

HEKA impulse 02

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Editorial

We are pleased to present the second issue of HEKA's newsletter, HEKAimpulse. In this issue, we highlight recent advances of the company's product developments that continue to set the standards in electrophysiology and electrochemistry and define the term "State-of-the-Art". A new generation of EPC amplifiers, the EPC 10, EPC 10 Double and EPC 10 Triple, continue the tradition of providing the world's best amplifier for electrophysiological recordings. Building upon the features of its revolutionary predecessor, the EPC 9, the EPC 10 series offers a vast array of improvements that have been implemented based directly on the requests and suggestions of our customers.

HEKA innovations do not stop at hardware improvements. The new multi-channel data acquisition software PATCHMASTER expands experimental versatility beyond expectation. This new software, available for Windows and Macintosh operating systems, retains all the flexible functions of PULSE, and adds a variety of novel procedures that make electrophysiological research even more efficient.

With corporate headquarters in Germany and Canada, and acting as official distributor of SUTTER products in Germany and Austria, HEKA recently opened a new office in Boston, MA, further expanding customer service and support. HEKA's know-how and innovative drive continues to provide its customers with cutting-edge tools that define the difference between a good and a brilliant experiment. Please visit HEKA website (heka.com) to stay informed about the true State of the Art.

P.S.: Oh, and don't forget to collect your free coffee mug (best used when designing that extra challenging experiment).

Scientific Note

Electrophysiology and Ion Channel Drug Discovery

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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. Early ion channel modulators, however, were identified by non-electrophysiological means and only after the initial discovery did mechanistic studies define specific interactions with channel proteins. Good examples of such compounds are perhaps local anesthetics and calcium channel blockers. The landscape has changed dramatically since the early days with significant methodological developments and the unveiling of the human ion channel genome. Historically, though, ion channel targets for drug discovery have been particularly interesting to those trained in the art and particularly frustrating to just about everyone else. This frustration arose in part from the difficulties associated with developing structure-activity relationships using relatively low throughput assay systems. Today this may be changing as the role of channels in almost all cell types becomes better defined and technological advancements bring the science of ion channels into the labs of more investigators in both the public and private sector.

The pharmaceutical and biotech industries have followed the advances of the scientific community at large with respect to the study of ion channels with two great leaps defining the modern age of ion channel biophysics. The first was the seminal work of Hodgkin and Huxley unraveling the molecular mechanisms involved in regulating cellular excitability. Reading their papers today one can still marvel at the level of insight. With a quantitative understanding of channel function in hand, the effects of compounds and drugs that modulate channels can be placed into proper context. For example, by studying the effect of resting membrane potential on calcium channel blockers it became clear why these compounds were safe and efficacious. Compounds interacted with the inactivated state of the channel, which was

prevalent at more depolarized membrane potentials. The same Ca^{2+} channel gene could be expressed in the heart and smooth muscle, but in the ventricle where the resting membrane potential is quite negative, Ca^{2+} channel blockers bind with low affinity since population of the inactivated state is low. In smooth muscle, however, with a more depolarized resting potential and greater population of channels in the inactivated state compounds bind with high affinity and block Ca^{2+} influx. Such compounds can relax smooth muscle but do not suppress ventricular contractility; a mechanistic understanding that followed from quantitative electrophysiological analysis. The second major leap in channel biophysics was the advent of the patch clamp technique discussed by Dr. Neher in an earlier issue of *Impulse*. This technique gives greater insight into pharmacological modulation of channel function, but perhaps more importantly allows electrophysiological recording from many previously inaccessible cell types. This opens the door to therapeutic opportunities outside of regulating cardiac and/or neuronal excitability.

