Electrophysiology Electrochemistry

HEKA impulse 03

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Editorial

Did you ever dream of

- *patch-clamping 100 times more cells per day?*
- *hundreds of whole-cell recordings with state-of-the-art quality?*
- *perform automatic patch-clamp experiments with excellent ease-of-use?*
- *establish dose-response curves in a breeze?*
- *speed up ion channel drug discovery beyond its current limits?*
- *free up valuable laboratory space?*

Do you have

- *substantial equipment budget?*

If you answered yes to all of the above, HEKA has something for you that will change the way you do experiments forever and you will definitely want to read the articles in this and the next issue of HEKA impulse. The last few years have seen unrelenting efforts undertaken by many biotech companies to establish automated patch-clamp systems homing in on high-throughput drug screening of ion channels. The need is high, especially since it has become clear that ion channels are not only excellent drug targets in their own right, but they can also be adversely affected by drugs that are unrelated to ion channel targets. Regulatory agencies such as FDA strongly recommend drug testing of known ion channels to rule out possible side effects before any drug-related clinical trial should be started.

HEKA has been collaborating with several companies (flyion GmbH, Nanion Technologies GmbH and Xention Discovery Ltd.) to integrate its years of technical know-how on electrophysiological data acquisition hard- and software systems with new and exciting medium- to high-throughput screening methods. The results are innovative instruments that are stunning and promise to revolutionize the drug discovery and mechanism-based testing needs of academia and industry now and in the near future.

Some of you, particularly those in academia, may have answered "no" to one of the questions above. However, creative financing and budgeting (individually or as a group of investigators) or a compelling grant proposal may get you closer to your dreams. Also, don't forget that Patch-clamp robots are well suited as a shared facility instrument and could be incorporated into your institution's capital budget and/or your next Program Project grant.

The Port-a-Patch: The Smallest Patch Clamp Setup for High Quality Electrophysiology

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In evaluating ion channel function, patch clamping, provides the highest information content but every electrophysiologist knows the draw back of the size and complexity of a patch clamp rig. We present here patch clamp recordings in the whole cell configuration performed with planar patch clamp chips, which are micro-structured from borosilicate glass substrate. The chips are used in the Port-a-Patch©, a simplified and miniaturized patch clamp setup that enables automated patch clamp experiments on a single cell. The PatchMaker software performs the experiment by executing user-determined protocols for cell positioning and in communication with the PULSE software also protocols for electrical stimulation and current readout. In various electrophysiological experiments, the high quality of recordings and the versatility of the perfusion of the recorded cells are demonstrated.

Ion channels have been drug discovery targets in the pharmaceutical industry for decades, largely due to the central role these proteins play in excitable cells, as discussed by Dr. Krafte in the last issue of impulse. The patch clamp technique is still the gold standard for ion channel characterization and pharmacology but its draw back is the low throughput as well as the size and the complexity of a patch clamp rig. This is the reason for the recent development of automated electrophysiology by several research groups and companies. The first advances were realized by the automation of two electrode voltage clamp experiments of Xenopus oocytes [1]. The second step was the automation of patch clamp measurements of mammalian cell lines. Most of the approaches are based on planar substrates using materials like silicon, PDMS, plastic or glass [1,2]. The method described in this article is using borosilicate glass, a proven material in the field of electrophysiology with sound electrical and optical properties [3].

Replacing the Conventional Patch Clamp Setup with the Port-a-Patch©

In academia and industry, the experimental setup for patch clamp recordings 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 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. In contrast to this, figure 1a shows a complete and straightforward ion channel screening station, the Port-a-Patch©. The Port-a-Patch© consists of a chip mounting station, where the signal amplification electronics is integrated, a separate suction control unit, an EPC-10 amplifier (HEKA Elektronik) and a software package that runs on the system computer.

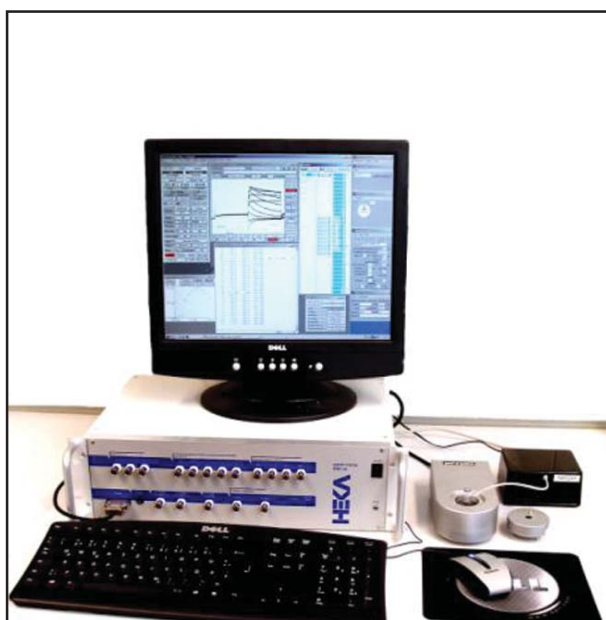


Figure 1A:
The Port-a-Patch© as an integrated patch clamp setup. Next to the EPC10, the chip mounting station is placed together with the suction control unit for automated cell positioning and measurement. This setup basically replaces a common patch clamp rig. Panel B displays the disposable, the NPC©-1 chip. The upper panel of figure 2b shows NPC©-1 chips in a production device, the lower panel a single chip upside down. The patch clamp chips are supplied on small screw caps, which are easily mounted onto the holder. This design enables a quick cycling time for the chip exchange between experiments.

