Ion Channel Drug Discovery and Research: The Automated Nano-Patch-Clamp Technology

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Abstract: Unlike the genomics revolution, which was largely enabled by a single technological advance (high throughput sequencing), rapid advancement in proteomics will require a broader effort to increase the throughput of a number of key tools for functional analysis of different types of proteins. In the case of ion channels - a class of (membrane) proteins of great physiological importance and potential as drug targets- the lack of adequate assay technologies is felt particularly strongly. The available, indirect, high throughput screening methods for ion channels clearly generate insufficient information. The best technology to study ion channel function and screen for compound interaction is the patch clamp technique, but patch clamping suffers from low throughput, which is not acceptable for drug screening. A first step towards a solution is presented here. The nano patch clamp technology, which is based on a planar, microstructured glass chip, enables automatic whole cell patch clamp measurements. The Port-a-Patch© is an automated electrophysiology workstation, which uses planar patch clamp chips. This approach enables high quality and high content ion channel and compound evaluation on a one-cell-at-a-time basis. The presented automation of the patch process and its scalability to an array format are the prerequisites for any higher throughput electrophysiology instruments.

AIMS AND SCOPE

The need for fast and sensitive drug testing in the pharmaceutical industry requires the implementation of rapid, highly parallel screening techniques [1, 2, 3]. Ion channels are excellent drug targets as they play a major role in many common diseases [4]. The most sensitive technique available for screening ion channel active drugs (ICADs) is patch clamp recording from cell membranes, which allows even single ion channels to be probed with great accuracy [5, 6]. The major drawbacks of this technique for industrial applications are the low throughput and the high personnel costs due to the labor intensive evaluation of individual drugs. Given the large number of drugs generated by combinatorial chemistry and the high degree of selectivity required, a rapid and efficient screening method is particularly important. Recently, different instruments for the automated analysis of ion channel function have been developed for ion channels expressed in Xenopus Oocytes [7] as well as mammalian cells [8, 9].

By gating the movement of charged particles (ions), ion channels mediate the flow of electrical current over the cell membrane [10]. Ion channels, therefore, essentially determine the ways cells exchange information in all tissues of the human and animal body, including those of the nervous, the cardiovascular, the intestinal and the reproductive systems. Ion channels are easily accessible for drugs, because they are membrane proteins exposed to the extracellular, the intracellular and the lipid membrane compartments alike.

Several methods are available today to test candidate ICADs: Electrophysiology (patch clamp), binding assays, radioactive flux assays, membrane potential sensitive fluorescent dyes, ion-sensitive dyes, and voltage sensing based on fluorescence resonant energy transfer (FRET). It is widely acknowledged that electrophysiological analysis, i.e. patch clamping, represents the gold standard for the evaluation of compound actions. In the configuration that is most useful for drug research (whole-cell patch clamp), an electrolyte-filled glass pipette is used to establish a low-resistance current path into the interior of a single cell. It is then possible to directly record current that is flowing through ion channels in the membrane with very high amplitude- and time-resolution.

Nanion's Patch Clamp Chip NPC -1

By using a new approach based on microstructuring techniques, we have developed chip-like devices for patch clamp recording. Indeed, there is at least one successful precedent for using a planar structure for electrophysiology [11]. Recordings of single ion channels [12] as well as whole cell currents [13] are performed with a chip made from glass substrate. The technique has the potential of revolutionizing