Many other significant advances have been made as well and today's pace is rapid. One need only examine the field of genomics or study the crystal structure of ion channels, which allow investigators 'see the biophysics', to gain an appreciation for the newest advancements in our understanding. Still, while the biophysical interpretation of cellular excitability has greatly improved, the way ion channel active drugs have been discovered and optimized has lagged behind. For all the quantitative insight the newer frameworks and methods provide, the experimental techniques themselves remain slow and far from optimal for either lead identification or optimization in a modern drug discovery program. The modern process of identifying a new ion channel drug follows some variation on the theme illustrated below along with the relative efficiency of applying electrophysiological methods to each stage (Figure 1). The process begins with a gene and ends with a clinical candidate (In the truest sense of the word a compound is not a 'drug' until it is on the shelf in a pharmacy and can be prescribed by a physician, but for the purposes of this discussion reaching the stage of an IND

submission is sufficient). There are, of course, exceptions and one need not always begin programs with a gene, but in the majority of cases the gene is the starting point.

The process typically involves cloning and functional expression of the appropriate gene or combination of genes in a mammalian cell line followed by high throughput screening (HTS) of anywhere from a few thousand compounds to >1 million compounds depending on the particular philosophy of the organization involved. 'Hits', or active compounds identified in the HTS process are characterized for their 'drugability' and selected leads further optimized for in vivo efficacy. Optimization includes assessment of activity in relevant models and measuring pharmacokinetic and pharmacodynamic properties.

Electrophysiological techniques, most notably whole-cell patch clamp, fit well in certain phases and less well in others. At the initial stage when one is characterizing the gene, the premium is on quantitative information and high throughput is not necessarily required. Electrophysiological methods and transient expression systems are well suited for such studies and have served many drug discovery programs well. In the next phase of assay development for HTS, electrophysiological methods are often used to understand and optimize particular assays. From this point on, however, electrophysiological throughput is lower than desirable and HTS along with the immediate follow-up is performed with other methods (e.g. fluorescence, binding, tracers, etc.). If the quality of the HTS assay is high and predictive then it may not be necessary to utilize any additional electrophysiology recording. Most often, however, even in the best cases there will be a need for additional electrophysiological checking to track structure-activity relationships (SAR) as the lead optimization process proceeds. Ideally, given enough throughput, all SAR development for ion channel drug discovery programs would be performed with electrophysiological recordings since these methods provide the most direct assessment of ion channel activity. The lead optimization phase is also designed to minimize activity at any problematic target. With respect to ion channels, the most prominent selectivity targets are cardiac potassium channels involved in repolarization (e.g. hERG). Prior to submitting an Investigational New Drug (IND) application, each candidate compound will be fully characterized for interactions at the channels using electrophysiological methods. While many compounds have been and continue to be successfully advanced in ion channel drug discovery programs, improvements in process and technology are needed to maximize the opportunities presented by the newest genomic data.

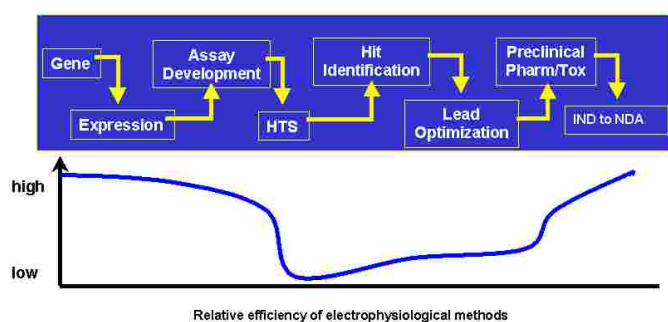


Figure 1

No new revolution has yet occurred with respect to ion channel drug discovery, but the sense is we may be very close. This optimism arises from advances in our understanding of the human ion channel genome, incredible insight into the structure of channels, novel computational approaches and applications of planar patch clamp methods, which will likely affect many of the stages in the process. In the schema shown above one can anticipate a greater number ion channel targets entering drug discovery programs and broader use of electrophysiological methods at all stages of the process. It should be a fun and exciting time.

Amplifier Cluster under Control of PATCHMASTER

The Ideal Solution for Automated Parallel Patch Clamp Machines

HEKA introduced the EPC 9 Double and EPC 9 Triple amplifiers to enable investigators to conduct technically complex experiments, such as the study of cell-to-cell interactions. Although either two - or three - amplifiers are combined in one housing, the individual amplifiers are completely independent from each other both on the operational and the software level. Furthermore, all that is needed to control each amplifier is the newly introduced HEKA software package PATCHMASTER.