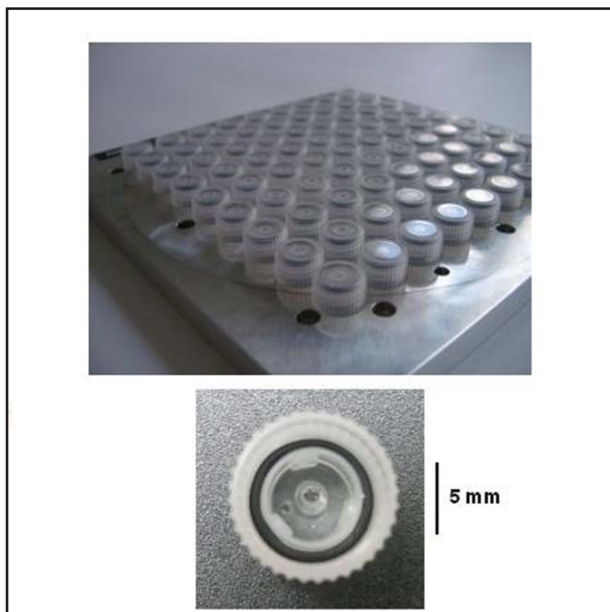
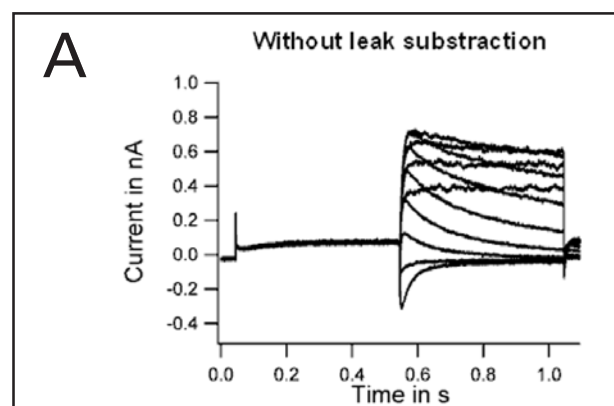


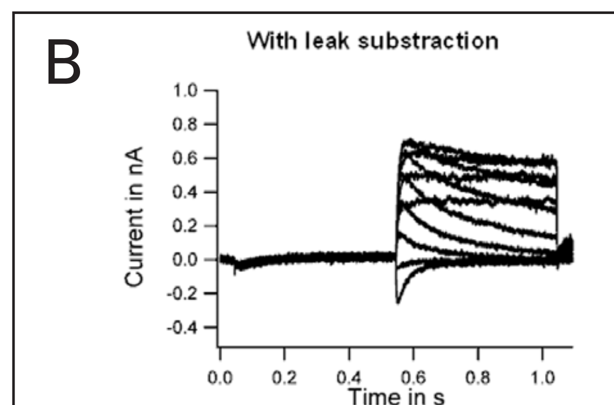
Figure 1B:
Panel B displays the disposable, the NPC©-1 chip. The upper panel of figure 2b shows NPC©-1 chips in a production device, the lower panel a single chip upside down. The patch clamp chips are supplied on small screw caps, which are easily mounted onto the holder. This design enables a quick cycling time for the chip exchange between experiments.

Nanion's Patch Clamp Chip NPC©-1
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 chips contain an aperture with a diameter of about one micrometer, on 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 3 M.
The micro-structured chip replaces the glass pipette, improves the experimental situation and greatly facilitates the experimental procedure. The NPC-1 chips are mounted on small screw–on caps, which have a built-in O-ring (Fig. 1b) and are screwed onto the holder, so that suction/pressure can be applied to the chip. Cells in suspension are simply pipetted onto the patch clamp chip. The positioning of cells is realized by applied suction, which an automated process without the use of micromanipulators or visual control. Furthermore, the principle is easily scaled up to an array format, enabling automated, rapid and parallel screening of ion channels.

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. Based on these experimental parameters the software is able to make decisions for the continuation of the experiment. For different cell types parameters can be optimized according to e.g. cell type, cell size or ease of getting whole cell access. Various standard settings are supplied with the system.
The PULSE software from HEKA Elektronik 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, the features of PULSE are available to the user. In addition, PatchMaker generates a log-file containing all relevant parameters of an experiment, like chip resistance, membrane resistance, whole cell capacitance etc.

Whole Cell Patch Clamp Recording with the Port-a-Patch©
Electrophysiology with the Port-a-Patch© is possible on the whole cell [4] as well as on the single channel level [3]. In figure 2a and b we show a typical recording from a CHO cell expressing the potassium channel hERG. The two upper panels compare the same data with and without the subtraction of a leak trace generated by a P/4 protocol. Figure 2c demonstrates the stability of the recording during the time course of 10 minutes, using the same cell type as above.





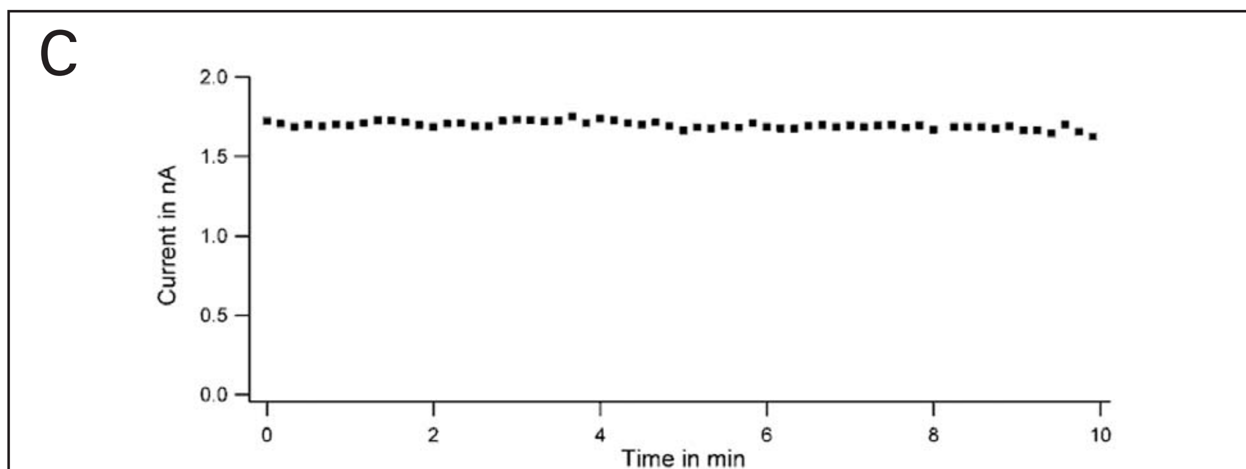


Figure 2:
hERG channels expressed in CHO cells were activated by a tail protocol. The protocol consisted of a depolarizing pulse to +40 mV for 500 ms followed by hyperpolarizing steps ranging from –100 mV to 0 mV for 500 ms. Panel A and B compare current traces with and without leak and zero subtraction. Panel C demonstrates the stability of the current amplitude of this hERG currents activated by a tail protocol to –40 mV.