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A

B
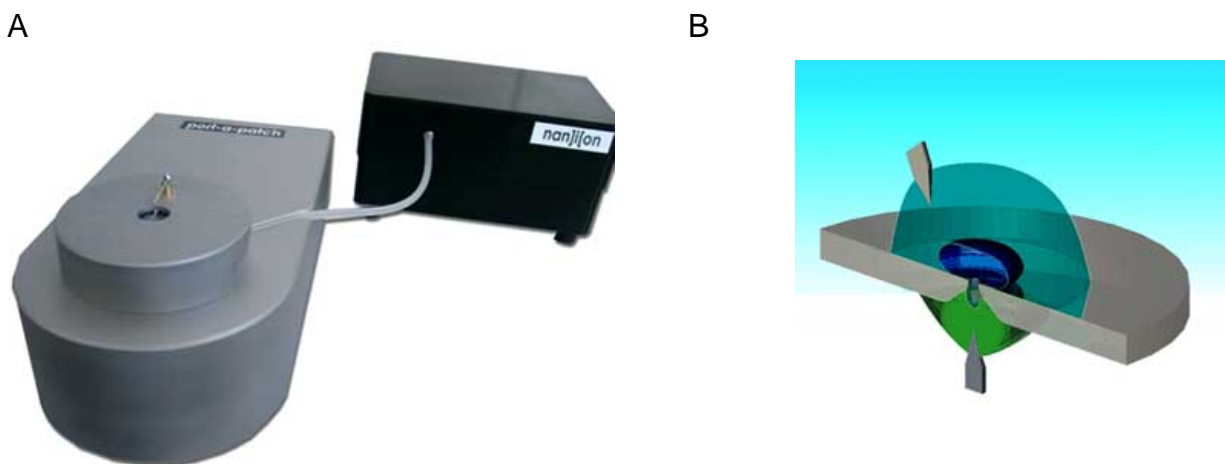
Fig. (1). In **A**, the integrated patch clamp unit which uses the planar patch clamp chips is shown. The chip mounting station and suction control unit together with EPC-10 amplifier are computer controlled by the PatchMaker software package. With the chip-based approach, no vibration isolation table or optical control is necessary. The resistance across the aperture in the chip is permanently monitored and used for the feedback of the software controlled suction applied to the patch clamp chip. Once a cell is acquired the software adapts the suction and establishes a whole cell configuration in a automated manner. The schematic in **B** shows the positioned cell on the aperture in the chip. The default situation is the cell attached configuration, suitable for single channel recordings. Via the application of a software controlled suction pulse, the cell membrane can be ruptured to achieve whole cell access. Also a brief voltage pulse (zapping) can be used.

(for more information on the commercial device and the consumable, please visit our website at www.nanion.de)

the way in which ICADs are evaluated by the pharmaceutical industry today, as it enables higher throughput screening with patch clamp accuracy.

While we have earlier reported on recordings from planar lipid bilayers using similar devices [14], the success in recording from mammalian cells represents a significant step for the development of automated electrophysiology [13,15]. This novel device has important applications not only in proteomics and pharmacological compound evaluation (drug screening), but will also facilitate biophysical and neurobiological experiments on ion channel proteins, e.g. combined patch clamp and (single molecule) fluorescence microscopy experiments [16].

The microstructured chip replaces the glass pipette, improves the experimental situation and greatly facilitates the experimental procedure. Cells in suspension are simply pipetted onto the patch clamp chip and using a software controlled suction protocol, a single cell is automatically positioned and sealed onto the aperture. The positioning of cells for patch clamp recording with suction applied to the chip is automated without use of micromanipulators or visual control. Furthermore, the principle is easily scaled up to an array format, enabling automated, rapid and parallel screening of ICADs.

The performance of the automated, planar patch clamp approach strongly depends on the quality of the microstructures used for the recording. For optimal low capacitance and, hence, low noise, we use borosilicate glass as a substrate. The capacitance of patch clamp chip is less than 1 pF, also enabling recordings from very rapidly gated or inactivating ion channel proteins, such as sodium channels. The chips contain an aperture with a diameter of

about one micrometer, in which a single cell is positioned by the application of suction. The open tip resistance across the aperture measured in standard saline solution is about 2-5 MΩ. In Table II, the typical parameters of the glass patch clamp chips are described.