1. Amplifier		DA:0 to Stim-1: OFF
0.00 A	0 mV	---
2. Amplifier		DA:0 to Stim-2: OFF
0.00 A	0 mV	---
3. Amplifier		DA:4 to Stim-3: OFF
0.00 A	0 mV	---
4. Amplifier		DA:4 to Stim-4: OFF
0.00 A	0 mV	---
I-mon	V-mon	R-memb

Within PATCHMASTER, the amplifiers and headstages are clearly identified (see figure to the left) making it very easy to assign each particular amplifier to each respective cell under study. Although independent, the amplifiers can be stimulated simultaneously. Furthermore, current and voltage signals from multiple amplifiers can be recorded, displayed and even analyzed online, whereby everything can be preconfigured in the Pulse Generator of PATCHMASTER. This versatility makes the EPC 9 Double and EPC 9 Triple ideal instruments for experiments such as the study of pre- and postsynaptic events, gap junction potentials or combined patch-clamp and amperometric measurements.

HEKA's newly developed EPC 10 amplifier series adds another dimension to the history of our digitally integrated amplifiers:

Clustering of EPC 10 Double or EPC 10 Triple amplifiers.

The EPC 10 offers the convenience that two interfaces can be connected to and controlled by a single PCI board in the computer. All input and output channels of the two interfaces are fully synchronized. Consequently, one can connect two EPC 10 amplifiers to one PCI interface card. The new PATCHMASTER software detects both interfaces and uses e.g. the two amplifiers in the same way as an EPC 10 Double amplifier.

2	DA	Unit	Stimulus -> DA	Leak	AD	Y	Link	Comp.	Points	Store	Zero	Leak
Ch-1	Stim-1	V	StimScale	<input type="checkbox"/>	Imon-1	A	1	1	700	<input type="checkbox"/>	Off	No Leak
Ch-2	Stim-3	V	StimScale	<input type="checkbox"/>	Imon-2	A	1	1	700	<input type="checkbox"/>	Off	No Leak
Ch-3	off	V	StimScale	<input type="checkbox"/>	Imon-3	A	1	1	700	<input type="checkbox"/>	---	No Leak
Ch-4	off	V	StimScale	<input type="checkbox"/>	Imon-4	A	1	1	700	<input type="checkbox"/>	---	No Leak

One could even connect two EPC 10 Double or EPC 10 Triple amplifiers to form a cluster of four or six amplifiers. PATCHMASTER software can access all eight DA outputs and acquire data from all sixteen available DA inputs.

The versatility of the PATCHMASTER software does not stop here:

PATCHMASTER controls multiple PCI cards

The PATCHMASTER software represents the control center of an amplifier cluster. Due to PATCHMASTER's ability to control multiple PCI boards, the cluster can be expanded to e.g. 16 and more fully functional and independent amplifiers.

The above features of HEKA's new generation of hardware and software are perfectly suitable for future automated parallel-patch clamp rigs. Why not take advantage of these features in your own lab? Improve single-handedly the turnover rate of your data acquisition by running multiple patch clamp experiments simultaneously - without compromising the quality standards you've come to expect from HEKA.

EPC 10 Single, Double and Triple – HEKA's newest patch clamp amplifiers.

Continuing the tradition of providing the world's best electrophysiology amplifier, the EPC 10 family has all of the features of the EPC 9 but offers many improvements.

Implemented EPC 10 Improvements:

- Integration with the built-in ITC-1600 A/D D/A interface. 8 analog input, 4 analog output, 16 digital input and 16 digital output channels. The interface has two 16 bit A/D converters enabling signals to be converted from two channels at a sampling frequency of 200 kHz / channel.
- New Low Frequency Voltage Clamp (LFVC). No more readjustment of holding current while conducting current clamp (CC) experiments. Small voltage drifts while in CC mode are controlled and adjusted to a fixed value. The membrane potential (V_m) is measured and used to compute a current waveform, which is fed back into the recorded cell.
- True CC experiments. The EPC 10 headstage acts as a voltage follower circuit resulting in rapid and accurate membrane potential recordings.
- Additional CC range with commanded CC 100 pA/mV input; up to ± 100 nA.
- No more short bulky cable! The interface is now connected to a PCI card in the computer via a pair of 5 meter fiber optic cables.
- Was accessing work space an issue? The EPC 10 headstage has a new ultra slim-line design.



