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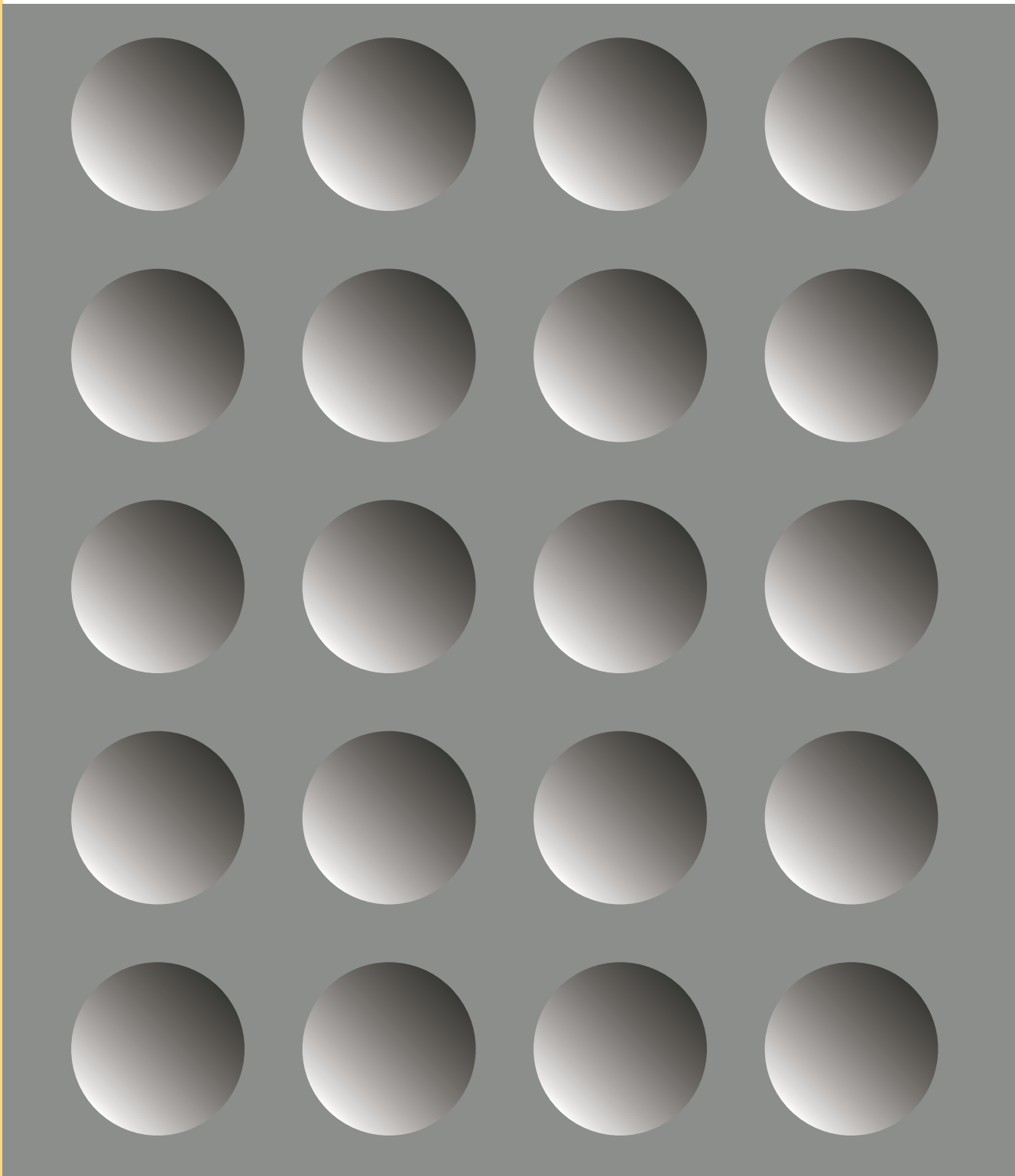
robocyte2

Automated Voltage-Clamp Screening for *Xenopus* Oocytes



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Introduction

Oocyte Screening Goes Automatic

Oocytes of the toad *Xenopus* laevis are widely used as an expression system for ion channels, transporters, and receptors in drug development. *Xenopus* oocytes are big, robust cells (about 1 – 1.2 mm in diameter), can be obtained in large numbers, and are easy to handle.