Replacing the Conventional Patch Clamp Set Up with the Port-a-Patch[©]

In academia and industry today, the experimental setup for patch clamp recording typically consists of the following parts: a microscope to optically control the positioning of the patch pipette, a micromanipulator to move the pipette, a vibration isolation table onto which the microscope is mounted, a faraday cage surrounding the microscope and table, a patch clamp amplifier for recording the signals from the cell and a computer for data acquisition. The necessary equipment is, therefore, rather bulky and can easily fill half a laboratory room. As the different components are only offered by different suppliers, no all in one solution can be bought and set up in a straight forward manner. The Port-a-Patch[©] (Fig. (**1a**)) consists of a chip mounting station, where the signal amplification electronics is integrated, a separate suction control unit, an EPC-10 amplifier (HEKA Electronics) and a software package that runs on the system computer.

The Port-a-Patch[©] uses the planar patch clamp chips, which are mounted on small screw–on caps. The caps have a built-in O-ring and are screwed onto the holder, so that a suction/pressure can be applied to the chip. The positioning of a cell is done automatically and is monitored by an electrical readout, therefore, no optical control is necessary. Due to the fact that the cell is fixed onto the aperture in the

Table I: Comparing the classical patch clamp technique with electrophysiology using the Port-a-Patch©.

Conventional Patch Clamp	NPC©-1 Port-a-Patch©
• Composed of many pieces	• One entity device
• Trained personnel necessary	• Technician operated
• Anti-vibration table necessary	• Inherently stable
• Large liquid consumption: >50 ml	• Small liquid consumption: <10 µl
• Elaborate fast fluid exchange	• Integrated fast fluid exchange
• Low throughput: <10 data points per day	• Higher throughput (up to 50 data points per day)
• Exchange of internal solution only with high effort possible	• Effortless exchange of internal solution

Table II: Performance of the NPC©-1 Chips.

• Chip resistance:	• 2-5 M , n = 300
• Chip capacitance:	• < 10 pF
• Seal resistance:	• > 1 G
• Access resistance:	• 6 -15 M
• Whole resistance:	• > 200 M
• Whole cell recording success rates:	• HEK 293 cells: 30 - 50% CHO cells: 30 - 50% RBL cells: 50 - 70%
• Whole cell stability:	• ~ 20 min
• Full dose response curve possible on one cell	

chip, no vibration isolation is required. This allows the significant miniaturization of the whole setup, which includes a pump, a valve block, a small micro-controller for the suction control and a miniaturized faraday cage. The chips can be easily changed with a cycling time of less than a minute. After a chip is installed in the instrument, the small faraday cap is placed above the holder for electrical shielding. Fig. (**1b**) shows the NPC©-1 chip and the experimental situation of a cell on the chip.

Table I summarizes and compares the features of a conventional patch clamp rig and the Port-a-Patch.

Materials and Methods

An important requirement for obtaining high quality recording with the patch clamp chip is the preparation of a clean suspension of well-isolated cells. All cells are grown in their medium described in the ATCC catalogue. Typically, we grow cells to 60-80% confluency and isolate them by the application of PBS or other enzyme free dissociation buffer. The cells are then centrifuged twice and the resulting cell pellet is resuspended in the recording solution, the extracellular solution. Cell concentrations that work best are in the range of $5x10^5$ to $5x10^7$ cells/ml. For an experiment, 5-10 µl of cell suspension is pipetted onto the chip. If the cells have already settled to the bottom of the eppendorf cup, they can be resuspended by withdrawing and releasing the cells with a 1 ml pipette before the measurement.

For the recordings, a commercially available amplifier and data acquisition software (EPC-10, HEKA, Germany) was used. The recorded data were filtered at 3 kHz and sampled at 10 kHz. Electrolyte solutions used had the following ionic compositions (mM): extracellular (top of chip): NaCl (140), KCl (3), $MgCl_2$ (1), $CaCl_2$ (1), HEPES (10), pH = 7.4, 270 mOsm; intracellular (underside of chip): for HEK cells: CsCl (130), NaCl (10), HEPES (10), EGTA (10), pH=7.2, 240 mOsm; for RBL cells: K-Aspartate (120), EGTA (10), HEPES (10), NaCl (10), pH=7.2; for CHO cells: KCl (120), NaCl (10), EGTA (10), Hepes (10), pH = 7,2.