Other EPC 10 Features:

- 3 TTL trigger outputs and 1 trigger input on the front panel.
- 2 telegraphing inputs on the back panel. These can be used to combine the EPC 10 with external devices, e.g. telegraphing gain amplifiers.
- Direct access to 16 digital trigger inputs and outputs on the back panel. Digital outputs could be used to control external devices, e.g. perfusion systems, video imaging.
- Complete software control with HEKA's PULSE or PATCHMASTER programs.
- The EPC 10 double and triple amplifiers contain 2 and 3 completely independent amplifiers within a single chassis. The desired amplifier is selected via software, each amplifier can be stimulated simultaneously and current and voltage signals can be recorded, displayed and analyzed online.
- Of the 4 D/A outputs provided by the built-in interface, one is always used to stimulate the amplifier. This results in 3, 2 and 1 free outputs for the EPC 10 single, double and triple respectively.
- The cost of an EPC 10 amplifier includes: amplifier, headstage(s), A/D D/A interface, PCI-1600 interface card, fiber optic cables, pipette holder(s) and model circuit.
- When combined with software, the EPC 10 system includes, not only a patch clamp amplifier, but also a digital storage oscilloscope, a variable analog filter, sophisticated pulse generator and a full featured data acquisition & analysis system.



- | | |
|------------|---|
| MAC | <ul style="list-style-type: none">• 64 MB RAM (or more for high speed-data acquisition via two channels)• Harddisk > 1 GB• Extended keyboard II• 17" colour monitor |
|------------|---|

- | | |
|-----------|---|
| PC | <ul style="list-style-type: none">• 450 MHz Pentium III processor• 64 MB RAM (or more for high speed-data acquisition via two channels)• Harddisk > 1 GB• Windows acceleration card with 4 MB RAM• Standard keyboard, mouse• 17" colour monitor• PCI bus with at least one free slot |
|-----------|---|

PATCHMASTER – the next generation of data acquisition software

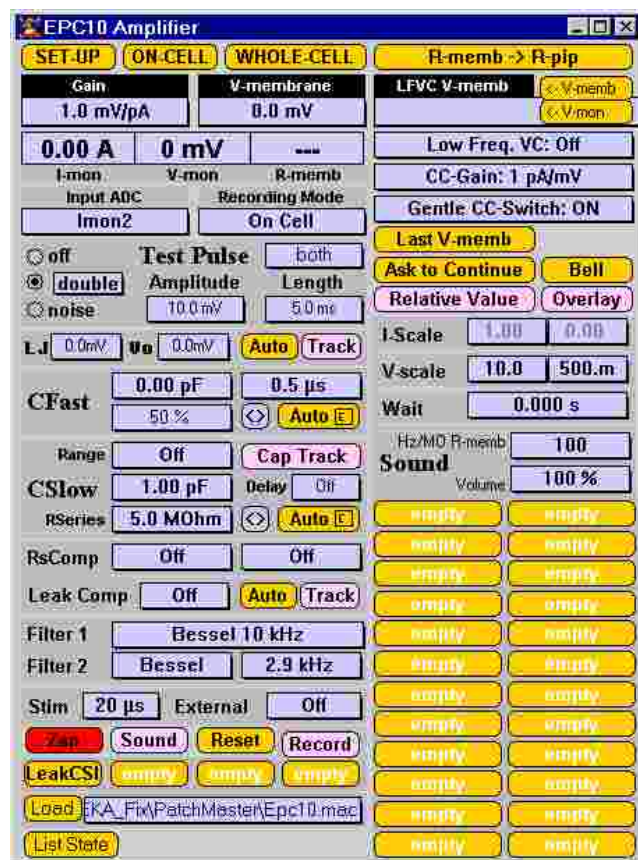
PATCHMASTER is a new multi-channel data acquisition software package offering all the features of PULSE but is even more advanced by enabling procedures that thus far were unattainable with other commercial software.