Nevertheless, the low throughput of manually performed electrophysiology prevents its use for secondary functional screening of drug targets.

More than ten years ago, Multi Channel Systems (MCS) presented the first commercially available fully-automated system for *Xenopus* oocyte screening, the Roboocyte. In 2011, it was time for the Roboocyte2.

The Roboocyte2 is a fully-automated all-in-one solution for medium-throughput screenings of ligand-gated and voltage-gated ion channels, as well as electrogenic transporters based on the well-established *Xenopus* oocyte expression system. All necessary tasks are accomplished by a single robot.

The automation of TEVC recording revolutionizes pharmaceutical drug discovery:

- A much higher throughput can be achieved at greatly reduced costs.
- The general conditions for an experiment can be standardized, enhancing reproducibility.
- It allows your highly qualified personnel to do away with routine work and focus on experimental design and data analysis.
- The Roboocyte2 can be operated overnight without supervision. You can start an additional experiment at the end of a working day and analyze the results the next morning.

The Roboocyte2 was designed not only for those familiar with the *Xenopus* oocyte expression system, but to also encourage others to step into an exciting and new technology for drug discovery.

Features

- TEVC recording of voltage-gated and ligand-gated ion channels and electrogenic transporters
- Flexible design of automated recording sequences
- Automated cell wash
- Automated compound application

Advantages

- 24 h operation
- Plug and play
- Easy to use
- Cost effective
- Time saving
- Increased throughput


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Hardware

Smart, Compact, and Easy-to-use System

The Roboocyte2's compact and functional design saves space on your work bench. It is compatible with standard lab equipment and can be easily integrated in your working environment. Software controls replace any knobs on the device. The Roboocyte2 is straightforward and easy to operate; handling does not require special skills or equipment.

The recording is performed using disposable standard 96-well plates, which are commercially available from several providers. The oocytes are plated into the wells in a couple of minutes. They quickly settle within the conical wells and adhere to the well bottom after a few hours. The oocytes no longer need to leave the plate; you can easily transfer the oocytes from the incubator to the Roboocyte2 device and back again.

The well plate carrier, powered by linear motors, hovers smoothly and noise-free on a cushion of pressurized air above a magnetic steel plate. It operates at 20 µm resolution. The complete system requires no maintenance other than occasional cleaning of the steel plate.

The vertically moving z-arm, which holds the TEVC probe, is designed for the high demand of speed and precision. The z-arm moves at a resolution of 20 µm; position and speed are computer-controlled. A quick adjustment process guarantees the precise impalement of the oocytes. We recommend using the ready-made measuring heads provided by Multi Channel Systems. If you prefer to produce your own, we strongly recommend blank measuring heads from MCS. You can then fix your own capillaries, perfusion tubing, and silver wire to the mounting support.

The new "ClampAmpC" high performance amplifier is specifically designed and manufactured for the two-electrode voltage clamp method by MCS hardware specialists. Ready-to-use TEVC probes make the handling quick and easy. The Roboocyte2 package includes everything you need to start right away, including a high performance computer with monitor, TEVC probes, tubing, the Roboflow liquid handler, and accessories.

Features

- Sequential recording of 96 oocytes without user intervention
- Neither special skills nor special equipment required
- Easy oocyte handling in disposable standard well plates
- Maintenance-free system
- High performance TEVC amplifier
- Support of the Roboflow liquid handler or an (optional) Gilson liquid handler

Hardware Details

Flexible and Automated TEVC Recording

Once the recording run has started, it proceeds automatically for all 96 oocytes – or for the selected oocytes – without supervision. The recording sequence of each individual oocyte can be flexibly designed exactly to your requirements. You can include reference values to trigger recording sequences based on the response of the oocyte. Automated controls identify unhealthy oocytes to eliminate unnecessary recordings. This means throughput is maximized while compound usage is minimized. Results obtained with the Roboocyte in the laboratories of Bayer AG show that up to 60 compounds, each paired with one positive control, can be applied to a single good oocyte. In theory, you can test several hundred compounds on a single 96-well plate before user intervention. The amount of compounds tested is in no way limited by the Roboocyte2 – it depends only on the properties of the compounds and on the viability of the cells. This provides a revolutionary increase in screening throughput.