Whole Cell Patch Clamp Recording with the Port-a-Patch©

Electrophysiology with the Port-a-Patch© is possible on the whole cell [13] as well as on the single channel level [12]. For the purposes of drug development, whole cell recording is by far the more relevant configuration and we will therefore, focus on this technique.

Recently, the hERG channel has gained great attention due to its role in cardiac arrhythmia and the LQT-syndrome. As the FDA requires testing of all new drugs on hERG channels for side effects, a large number of whole cell recordings is performed on this particular target. We have therefore validated our technology on a CHO cell line stably transfected with hERG. In (Fig. (**2**)) whole cell recordings performed with the chip from hERG channels expressed in a CHO cell are shown.

In order to further demonstrate the high quality of the patch clamp recordings performed with the microstructured glass chips, we chose a sodium channel, the rNav1.2a. The kinetics of this sodium channel are very rapid, the inactivation takes place within 2 ms. This is one of the most rapid ion channels that we know and is therefore, a good study object for validation of a new method. (Fig. (**3**)) displays whole cell recordings from the rNav1.2a expressed in a HEK cell.

Due to the low capacitance of the glass chip and the excellent voltage clamp made possible by the chip design, recording from even these fast ion channels is possible. Our chip based technology is not limited to a specific cell line nor to a specific channel type, but is as generally applicable as the pipette based patch clamp technique is. Of course some cells are easier to patch with the chip than others, as is the case with patch pipettes. The 'patchability' is very much correlated, i.e. a cell that is easy to patch with a pipette will most likely work fine with the chip as well.