Improvements over PULSE:

- The 2 A/D data acquisition channels of PULSE have been increased with up to 16 input channels with automatic data compression (different sample rates for different channels).
- The 1 D/A (stimulation) channel of PULSE has been increased with up to 8 stimulation channels.
- The versatility of the Pulse Generator within PULSE has been expanded by the addition of the Protocol Editor of PATCHMASTER. Virtually every imaginable protocol can now be generated and precisely timed with complex functions such as repeat loops, Macros and conditional statements.
- Online analysis features of newly acquired data have been enhanced. Analysis templates can be predefined and stored and there is a direct link between Pulse Generator sequences and analysis procedures.
- PATCHMASTER's I/O control monitor provides direct access to hardware where the status of input digital and analog channels can be monitored and output signals can be set.
- Enhancement of automatic leak compensation. Leak pulses are supported for all output channels with various leak pulse storage modes: none, average, individual.

Other PATCHMASTER Features:

- Supports all HEKA amplifiers or any other patch clamp amplifier or two-electrode voltage clamp amplifier.
- Runs on both PC-based and Macintosh computers.
- PATCHMASTER can control multiple PCI boards.
- Data acquired with PULSE are automatically converted to PATCHMASTER format.



- Easy exporting of data (e.g. ASCII, IgorPro, MatLab).
- Three D/A channels (auxiliary stimulation trigger channels).
- All amplifier settings can be controlled either by mouse or keyboard.
- Built-in oscilloscope with adjustable size.
- Automatic routines for capacitive transient compensation.
- Lock-In configuration for membrane capacitance measurements.
- Acquisition and update of values such as RMS noise, pipette resistance, seal resistance and other user-defined parameters.
- Control of external devices (e.g. perfusion systems, photometric equipment).
- PATCHMASTER license includes a copy of PULSE, software upgrades are always FREE and can be downloaded at www.heka.com!!

Q I am working with an EPC 9 Double and PULSE. I would like to record simultaneously from the two amplifiers to do paired recordings of neurons in slices. I understand how to visualize the traces coming from the two headstages in the EPC 9 window by selecting alternatively the fields of the two amplifiers, however it would be useful to display the two traces in the oscilloscope window at the same time. Is this possible and if yes how?

More importantly, I would like to create protocols for one amplifier and not the other and vice versa, so that, for example, to be able to inject a voltage pulse using one amplifier and applying no pulse in the other and again be able to see the two traces at the same time. Is that possible? This is necessary for the double-patch recording when one wants to activate one cell and see the synaptic response in another. Please let me know how to use the amplifier in this way.

A It is possible to record and to display current or voltage traces of both amplifiers simultaneously. The settings are made in the pulse generator dialog:

Please set the number of "Channels" in the 'AD/DA channels' section of the dialog to "2". The inputs for data acquisition can be specified in the same section. Selecting a specific AD for "Trace 1" and "Trace 2" will cause the stimulation and acquisition using those fixed channels, irrespective of which amplifier is the "active" one. For example, if you want to record the current of both amplifiers, set "Trace 1" to Imon 1 and "Trace 2" to 'Imon 2'.

By default, both current and voltage traces of the "active" (selected in the amplifier dialog) amplifier are stored and displayed in the oscilloscope.

Selecting a specific Stim-DA (in the same section) will cause the stimulation to use this fixed channel, irrespective of which amplifier is the "active" one. PULSE will use the known current- and voltage gains as well as the modes (On-Cell, Whole-Cell, etc.) of the addressed amplifier. For example, this allows keeping the first amplifier in cell-attached mode with a high current gain, while at the same time reading from the second amplifier in the whole-cell mode with a medium current gain setting.