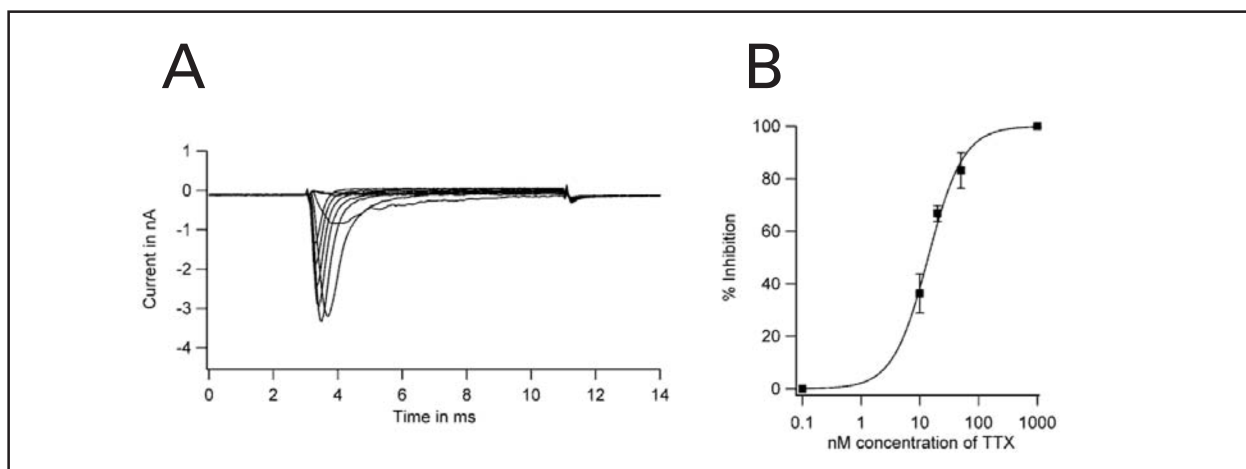


Figure 3:
In A rNav1.2a channels expressed in HEK cells were activated by depolarizing pulses with increasing amplitude from –60 mV to +20 mV in 10 mV steps. Panel B shows a pharmacological block of this voltage-gated sodium currents with increasing concentrations of TTX. For each concentration only 10 µl of the TTX containing solution were applied. Channels were activated by a voltage pulse from –100 mV to –20 mV.

Another example for the high quality of the patch clamp recordings performed with the micro-structured glass chips, are recordings of the sodium channel rNav1.2a. The kinetics of this sodium channel are very rapid, the inactivation takes place within 2 ms. It is therefore a good study object for validation of a new method. Figure 3a displays whole cell recordings from the rNav1.2a expressed in HEK cells. The data presented are not leak subtracted.

Pharmacology with the Port-a-Patch©
During the development and evaluation of new compounds one will typically want to measure the effect of drugs on its targets. To achieve a dose response curve, increasing concentrations of one compound are applied to one cell. As an example of such an experiment, we chose to apply increasing concentrations of TTX on the sodium channel rNav1.2a expressed in HEK cells. The corresponding dose-response curve is shown in figure 2b, resulting in a half maximal block (IC_{50}) of the sodium current at a TTX concentration of 14.9 nM (literature value 12 nM).

Conclusions
As the micro-structured glass 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 NPC-1© chips, has been shown for different cell types and ion channels. Cell types that work well with the Port-a-Patch© include CHO and HEK cells, which are widely used as expression systems for generating stable transfected cell lines. The presented data show the performance of the technology, which enables patch clamp recording from fast inactivating sodium

channels and also stable recordings as a bases for pharmacological experiments. All data presented were acquired with the Port-a-Patch©.
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 requirements is a goal long sought by the pharmaceutical industry. Highly integrated chip-based electrophysiology provides a solution for this pressing need.

Acknowledgement
The CHO cells expressing the hERG channel, as well as the data shown in figure 2c, were kindly provided by IonGate Biosciences GmbH, Frankfurt. The HEK cells expressing the sodium channel were kindly provided by Neurosearch A/S, Copenhagen. This work was supported in part by the BMBF (13N8364).

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[4] Fertig, N., R.H. Blick & J.C. Behrends. Whole cell patch clamp recording performed on a planar glass chip. Biophys. J, 82(6):3056-3062 (2002).

Flyscreen® 8500: A Fully Automated Ion Channel Screening Robot Introducing FlipTips® Technology

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Ion channels are becoming an increasingly important focus in drug research. Ion channel blockers and openers have significant potential as therapeutics for a variety of diseases. Furthermore, testing guidelines now require cardiac ion channel screening for pharmaceutical safety. The most advanced method to study ion channels directly is the patch clamp method. But while it facilitates complete, microsecond-scale control over the electrical and chemical environment of the channel pore, traditional patch clamp requires considerable skills, expensive tools and plenty of time. These obstacles are now overcome by the patch clamp robot Flyscreen® 8500. The robot is able to produce true gigaseals and whole-cell preparations at a high rate using the FlipTips® technology, where glass micropipettes are filled with cell suspension and the cells are subsequently flushed toward the tip of the glass. Integrating this method with HEKA's EPC 10 technology and PATCHMASTER® software, Flyscreen® 8500 is able to perform fully automated sealing, whole-cell break-in and recording tip/liquid handling.

Developing the automated patch clamp robot Flyscreen® 8500
One of the obstacles in creating an automated patch clamp device is to prepare an ultraclean surface on a suitable – and affordable - substrate to obtain the necessary high resistance seals. Furthermore, a single mammalian cell needs to be correctly positioned on a micron scale without microscope or micromanipulator. The third challenge is to successfully integrate the automatic handling of the complex fluidic and electronic procedures involved in the patch clamp process.
To this end the developers at flyion designed the FlipTips® technique using glass micropipettes. Years of experience in the field of patch clamping have shown that glass is an inexpensive and proven gigaseal substrate. The melting of glass produces ultraclean and smooth surfaces. Glass tips provide a reliable and cost effective structure for single cell screening in a micrometer scale. FlipTips® technique employs borosilicate glass pipettes whose recording tips are filled with cell suspension flushing the cells towards the glass tip (Figure 1). With this new method flyion was successful in producing gigaseals at a high rate. The seals formed between the outer circumference of the rounded cell and the

smooth inner surface of the glass tip. It turned out that seal resistances were as high as in traditional patch clamp experiments: the seal rate achieved with Chinese hamster ovary cells showed 82% and the seal resistance 9.3 ±1.7 GOhm (mean ± SEM, n=378). Furthermore, the giga-seals inside glass tips proved extremely robust.