Ready to Use TEVC Probes

The automated microelectrode impalement into the oocyte is fast, precise, and gentle, thereby minimizing cell damage. Repeated impalements of a single oocyte are often possible. After filling the glass electrodes with KCl solution, the supplied TEVC probes are ready to use. Simply connect the probe to the z-arm and the perfusion manifold and you are ready to go. The probe can be stored overnight and typically reused for several days.

The perfusion inlet leads to either a manifold that is connected to the Roboflow pinch valve system or to the Gilson GX-271 external liquid handler. In both cases, peristaltic pumps transport the solution to the oocyte and aspirate the fluid via the outlet to the waste receptacle. The probe design ensures a steady and pulse-free flow.

ClampAmpC

The compact, high-performance digital amplifier is integrated within the Roboocyte2 robot. It operates in either current clamp or voltage clamp mode and uses a PI-based negative feedback technology. The ClampAmpC records currents up to ±107 µA at a resolution of 1 nA. It is fully computer controlled. The clamp speed is as fast as can be achieved with a large cell like the oocyte: Typical rise times are below one millisecond. Features such as automated checks of electrode resistance and automated clamp optimization are part of the Roboocyte2 software.

Status Display

The integrated LCD display shows all relevant parameters such as electrode voltage offsets, electrode resistances or the membrane potential of the cell after impalement.

Roboflow Perfusion System

The Roboflow Perfusion System is simple and easy-to-use and is ideally suited for rapid standard tests, dose response analyses, and small screens. The Roboflow is equipped with twelve pinch valves and two peristaltic pumps and is fully controlled by the Roboocyte2 software. Using a highly precise peristaltic pump instead of gravity to transport the solution to the oocyte gives you full control over the speed of solution flow: The perfusion rate can be adjusted between 0.01 and 10 ml/min. For more demanding tasks, such as larger compound screenings, the Roboocyte2 easily interfaces with the widely used Gilson GX-271 liquid handler.

Automated Compound Application

Although perfusion is usually continuous, it can be paused during the recording process to save compound. The small volume of a well ensures that a flow rate of about 5 ml/min is enough for rapid and efficient solution exchange with minimal compound usage. You can easily implement your own automated drug-saving strategies into your experimental setup, for example, by testing the viability of an oocyte automatically each time before delivering a compound.

If you use either the integrated twelve pinch valve Roboflow or the external Gilson GX-271 liquid handler, all perfusion protocols are automated and controlled by the Roboocyte2 software.

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Software

Flexible and Intelligent Solution

Oocyte impalement, compound application, TEVC recording, and online analysis are all performed automatically under computer control. The main characteristic and primary advantage of the Roboocyte2 is its full automation. You design the experiment and define all parameters in advance. At run time, you start the session with a single mouse click. The Roboocyte2 controls the recording for all 96 oocytes in a well plate automatically, even including a wash cycle. Thus the recording can go on 24 hours a day, unsupervised.

The Roboocyte2 software's easy-to-use graphical user interface makes daily work with the Roboocyte2 quick and easy. Automated software controls replace any knobs on the robot. Customizable response-dependent recording sequences and control routines replace personal supervision.

How is such a degree of automation possible for such a demanding task?

It is achieved through JavaScript-like text files containing commands. Test scripts and scripts for standard experiments are provided by Multi Channel Systems. Each user can write their own scripts with the included editor. All experimental settings are defined within the script. You can write a script for any kind of experimental setup. Once the appropriate script is loaded into the Roboocyte2 software, simply click the Start button to start the robot. The script is then performed without the need for further customization and supervision.