The new PATCHMASTER software is even more versatile. It allows simultaneous stimulation of both amplifiers with different stimulation pattern and can acquire data from up to eight AD input channels.

Q I will have to do a lot of electrical activity recordings. Most patch-clamp amplifiers, including the EPC 7, introduce substantial distortion when recording action potentials in the CC mode (Magistretti et al., Trends. Neurosci. (1996) 19, 530-534). In order to minimize this problem Axon Instruments has introduced a 'fast CC mode' on its Axopatch200A. Does HEKA provide a similar solution with the EPC 9?

A Yes. The EPC 9 has two feedback circuitries for current clamp recordings. The so-called "fast" current clamp mode was introduced with the "C" version of the hardware in 1995 and is also incorporated in the EPC 8. The EPC 7 and older EPC 9 amplifiers ("A" and "B" version) lack the fast current clamp mode. The board version of your EPC 9 amplifier is displayed in the last menu item of the EPC 9 menu. If your amplifier supports the fast current clamp speed, it will be activated by default. To turn this mode off close the oscilloscope, click the red button labeled CC Fast Speed and then open the oscilloscope again. Now, you will see a much slower signal.

The current clamp circuit of the new EPC 10 amplifier was further improved. A new design of the pre-amplifier allows to perform "True CClamp" experiments. In CClamp mode, the headstage acts as a voltage follower circuit, which guarantees very fast and accurate membrane potential recordings. Similar to a classical microelectrode amplifier, the cell voltage is measured on a very high input resistance and C-fast compensation is no longer necessary.

Q How can I select the second trace ("Trace 2") when using the measure or scan data controls of PULSE? I tried a couple of things but nothing seemed to work.

A Just switch in the Online Analysis dialog from "First Trace" to "Second Trace".

Q Another question about my new HEKA EPC10. When I load PULSE, I get a message saying that the file SCALE.520144 cannot be found. Sure enough. I can't find it either. Then when I continue, with the model circuit in the "on-cell" mode, my Rmemb is 20 megohms, not 10 as it should be.

A The file, PULSE is looking for, is the calibration file of your EPC 10 amplifier. It is shipped with the EPC 10 on a floppy disk. These Scale files are very important for the proper function of the EPC 10 amplifier, since all calibration settings are stored in that file. If you should

have lost the floppy disk, or if you want to re-calibrate the EPC 10, you can create a new scale file in the "Test and Calibrate" menu of the PULSE software.

Is there a limit on the number of data points that can be displayed on the Oscilloscope window?

The maximal number of data PULSE+PULSEFIT can display corresponds to the maximal number of samples in the stimulus buffer. For example, if you increase that value (buffer allocation), you also increase the other limit.

Why does PULSE+PULSEFIT sometimes not display the complete sweep?

This occurs if the sweep is longer than the presently allocated stimulus buffer. This can happen e.g. if you acquired the data on a computer where you increased the stimulus buffer size and now try to analyze the data on another machine that has the default size. In this case, sweeps are only displayed up to the current buffer limit. Increase the buffer size appropriately.

It seems that I cannot edit existing TIDA stimulation files? Any ideas?

TIDA uses the Windows Notepad editor for editing text files like stimulation files (*.STM). The Notepad of Windows 95 has the peculiarity that it only accepts file-name extensions that are registered for it. That means, if a stimulation file has been opened by Notepad and saved under another name ('SAVE AS'), then Notepad will append the extension 'TXT' to the new name even if 'STM' has been specified explicitly. Therefore, you should register the file-name extension 'STM' to Notepad by executing the following steps:

- Start the Windows Explorer,
- select the menu item 'VIEW-OPTIONS',
- in the dialog select the tab 'FILE TYPES',
- press button 'NEW TYPE'.
- It is sufficient to enter 'STM' in the edit-box 'ASSOCIATED EXTENSION',
- to press the button 'NEW....',
- to enter 'OPEN' in the edit-box 'ACTION'
- and 'NOTEPAD.EXE' in the edit-box 'APPLICATION USED TO PERFORM ACTION'.
- Information about the other fields can be found in the Windows on-line help.
- Please confirm with 'OK'.