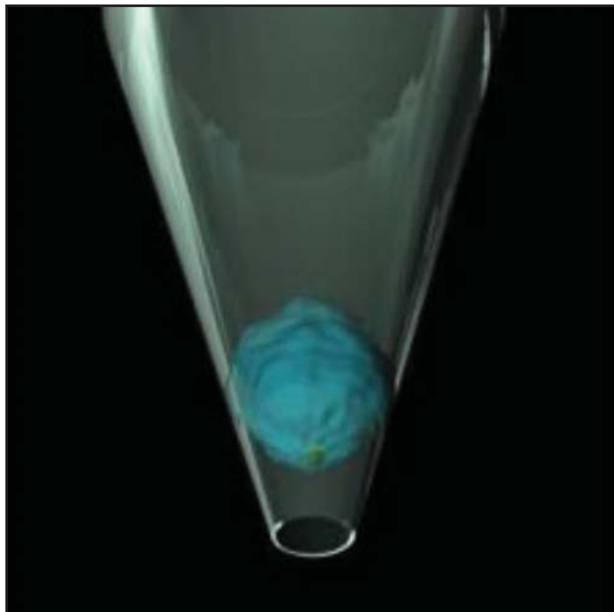

Figure 1: FlipTips® configuration. Schematic of a single cell located inside the patch pipette tip. Whole-cell configuration is established through applying repeated suction at the tip.

Another major advantage of this new patch clamp configuration is the possibility to add compounds to the extracellular membrane surface by using automated liquid handlers. The extracellular volume is small (15 µl) and compound solution can be easily added and flushed into the wide opening of vertically mounted recording tips from above.

Based on the above methods Flyscreen 8500® was developed (Figure 2). In the core of the machine, an array of receptacles holds recording tips. The glass tips are embedded in a molded plastic jacket that facilitates tip and liquid handling. They are placed and filled with cell suspension by the robot. Within the tips, seals and whole-cell preparations are automatically obtained. The tips then are replaced until every single position in the array contains a true whole-cell preparation. Subsequently, a control current is taken and compounds are added. If desired, this is followed by a washout period. The measurements are performed asynchronously at maximum throughput for each electrode position. The positions are completely independent, electrically separated and shielded. Thus, there is no electrical crosstalk between adjacent positions. The whole process is software-controlled and fully automated using amplifiers founded on HEKA's EPC 10 technology. The user interface is using HEKA's acquisition program PATCHMASTER®.

Patch Clamp Recording With Flyscreen 8500®

Whole Cell Access - In most screening scenarios, the whole cell configuration of the patch clamp method is preferable since currents are larger and the channel proteins behave in a more physiologically relevant manner than in other configurations. A whole cell preparation demands

Figure 2: The Flyscreen® 8500 ion channel screening robot.

that physical or chemical disruption of the cell membrane occur only on one side of the seal. This is done in order to minimize electrical access resistance and to optimize diffusional access to the remaining intact cell membrane.

A true whole cell configuration is achieved by applying suction pulses to the cell sealed inside a glass tip. Suction towards the tip disrupts the membrane area facing the tip. Since only individual constant suction pulses are needed instead of a feedback pressure control, automated seal and whole cell formation is reliable and reproducible without any operator action. The software monitors resistive and capacitive currents during a test pulse and controls the pressure. Typically, 1-5 suction pulses are needed for disrupting the membrane surface facing the tip opening. The seals are very stable and seal loss during suction pulses is a rare event (<10%). More than 80% of the whole cell preparations are still intact after >15 min. Another striking difference when comparing the new method to conventional patch clamping is the low and stable access resistance, resulting in negligible voltage error and high voltage clamp quality (Figure 3).

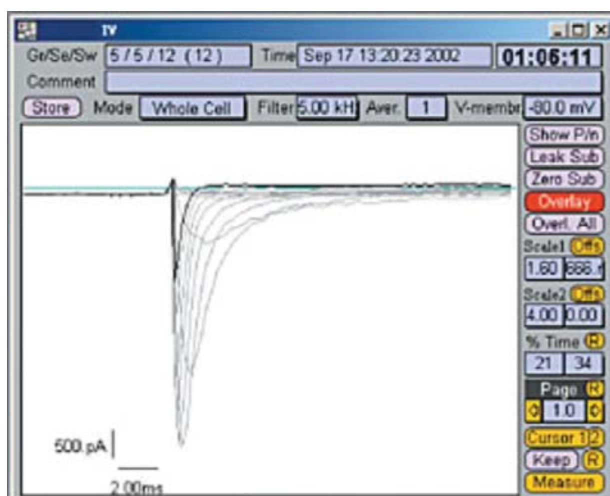

Figure 3: The high quality of the Flyscreen® voltage clamp is demonstrated using fast voltage dependent Na+ channels expressed in CHO cells. Kinetics and current-voltage behavior of overexpressed brain type Na+ currents in a typical cell acquired with the PATCHMASTER® user interface. Cells were held at -80 mV and voltage steps of 10 mV increments were applied to +30 mV.

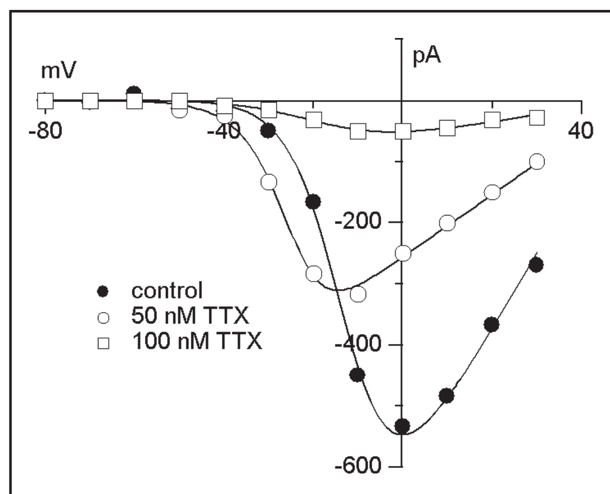

Figure 4: Block of VOC Na+ channels by tetrodotoxin. Example CHO cell overexpressing VOC sodium channels was sealed and opened by a pressure pulse with Flyscreen®. Tetrodotoxin (50 nM and 100 nM) was added following a control recording. Cells were held at -80 mV.