Pharmacology with the Port-a-Patch©

During the development and evaluation of new compounds one will typically want to measure the effect of a drug on its target in correlation to the amount of drug
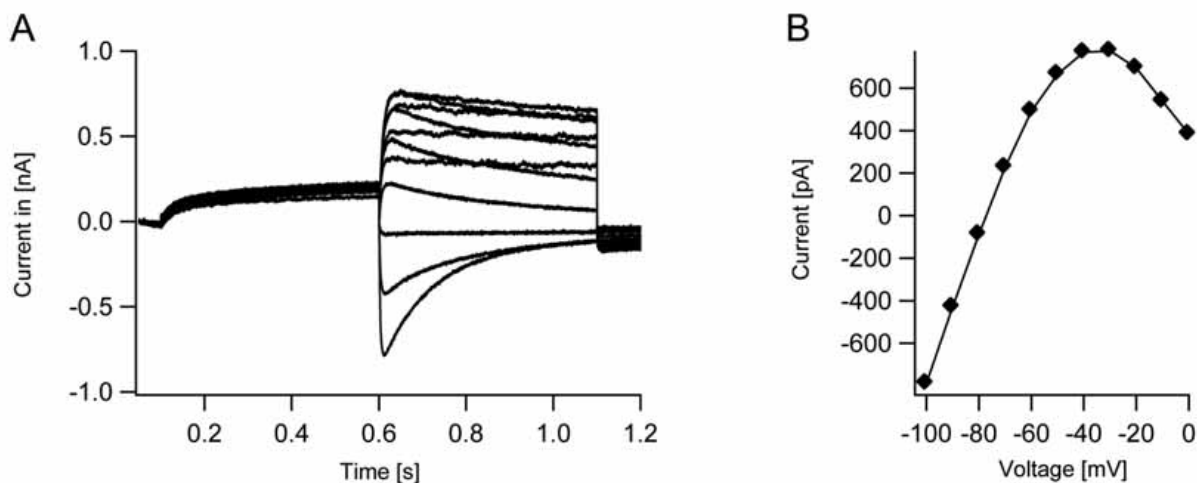
Fig. (2). hERG channels were activated by a depolarizing pulse from –80 mV to +40 mV for 500 ms. The current amplitude in B was measured as the peak amplitude of the following tail current. The voltage of the tail protocol ranged from –100 mV to 0 mV.
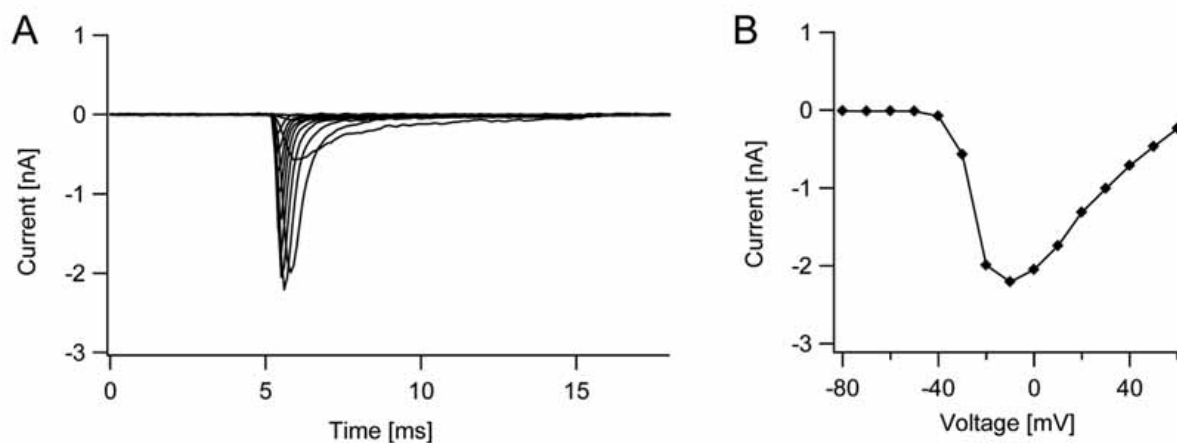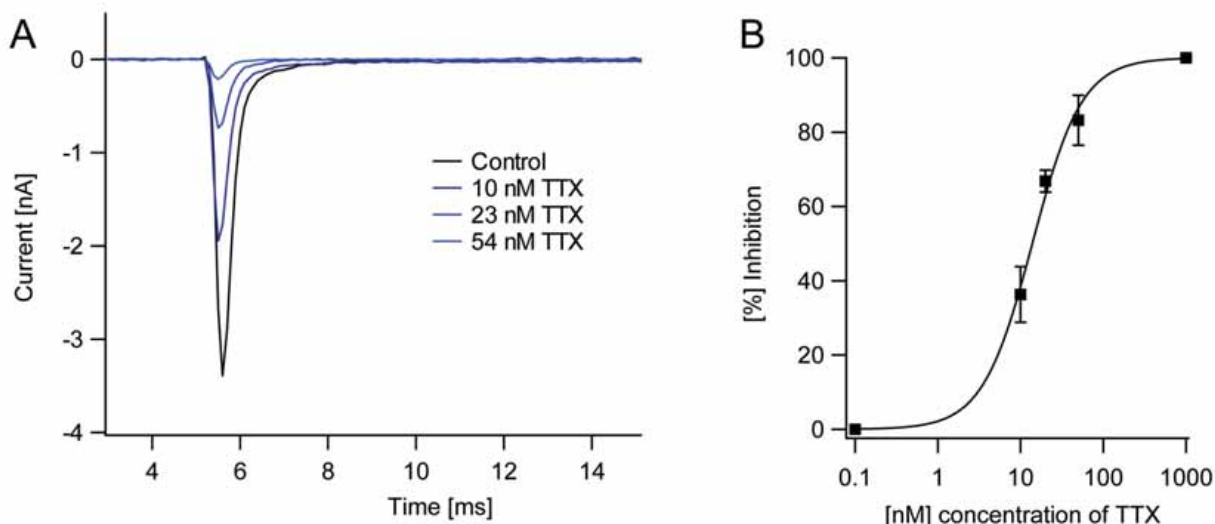