Events Electrophysiology

- 47th meeting of the Biophysical Society
March 1st-5th, 2003,
San Antonio, Texas.
- 82. Jahrestagung der Deutschen Physiologischen Gesellschaft
March 2nd-5th, 2003,
Bochum, Germany.
- 29th Göttingen Neurobiology Conference
June 12th - 15th, 2003,
Göttingen, Germany

Events Electrochemistry

- DECHEMA-Kurs: Moderne Meßmethoden in der Elektrochemie und Korrosionsforschung
Nov. 18th-19th, 2002,
Frankfurt am Main, Germany.
- ACHEMA 2003
May 19th-24th, 2003,
Frankfurt am Main, Germany.

HEKA Courses

Introductory courses to PULSE and Software LockIn (for electrophysiologists) or POTPULSE (for electrochemists) will be held in Lambrecht/Germany. These courses will cover the basic principles of the program and show first steps involved in performing standard measurements. Please contact us for more detailed informations.

Real-time monitoring of membrane capacitance in *Xenopus* oocytes with PULSE and X-CHART

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Membrane Capacitance (C_m) correlates closely with membrane surface area and is therefore exploited widely to follow plasma membrane dynamics. For instance, C_m measurements in secretory cells allow the resolution of exocytotic events on the level of single vesicles and a millisecond time scale. Herein, information about C_m is computed from the current response to a sine-wave voltage stimulus (i.e., “impedance” or “admittance analysis”, a “frequency-domain” approach).

In large cells such as the widely used *Xenopus* oocytes that are mostly studied using the two-electrode voltage clamp (TEVC) technique, this approach does not work as nicely, due to fundamental physical givens. Several approaches to C_m measurements via TEVC have been described that employ other stimuli such as ramps, saw-tooth or steps. These “time-domain” methods lacked the appeal and power of its frequency-domain counterpart, being relatively cumbersome, slow, and coarse. On the other hand, the time-domain approaches were still far from their theoretical limits, suggesting considerable room for improvement.

We therefore tried to make C_m measurement via TEVC simpler and better. First, we modified the stimulus and algorithm used to compute C_m , the so-called “paired ramps” approach (Fig. 1).

Second, we used PULSE and X-CHART to implement the paired ramps. The combination of these two software packages permits to do two things simultaneously that seem to be mutually exclusive: On the one hand, run fast stimuli and extract two crucial parameters from the current trace with a high repetition rate, on the other hand, simultaneously translate these parameters into plain C_m values, collect and display them online over hours. Technical details of our method, the results of extensive performance testing, and background information can be found in a recent article in the *Biophysical Journal* (see below).

Implementing C_m measurements for TEVC boils down to installing a few short files with the proper instructions for PULSE and X-CHART; no extra hardware and no alteration of the recording arrangement are required (Fig. 2). At the push of a menu button, C_m can be measured with high time resolution, precision and accuracy. In practice, C_m changes of 0.5 nF are resolved routinely at several Hertz. Both values can be improved further for either speed or precision. The test stimuli per se are not a challenge to voltage clamp amplifiers. As always with TEVC, however, large currents may compromise clamp fidelity and thus also C_m measurements. Possible countermeasures include maximizing proportional gain, use of amplifiers that afford integral feedback, and series resistance compensation.