Using tips with 0.9±0.1 MOhm tip resistance, the series resistance is only 2.7±0.5 MOhm (n=355, CHO cells) and remains completely stable throughout the entire recording. This is in contrast to conventional patch clamp experiments, where series resistance after break-in tends to increase from 5 to 20 MOhm within 15 min. depending on cell type and intracellular saline composition. This advantage over other patch clamp methods is due to the different geometry of the glass in which the seal is formed. When using Flyscreen®, no disrupted membrane fragments are left floating inside the narrow opening. Therefore, Flyscreen® is perfectly suited to compare current readings before and after the addition of a drug during a screening run. Typical concerns about voltage errors and other artifacts developing over time with conventional whole cell experiments are virtually eliminated.

Perforated patch - In some instances electrical access to the cytosolic compartment is required in a manner that prevents wash-out of second messengers. This is typically achieved with ionophores, like nystatin or amphotericin, and provides access for small ions only. The disadvantages of this approach compared to traditional whole cell preparations are the larger access resistance and the lack of chemical control over the intracellular compartment. For example, with perforated patches, the application of K^+ channel blocking ions for measuring Ca^{2+} channels is not feasible.

When using Flyscreen®, amphotericin produces perforated patch access within 200 ms. Access resistance ranges from 5-12 MOhm, and seal stability is unaltered when compared to whole cell preparations.

Liquid Control - The quality of data gained from a screening process is dependent upon assay precision. Within the Flyscreen® robot, compounds are added to the extracellular membrane surface using automated liquid handlers. The extracellular volume is small (15-20 µl) and compound solution can be simply flushed into the wide opening of vertically mounted tips. Figure 4 shows a highly reproducable inhibition of whole cell Na^+ currents.

Conclusions

The patch clamp robot Flyscreen® 8500 automatically obtains gigaohm seals and whole cell preparations. The entire patch clamp process is software controlled and fully automated. Flyscreen® 8500 offers multiple benefits including simplicity of operation and reduced running costs due to the implementation of the robots unique disposable recording FlipTips®. The machine can perform hundreds of independent whole-cell screens per day, much more than achieved with traditional patch clamping, and at significantly lower cost. In contrast to the considerable skills needed for the traditional method, the operator of the machine only needs to replace compound plates and trays containing the tips, as well as to refill cell suspension at given intervals. Thus, the robot eliminates many of the obstacles encountered by researchers using the traditional patch-clamp method.

PATCHMASTER for PULSE Users.

Although in some respects PATCHMASTER ”looks” similar to PULSE, it is a completely new program, following an improved strategy for process handling and programming. Therefore, sometimes PULSE users may miss some typical PULSE behavior of the program until it becomes clear what the benefits of the new features are. Obviously, a substantially increased realm of functions and flexibility comes at some price. In most cases, this ”price” is to set some definitions before using PATCHMASTER in order to customize the program according to the individual needs. Thus, this tutorial tries to explain why some things are different ”although the old way worked very well”.

PULSE could take data from two AD channels and stimulation was limited to one DA channel. Additionally, up to three separate trigger channels could be defined. PATCHMASTER supports a (theoretically) unlimited number of DA and AD channels. This number is only limited by the hardware used. Given an EPC10 with a built-in LIH1600 interface, there are 4 DA channels plus 16 digital (TTL) output lines. The number of software AD channels corresponds to the number of available hardware ADs, in most cases 8. The extended number of channels requires specifying which channels are used, displayed, analyzed etc. Furthermore, it required a substantial redesign of pulse generation and data acquisition.
With the new Pulse Generator of PATCHMASTER, independent pulse patterns can be output via the selected DA channels and thereby allows simultaneous stimulation of multiple amplifiers with distinct stimulation patterns. Triggers are now handled in the same way as ”regular” DAs used for stimulation of cells. This removes all limitations regarding number and patterns of triggers. Please let me draw your attention to some more improvements of the new design of PATCHMASTER data acquisition:

- A compression factor can be specified for each channel in order to reduce the amount of stored data.
- Acquired data traces can be processed by mathematical functions to compile additional derived data traces.
- Zeroline subtraction can now be performed on any segment.
- Leak pulses can now be generated for individual output channels.
- For each segment it can be specified whether or not it is to be stored. ”Store=off” segments largely behaves like the previous ”conditioning segment”. The implementation and details, however, are different. Unlike in PULSE, the ”Store=off” segments are always explicitly output and sampled. Only after sampling the corresponding data are removed from the traces. The big advantage is that the durations of the ”Store=off” segments are now precise like all other segments.
- Simultaneous capacitance measurements on multiple headstages of a patch clamp amplifier (EPC 10 Double or EPC 10 Triple) are now possible.

The Online Analysis also has been redesigned completely. As a result, the analysis has become much more flexible and powerful. The immediate consequence for PULSE users is that there is no default analysis anymore. Thus, without definition of online functions, there will be no online analysis. The major improvements with respect to PULSE are:

- Arbitrary number of analyses and a much greater set of analysis functions.
- Generation of derivative data.
- It can be specified for each analysis parameter whether or not it is to be written to the notebook during data analysis.
- Analysis results can be plotted in multiple graphs placed in up to two windows.
- An unlimited number of analysis protocols can be stored.
- Online Analysis can be directly triggered by the incoming data (Analysis method can be specified in the Pulse Generator).

The Protocol Editor is completely new. It allows for a versatile definition of complex protocols. Experiments can be automated and standardized, including incorporation of feedback from online analysis or external devices. Several PULSE features have been moved from the Pulse Generator to the Protocol Editor. Instead of ”linking” PGF sequences in PULSE, the REPEAT loops in the Protocol Editor provide a much more flexible sequence handling. Amplifier adjustments such as updates of C-fast, Cslow, G-series etc. can now be also called from the Protocol Editor.

Several tutorial files are available for download from our web site (www.heka.com - Support). These PDF documents will quickly summarize the most relevant differences between PULSE and PATCHMASTER and will guide you through the main features of the PATCHMASTER program.