The Roboocyte2 Java scripting language includes all important commands and functions in a nutshell. This concept makes the Roboocyte2 scripting language sophisticated and powerful, but not redundant or unnecessarily complex. The structure and command names are designed to be very intuitive and user friendly. You do not need any programming skills; a very basic understanding of the general concept is sufficient to harness the full power of scripting.

For quick tests, a convenient manual mode is provided as well. You can manually control all Roboocyte2 actions and parameters that are otherwise automated.

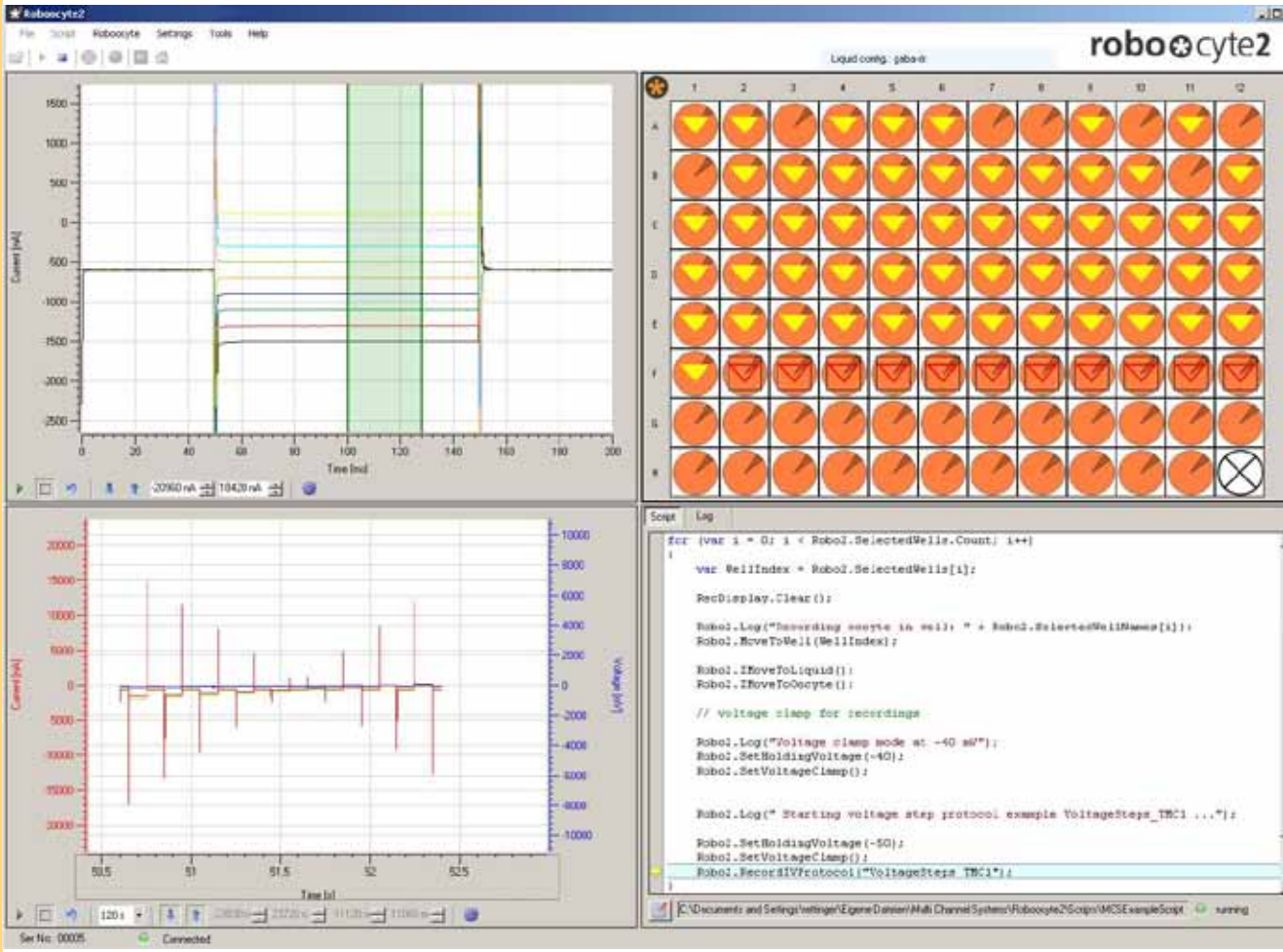
Automated Features

- Two-electrode voltage clamp
- Oocyte impalement
- High throughput compound screening
- Generation of dose-response curves
- Semi-automatic calculation of EC/IC50 values
- Perfusion paradigms
- Response-dependent recording sequences save time and compounds