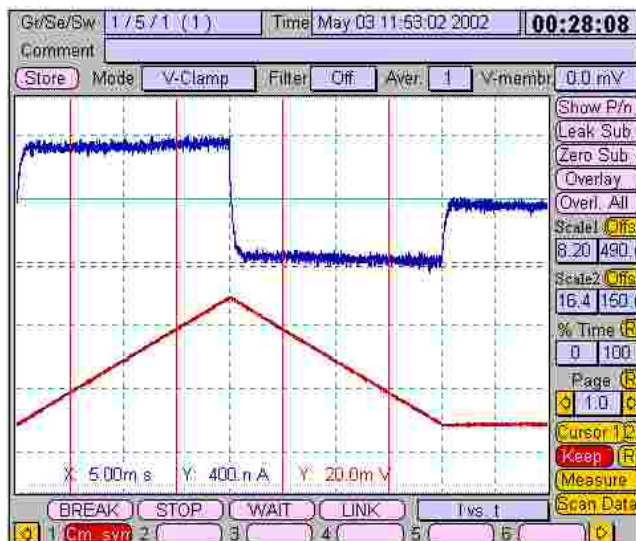


Figure 1: C_m measurement using paired ramps (screenshot oscilloscope window). In the two-electrode voltage-clamp mode, a specific stimulus (“paired voltage ramps”, brown trace) is applied to a *Xenopus* oocyte. From the observed current response (blue trace), the “online analysis” function of PULSE determines two current integrals (time windows indicated by red vertical lines). These integrals correspond to capacitive charges Q_A and Q_B that allow one, together with the known height V of the ramp stimulus, to calculate membrane capacitance according to $C_m = (Q_A - Q_B) / 2V$.

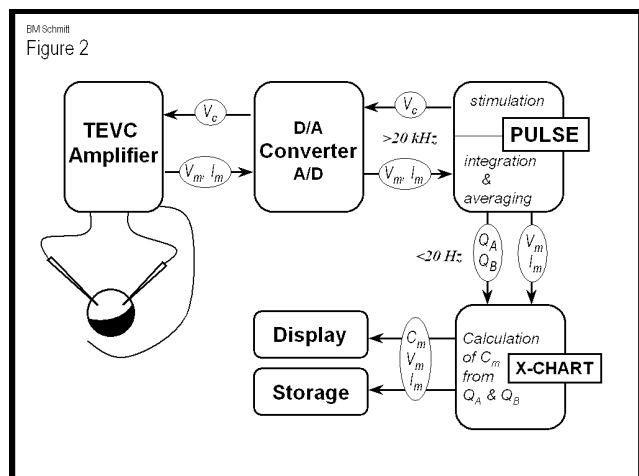


Figure 2: Integration of hardware (amplifier and D/A converter) and software (PULSE and X-CHART) for C_m measurements using paired ramps. A crucial feature is the ability to run one fast and one slow program simultaneously. PULSE acquires the high-frequency data, breaks them down to two relevant figures that are handed over to X-CHART, and then discards them. X-CHART computes C_m values, displays a continuous C_m -trace on-line (together with V_m , I_m), and stores the data.

Taken together, the “paired ramps approach” offers a straightforward yet powerful method that should be useful for studies of exo-/endocytosis, of the regulation of channels and transporters via insertion/retrieval, and for C_m measurements for high-throughput screening. Last but not least, there may be novel applications waiting, such as the analysis of depolarization-induced secretion reconstituted via heterologously expressed proteins in *Xenopus* oocytes.

Offer for download: This article and the used PULSE and X-Chart files for C_m measurements can be downloaded from:
www.heka.com/support/tuto.html.

Reference:

An Improved Method For Real-Time Monitoring of Membrane Capacitance in *Xenopus laevis* Oocytes. B.M. Schmitt & H. Koepsell. *Biophysical Journal* 2002, 82:1345-1357.

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. Within minutes driving time, one can visit the famous vineyards along the "Deutsche Weinstrasse". 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. Mahone Bay is a beautiful seaside town located about an hour's drive south of the Halifax airport. Activities in the Canadian branch of HEKA include assembly of EPC 7, EPC 8 and EPC 10 Single, Double and Triple amplifiers. Repairs of HEKA equipment for North American customers are also carried out in Canada.

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. USA customers should contact HEKA in Southboro for support and equipment needs.

In addition to HEKA's three branches listed above, HEKA products are available through the following distributors:

Australia and New Zealand:
SDR Clinical Technology
213 Eastern Valley Way
Middle Cove NSW 2068
Tel: +61 - (0) 2 9958 2688
Fax: +61 - (0) 2 9958 2655
sdr@sdr.com.au

China:
InBio Life Science Instrument Ltd.Co.
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Wuhan City, 430074, Hubei
P.R.China
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ibb@hust.edu.cn

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