Fig. (3). rNav1.2a channels expressed in HEK cells recorded in whole cell mode with the patch clamp chip. **A:** Very rapidly inactivating currents corresponding to depolarizing pulses with increasing amplitude from –80 mV to 60 mV in 10 mV steps. **B:** The corresponding IV relation with the typical features of a sodium channel current.

applied. To achieve such a dose response curve, different concentrations of the compound under evaluation need to be applied to the ion channel protein. For each concentration, a specific parameter is monitored, for example the maximum amplitude of whole cell current. In (Fig. (**4**)), one such concentration-dependent effect is shown. Again, whole cell recordings from the sodium channels expressed in HEK cells are performed and increasing concentrations of the well-known sodium channel blocker tetrodotoxin (TTX) are applied to the extracellular solution. The current amplitude is recorded for different TTX concentrations, which are simply pipetted onto the chip. The corresponding dose-response relation is also shown in (Fig. (**4**)), resulting in a half maximal block (IC50) of the sodium current at a TTX concentration of 14.9 nM (literature value 12 nM).

Perfusion with the Port-a-Patch©

The cell on the chip is accessible for ligands and can be perfused by a simple solution exchange from top or bottom side of the chip. Solution exchanges and test compound applications can be performed very rapidly. In Fig. 5 whole cell recordings from perfusion experiments on RBL cells are presented. The RBL cells endogenously express inward rectifying potassium channels. The cells were voltage clamped at -80 mV, where these channels have a high open probability, and potassium concentration in the external solution was varied. The addition of high concentration potassium solution increased the conductivity as more ions could permeate the channels. The resulting increase in the holding current is completed within less than 10 ms. The reverse effect is achieved by reducing the external potassium concentration again. A complete perfusion cycle is shown in (Fig. (**5**)). The resulting time constants for increasing and decreasing the potassium concentration is $t_{in} = 6.7 \pm 3.3$ ms and $t_{out} = 8.0 \pm 2.0$ ms (n=8). The fast perfusion makes the planar patch clamp approach applicable for studying even fast ligand-gated ion channels.

Fig. (4). Pharmacological block of voltage-gated sodium currents mediated by rNa1.2a channels expressed in HEK 293 cells recorded with the Port-a-Patch[©]. Panel A displays the raw rNa1.2a data with increasing concentrations of tetrodotoxin (TTX). Channels were activated by a voltage pulse from –100 mV to –20 mV. Panel B shows the corresponding dose-response curve with a fit of an Hill-curve resulting in an IC50 of 14.9 ± 5.3 nM (a data point at high TTX concentration was added for the fit).
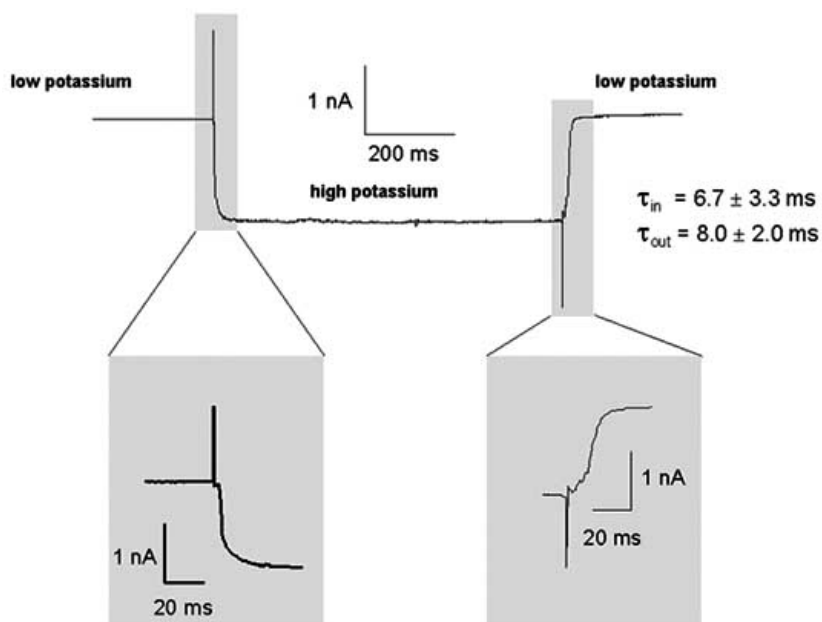
Fig. (5). Rapid perfusion of RBL cells patch clamped with the NPC[©]-1 chip. The endogenous inwardly rectifying potassium channels of the RBL cells result in a steady state whole cell current at a negative holding potential of –80 mV as applied in this experiment. By varying the potassium concentration in the extracellular solution the whole cell current is increased or decreased accordingly. An increase of more than 1 nA in current is achieved by changing from a low to a high potassium concentration. Upon removal of the high potassium concentration back to the former low concentration, the whole cell current drops again to the former steady state level. In the two insets the timescale is blown up to emphasize the rapid solution exchange achieved with this chip perfusion approach. The time constants for the addition and washout of solution are less than 10 ms.