PULSE / PATCHMASTER
Hardware/Operating System Compatibility Chart

Windows 95/98/2000/NT4/ME/XP Supported dongle: parallel (printer) port dongle			
PULSE familiy[1]		PATCHMASTER	
EPC9/ITC-16/ITC-18	EPC10/LIH 1600	EPC9/ITC-16/ITC-18	EPC10/LIH 1600
yes	yes	yes	yes

Mac OS 9.x Supported dongles: ADB-EVE3 and USB-EVE3			
PULSE familiy[1]		PATCHMASTER	
EPC9/ITC-16/ITC-18	EPC10/LIH 1600	EPC9/ITC-16/ITC-18	EPC10/LIH 1600
yes	no (PULSE/X - CHART/FURA)	yes	yes

Mac OS 10 Supported dongle: USB-EVE3			
PULSE familiy[1]		PATCHMASTER	
EPC9/ITC-16/ITC-18	EPC10/LIH 1600	EPC9/ITC-16/ITC-18	EPC10/LIH 1600
no	no	yes	yes

1) PULSE family = PULSE, PULSEFIT, PULSETOOLS, PULSESIM, X-CHART, FURA

In summary, please note the following points:

- PULSE is not going to support the Macintosh OS X platform. (Users of PULSE on the Macintos OS X platform will have to upgrade from PULSE to PATCHMASTER.)
- Macintosh G5 computers are not yet supported by our hardware. A new PCI board for the PCI-X Bus is in preparation.
- PULSE is not supporting the EPC 10/LIH 1600 on Macintosh OS 9.x platform. (Users of PULSE in combination with an EPC 10 or LIH 1600 on the Macintosh OS X platform will have to upgrade from PULSE to PATCHMASTER.)
- Dongle support:
  - Windows platform: Parallel (printer) port
  - Mac OS 9.x: ADB-EVE3 and USB-EVE3 (Users of ADB-EVE2 dongles have to change to a newer dongle type)
  - Mac OS 10: USB-EVE3 (Users of ADB-EVE2 and ADB-EVE3 dongles have to change to USB-EVE3 dongle type)

PROBE SELECTOR · Headstage Multiplexer

HEKA is pleased to introduce the new PROBE SELECTOR. This headstage multiplexing device turns a single EPC 10 patch clamp amplifier into an eight or twelve channel serial patch clamp device. Patch clamp systems with up to 96 channels can be set-up using several probe selectors in combination with a set of EPC 10 DOUBLE or EPC 10 TRIPLE patch clamp amplifiers. Special versions of the PROBE SELECTOR matching your special requirements are available on request.

The multi-patch system is controlled via the PATCHMASTER software and can be built-in your custom automated patch clamp system e.g. via the Batch Mode offered by the PATCHMASTER software.

HEKA's fully computer controlled, digitally integrated patch clamp amplifier EPC 10 revolutionized patch clamp technology by providing unprecedented accuracy, highest degree of automation and versatility. HEKA's headstage multiplexing device further increases throughput and allows setup of multi-channel patch clamp systems on the basis of the fully computer controlled patch clamp amplifier EPC 10.

Main Features

The PROBE SELECTOR is available with 8 or 12 channels allowing up to 8 or 12 probes (headstages) to be connected to a single EPC 10 patch clamp amplifier.

Special versions of PROBE SELECTOR devices to be used with EPC 10 DOUBLE or TRIPLE patch clamp amplifiers are available on request.

Each independent amplifier of an EPC 10 DOUBLE or EPC 10 TRIPLE can be extended by a single PROBE SELECTOR allowing to set-up systems with 16/24 or 32/48 channels.

The "active" probe of the PROBE SELECTOR behaves like a probe connected directly to the EPC 10, and is indicated by a LED on the front panel.

"Non-active" probes are held at a common holding potential in the medium gain range. Upon selection of a probe, the gain and holding potential specified of the corresponding amplifier are set.

PATCHMASTER PRO supports GLP and FDA Regulations

Good Lab Practice (GLP) is becoming an increasingly more important aspect in the organization and daily life of commercial laboratories. This is especially so since the Food and Drug Administration (FDA) is requiring conformity to a set of GLP rules for data used in FDA applications (21 CFR Part 11). Any research entering the commercial world will be faced with the question of how reliable data and results were acquired and stored. Is it still possible to know exactly how the data were gathered? Who was the person doing the experiment? What were the exact parameters (e.g. amplifier gain? last calibration of instruments?).

Suddenly, it becomes apparent that it would be a big advantage if one had applied at least a minimal set of GLP rules. HEKA has always set the highest standards for electrophysiological data acquisition software. Currently, academic laboratories around the world are utilizing numerous features built within our software that enable them to conduct experiments and manage data confidently and with good lab practices in mind.

To comply, however, with new FDA regulations and satisfy the requirements of commercial customers, HEKA has designed the PATCHMASTER PRO software. PATCHMASTER is a data acquisition and analysis program package for patch clamp experiments which offers features meeting the highest standards of modern electrophysiology. This new software, running on PC-based and Macintosh computers, offers a variety of novel procedures aimed to make electrophysiological research more versatile and efficient. With PATCHMASTER, experimental design, performance, and analysis become much more flexible, giving rise to a high degree of automation and standardization. (Please refer to the Technical Note in this issue of HEKA impulse)

PATCHMASTER PRO is designed to support, in addition to PATCHMASTER, a set of Good Lab Practice (GLP) standards for data acquisition and handling, as established by the Food and Drug Administration (FDA) (21 CFR Part 11).

The following features to support stricter GLP and FDA rules are provided by PATCHMASTER PRO:

Electronic Signature
- The log-in and log-out with signature and password is enforced, and its entry will be validated.
- The signature is stored in the data file.
- The signature can be used to automatically generate the filenames according to a set of rules, e.g. [date][user ID][test no].
- The signature is always exported during data export as an additional field. In case of print-outs, the signature is printed on each page.
- Login actions including timestamps are recorded in a secured audit file

Audit
- An audit recording is generated, e.g. by storing the sequence of executed protocols in a separate audit file.

Access Control
- A log-in is required for any action.
- Three levels of access can be specified for each user.
- The required access level for most functions can be defined by an administrator.

Data Integrity
- All files can be checked using CRC (cyclic redundant checksum) support.
- All files generated in a session will be combined into one "bundle" file.
- Blocking of the possibility to delete data records during data acquisition, i.e. when the data file is opened with write permission ("New" and "Modify").
- Data can be encrypted.

Hardware Validation
- At the start of the program, the connected amplifier and its functionality can be validated.
- The identity of the amplifier is stored with the parameters in the data file.
- Validating that the calibration files and the field test protocols are newer than a user defined time span.

Although PATCHMASTER PRO can be run with any patch clamp amplifier, full functionality is best achieved when run with HEKA's computer controlled and programmable EPC 9 and EPC 10 family of amplifiers.

