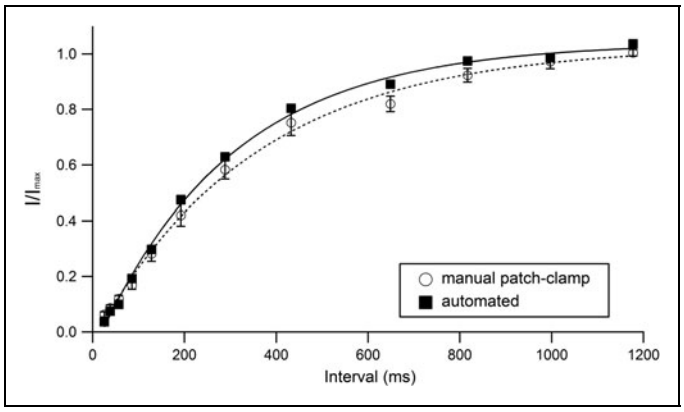

Fig. 10. Comparison of recovery curves done with pure manual patch clamp ($n=10$) versus automated operation ($n=1$). Error bars represent mean ± SEM.

of the concurrent automated systems use rather slow robotic pipettors for compound applications, minimum exposure times can extend to several seconds, introducing systematic errors by desensitization of the receptors. For a remedy, some pipettor-based systems do stacking of agonist and washout solutions inside the pipettor needle to reduce exposure times.[25–27]

Another common workaround is the addition of allosteric modulators that slow down the desensitization kinetics or enzymes to chemically reduce agonist concentration after washout, even for simple assays.[26,27] A notable exception is the Ionflux™ instrument by Fluxion technologies, which integrates the microfluidics for compound application into the disposable plates, allowing for shorter exposure times and even double applications with interval times of less than a second.[29]

Table 1. Comparison of Patch Clamp Methods

	Manual patch clamp	Catch and patch	Planar patch clamp
Number of channels	1	4[a]	16–768
Throughput (conventional drug application)	~10 cells/day	~30 cells/day[a]	100–10,000/day
Number of cells needed per successful recording	<100	<1,000	1,000 …. 150,000
Cell selection	Yes	Yes	Not possible
Costs per pipette/chip site	0.30 €	0.30 €	4–8 €
Compound washin time (whole cell)	~5 ms	~5 ms	~30 ms to >200 ms
Shortest double application interval (whole cell)	<20 ms	20 ms	Not reported
Compound washin rise time (outside out)	8 µs	Planned	Not possible
Shortest double application interval (outside out)	26 µs	Planned	Not possible

[a]Throughput based on a four-channel setup that is currently under development.

However, for many advanced experiments in LGIC research, compound application performance on a completely different level is required. Short agonist exposure times in the millisecond, sometimes even submillisecond, range are being used for synaptic neurobiology, biophysical characterization of LGICs, and mode of action analysis in LGIC pharmacology.[8,30,31] For the study of recovery kinetics, interval times between double applications in the millisecond range are frequently required.[5]

In the manual patch clamp realm, the piezo-driven theta-tube is undisputedly the gold standard for this type of research.[8] This method has so far remained bound to pipette-based, and hence manual, patch clamp. In the experiments shown in *Figure. 9A*, we were able to use exposure times as well as double application intervals down to 20 ms and still could clearly resolve deactivation from desensitization. Comparable performance has not been demonstrated so far with chip-based systems.

In whole cell experiments on real cells, unstirred layer effects on the surface of the cell are supposed to slow down the compound exchange rate that is actually seen by the receptor. If extremely fast compound exchange rates are required, this effect can be minimized by recording from small membrane vesicles instead of whole cells. The theoretical and practical limits of the theta tool method on these so-called outside out recordings have been explored by Auzmendi *et al.*[7] who were able to produce agonist pulses with 8 µs rise time and 26-µs duration. In preliminary experiments, we were able to generate such outside out recordings with our method. To achieve this, the catch pipette pressure is simply adjusted to hold back the cell while retracting the recording pipette from the catch pipette after whole cell formation. With some further optimizations, we believe that our system could also become the first automated patch clamp system that is able to routinely generate outside out patches.

In conclusion, here we present a completely new concept for automated patch clamp based on classical recording pipettes, offering high-quality gigaohm seals, optical control with intentional cell selection, low cell consumption, and the possibility to easily integrate advanced methods and extensions from the manual patch clamp world. As a demonstration of the power of our approach, we showed for the first time the integration of a state-of-the-art method in

LGIC research, the theta tool ultrafast compound application system, into an automated patch clamp device.

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DISCLOSURE STATEMENT

The Patent US 20140137396 A1, which is cited in the text and covers central parts of the method described in this article, is currently owned by NMI. The patent has been licensed to Multichannel Systems GmbH (Reutlingen, Germany) that is developing a commercial version of the system under the brand name, Patchserver.

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Abbreviations Used

Ach = acetylcholine
AChR = acetylcholine receptor
AUC = area under the curve
LGIC = ligand-gated ion channel