The Software of the Port-a-Patch[©]

The software running the Port-a-Patch[©] device is called PatchMaker. It allows the user to use predefined parameter settings for the suction control to acquire a cell and get whole cell access. These parameters can be adjusted by the user until their values are optimally suited to a given experiment. PatchMaker automatically adjusts the offset

potential, reads out and compensates for the capacitance of the chip and the cell, reads out the seal and access resistance and, based on these experimental parameters, makes decisions for the continuation of the experiment. For different cell types parameters can be optimized according to e.g. cell size, ease of getting whole cell access etc. Various standard settings are supplied with the system.

The well known Pulse software from HEKA Electronics is integrated into the PatchMaker software. PatchMaker uses a batch interface to control Pulse for the readout of parameters and the electrophysiological experiments. For the definition of voltage stimulation protocols, data acquisition and analysis, all the features of Pulse are available to the user. In addition, PatchMaker generates a log-file containing all relevant parameters of an experiment, e.g. chip resistance, membrane resistance, whole cell capacitance etc.

The Port-a-Patch$^{©}$ uses an EPC-10 amplifier, offering the high quality and versatility of this excellent research instrument to the user.

CONCLUSIONS

As the microstructured chip replaces the glass pipette, the experimental situation and the procedure of patch clamping is greatly simplified. The positioning of cells for patch clamp recording with suction applied to the chip is automated in a straightforward manner.

The high quality of the recordings performed with the Port-a-Patch$^{©}$ and its consumable, the microstructured glass chips, has been shown for different cell types. Cell types that work well with the Port-a-Patch$^{©}$ include CHO and HEK cells, which are widely used as expression system for generating stably transfected cells. The presented data show the performance of the technology, which enables patch clamp recording even from very fast inactivating sodium channels and also allows for rapid perfusion of the cells. All data presented was acquired with the Port-a-Patch$^{©}$.

Electrophysiology in combination with a precise and rapid perfusion enables sophisticated experiments on ion channels. The planar patch clamp approach with its automated cell acquisition, recording and versatile perfusion allows the generation of high content data like an IC50 of a given compound at any potential on a single cell. Due to the favorable geometry of the chip only tiny volumes are consumed, making this technology very attractive for experiments with rare and expensive compounds.

Making advanced electrophysiological techniques such as patch clamping compatible with higher throughput require-

ments is a goal long sought by the pharmaceutical industry [17,18,19]. Highly integrated chip-based electrophysiology provides a solution for this pressing need.

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