Q I just updated my PULSE software to PATCHMASTER. The user interface is very similar to PULSE, however, I could not find out how to start a PGF sequence. In PULSE, clicking on buttons located in the oscilloscope screen could start the sequences. These buttons do not seem to exist any longer in PATCHMASTER.

A These buttons still exist, but they have been moved to the protocol editor dialog, which now is the "control center" for data acquisition. If you open the Protocol Editor, you can see all available sequences/pulse patterns in the top right sequence pool. This new dialog can not only be used to start PGF protocols, but one can assemble complex experimental arrangements by combining PGF-templates with other operations (e.g. breaks, IF-THEN loops, amplifier setting changes).

Q In PULSE, I could subsequently switch between two PGF sequences, by linking both sequences in the Pulse Generator dialog. Does PATCHMASTER offer such a feature?

A In PATCHMASTER a "linking" of PGF protocols is no longer required. Instead, a sequence of an arbitrary number of PGF protocols can be defined in the Protocol Editor. If you intend to alternate between two protocols, you can create a REPEAT loop and run the sequences within that loop. The same protocol can also be used to trigger bath perfusion, or to switch from voltage clamp to current clamp mode between two PGF sequences or even to manipulate the parameters of a PGF sequence.

Q I am using EPC 10 and PATCHMASTER. Please let me know how to perform an automatic C-slow compensation before each sweep.

A The answer is the Protocol Editor. The command "Acquire Each Sweep" inserts a repeat loop, which allows executing protocol events between acquisitions of sweeps. To perform a C-slow compensation, you can add the "amplifier" command with the option "Auto C-Slow".

Q At the very bottom of the pulse generator dialog in PATCHMASTER, I stumbled on ten new controls (p0 ... p10). What are these controls good for?

A These parameters are used to facilitate input for "Duration" and "Voltage" of segments in a pulse protocol. E.g., if a pulse protocol consists of a train of pulses to the same voltage, this voltage could be specified via a parameter. Editing of this parameter will then be of effect for many segments without extra editing of segments. In the Pulse Generator the names for the parameters can be specified in order to remember what they are supposed to be used for. The parameters p1 to p10 are stored in a PGF pool file. They can be edited in the Pulse Generator or can be set via the Protocol Editor. Note that parameters to be used in the Pulse Generator have to be given in SI units, i.e. in volts and s (not mV and ms).

Events & Courses

Events Electrophysiology

- 83th Annual Congress of the "Deutsche Physiologische Gesellschaft e.V."
  March 14 - 17, 2004, Leipzig, Germany
- Synaptic Transmission: From ion channels to synaptic network functions.
  June 3 – 5, 2004, Göttingen, Germany
- 4th Forum of European Neuroscience (FENS)
  July 10 - 14, 2004, Lisbon, Portugal
- Canaux Ioniques
  September 2004, France
- 34th Annual Neuroscience Meeting
  October 23 - 27, 2004, San Diego, CA, USA
- Göttingen Neurobiology Conference
  February 17 – 20, 2005, Göttingen, Germany

Events Electrochemistry

- 55th Annual Meeting of the International Electrochemical Society ISE
  September 19 - 24, 2004,
  Thessaloniki, Greece

Capacitance monitoring in Xenopus oocytes: Online-correction of series-resistance errors using PULSE, X-CHART and a novel hardware

Bernhard M. Schmitt

In the preceding newsletter (HEKA impulse 02), we presented an improved approach to monitor membrane capacitance (Cm) in large cells such as Xenopus laevis oocytes. This "paired ramps" approach works in the two-electrode voltage-clamp (TEVC) mode. Recordings with this method are simple, proceed in real-time, and offer high temporal resolution, accuracy and precision (Biophys. J. 82:1345-57, 2002). Here, we present a novel technique that allows to overcome the detrimental effects of the so-called "series resistance errors" in the context of C_m measurements.

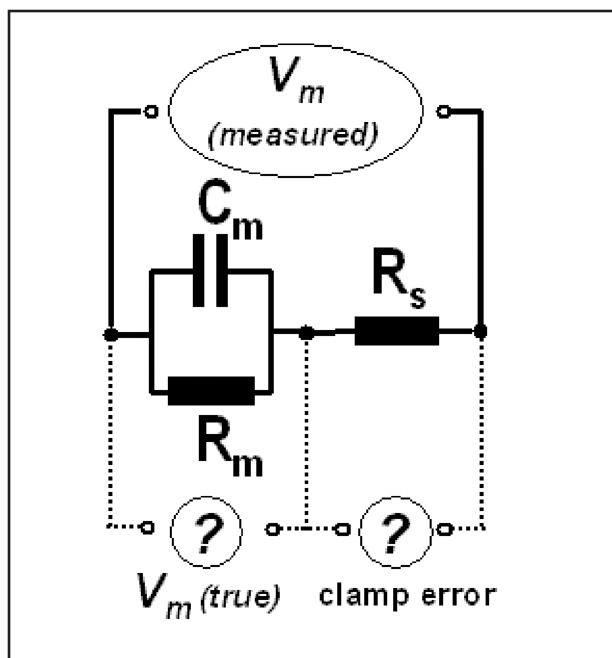

Figure 1: Series Resistance Errors. When studying the electrical properties of Xenopus oocytes using the two-electrode electrical voltage-clamp (TEVC) technique, the parameters of interest are membrane capacitance (C_m) and membrane resistance (R_m). The essence of the voltage-clamp technique is to facilitate their analysis by keeping the membrane potential (V_m) constant and at a known value. However, this approach is jeopardized by series resistance (R_s): any R_s takes away from the membrane proper an unknown, potentially significant fraction of the applied and measured potential. Thus, the measurements overestimate the true membrane potential (V_m true). Such R_s errors are a general handicap of the TEVC method, including C_m monitoring via TEVC. In order to overcome the problems caused by R_s, it is necessary to determine R_s directly, calculate clamp error and true membrane potential, and then re-scale the measured C_m value accordingly.

"Series resistance" (R_s) is a parameter that lumps together any resistance between the two voltage sensing electrodes (intra- and extracellular) other than the membrane resistance (R_m) proper (Figure 1). Clearly, the potential measured in this way can only provide a faithful reflection of the true membrane potential if R_s is absent or negligibly small as compared to R_m. Otherwise, the measured potential is higher than the true one. Such a clamp error (i.e., series resistance or R_s error) compromises the accuracy of any voltage clamp experiment. Unfortunately, however, R_s errors are difficult to detect, and hard to overcome. Moreover, we know lamentably little about the structural or biochemical underpinnings of R_s. It might be for such reasons that many experimenters are living in a "state of denial" vis-à-vis this fundamental problem of the popular TEVC technique. For the "paired ramps" measurements of C_m, a given series resistance R_s translates into an underestimation of the true C_m by a factor $2R_s/(R_s+R_m)$. As a rule of thumb, R_s problems are probably negligible when working with ion transporters that yield currents up to 500 nA, but should not be taken lightly at higher values.
Because the relationship between R_s and R_m is simple, it would in principle be straightforward to compute the true membrane potential, provided that R_s is known. Until recently, however, there were no TEVC methods available for the fast and precise monitoring of R_s in parallel with C_m. Now, H.-R. Polder and coworkers developed a novel hardware device that enables real-time monitoring of R_s in the TEVC mode (available from NPI electronic, Tamm, Germany). Together with PULSE and X-CHART, this "Rs box" allows to correct R_s errors automatically and in real-time. This feature provides a valuable useful "add-on" to our previously described C_m monitoring approach.
We implemented automated R_s correction for C_m monitoring via a "two-stroke cycle": First, the R_s box applies a test pulse to probe for R_s, samples and holds the measured R_s value. Second, the clamp amplifier (TEC-05, NPI electronic) follows with the C_m test pulse, controlled by PULSE software (HEKA Elektronik, Germany). After every such "two-stroke cycle", the X-CHART software (HEKA Elektronik) picks up the two preliminary results: from PULSE, the "online analysis" data that yield raw C_m; from the memory of the R_s box, the R_s value. Then, X-CHART solves in real-time for the approximated error $2R_s/(R_s+R_m)$ and rescales the raw C_m value accordingly. Communication with the R_s box and programming of the two-stroke cycle is comparably simple, thanks to the seamless cooperation between PULSE and X-CHART that was already crucial for the implementation of the C_m measurements. If desired, the sampling rate for C_m values may be increased at the expense of updating the R_s value less frequently. Because R_s fluctuations are usually much slower than potential C_m changes, this will not significantly affect the accuracy.

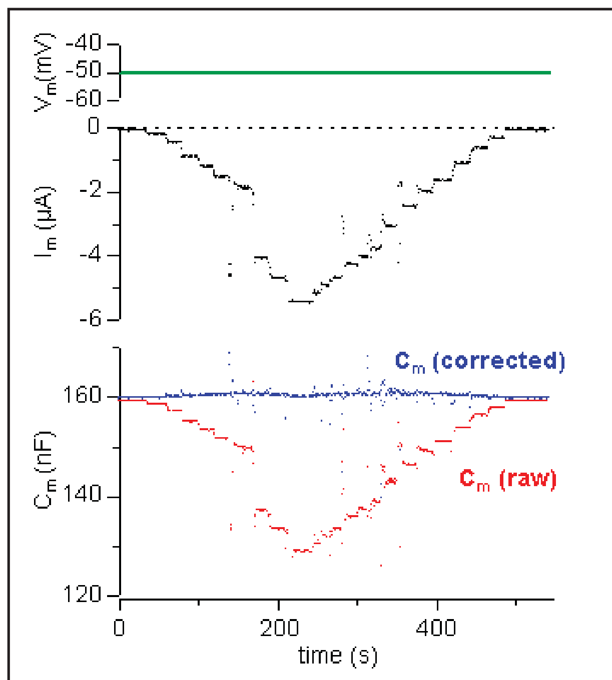

Figure 2: Automatic Correction of Series Resistance Errors. In a model circuit, membrane capacitance was monitored at –50 mV in the presence of a fixed series resistance (R_s=1 k), but variable membrane resistance. Without correction, greater currents (I_m, black trace) were associated with greater series resistance errors (not shown) that translated into greater deviations of the measured C_m values (red trace) from the true value (C_m=160 nF, fixed). With the aid of a novel hardware device that measures R_s directly ("R_s box") in conjunction with PULSE and X-CHART, series resistance could be measured and sampled continuously, and used to calculate the corrected C_m values (blue trace). The automatic correction of R_s errors extends the working range of the "paired-ramps" approach to C_m monitoring to situations with high currents of several µA.

Performance of this approach was tested in a calibrated electrical cell model with tunable R_s, R_m, and C_m (Figure 2). When the critical ratio $R_s/(R_s + R_m)$ was varied over a wide range at constant potential and C_m, membrane currents varied from –5 nA to –10,000 nA. Under these conditions, uncorrected C_m measurements underestimated the true C_m of 160 nF by up to 30% (apparent C_m ranging from 115-160 nF). After correction of the R_s error, C_m values accurately reflected the true value within 1 nF (<1%). In spite of an increased cycle duration as compared to C_m measurements without R_s correction, a high C_m sampling rate of several Hz could be maintained. When this approach was tested in Xenopus oocytes, the R_s measurements were somewhat more sensitive to clamp quality (non-linear electrode response, capacitive coupling, etc.) than measurements of C_m or I_m alone. Here, it was important to optimize clamp performance and to tune individually the parameters of the R_s stimulus by means of several dials on the front panel of the R_s box.

In sum, additional monitoring of R_s provides a simple and efficient means to obtain valid C_m measurements even in the presence of high R_s. Thus, this method extends the application range of C_m monitoring using the "paired ramps" approach to oocytes with overexpressed and activated ion channels. In a wider context, simultaneous measurements of R_s and C_m may prove useful for elucidating in Xenopus oocytes the largely unexplored biological underpinnings of these two electrical parameters.

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HEKA now offers customer support at three locations:

The headquarter **HEKA Elektronik Dr. Schulze GmbH** is located in Lambrecht/Pfalz, a little town in the Pfälzer Forest. The headquarter supports distributors worldwide and serves customers in Europe and in areas around the world where distributors of HEKA are not yet available.

In 1999, **HEKA Electronic Inc.** began operations in Canada to serve the Canadian and American market. The HEKA office is located in Mahone Bay, Nova Scotia. Activities in the Canadian branch of HEKA include assembly and repair of HEKA equipment for North American customers.

Recently, **HEKA Instruments Inc.** opened an office in Southboro, part of the famous business area of Boston, MA. This further expands customer and sales support in the USA.

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