Advantages

- Full automation and time saving: Once you have loaded a JavaScript, start a recording process with a single mouse click.
- Flexibility and power: Set up several recording protocols for various applications, with full control of all parameters.

Software Details



Easy-to-Use Software Interface

The easy-to-use Roboocyte2 software, with its intuitive graphical user interface, allows you to operate the Roboocyte2 and collect and evaluate data.

The well plate view gives a quick overview of the current state of the oocytes. Simply click any virtual wells to select all or specific oocytes for recording. The Roboocyte2 program provides an intuitive manual mode for trying out your experimental settings and for quick tests. Manual mode also allows new Roboocyte2 users to gain initial experiences without having to set up a detailed protocol.

The Roboocyte2 software allows DC offset correction, electrode resistance check, oocyte impalement, compound application, as well as current and voltage clamp under both manual and automatic control.

Data Handling

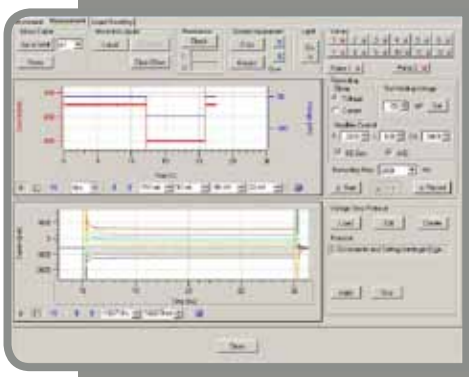
The recording and control windows display results online throughout testing. Acquisition and real-time analysis are performed automatically from 96 oocytes in a well plate. Compound information and results are automatically stored in a Microsoft Access compatible database file that offers all the conveniences of an industry proven database for creating reports, managing enormous numbers of compounds, and evaluating results. Alternatively, you can export the data in ASCII format and use your custom evaluation software.

Flexible Recording Protocols

Design your custom parameters and prompt them in a script. You can flexibly adjust these parameters in a dialog box at the beginning of a recording. For example, you can easily change the holding voltages in a voltage step series from run to run. The use of predefined recording protocols guarantees a quick setup time and high reproducibility of experiments, preventing handling errors.

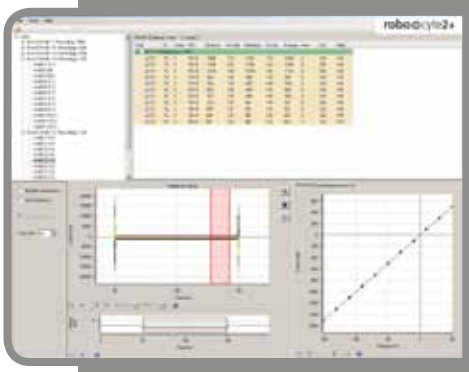
Manual Control Mode

You can test your protocols and perform quick TEVC recordings under manual control. Simply open the "Manual Mode" dialog and enter the well number to move the TEVC probe to the selected oocyte. Control oocyte impalement by checking the membrane potential. Switch from current to voltage clamp and start voltage step protocols with the easy-to-use graphical user interface. Apply compounds by clicking the corresponding buttons in manual mode.



Data Analysis with Roboocyte2+ Software

Data can be analyzed with the Roboocyte2+ program. After loading the plate file, recordings can be displayed and analyzed with a few mouse clicks. Analyzed data, such as maximum, minimum, extremum, average, or area under the curve are immediately plotted into the result window, i.e., either IV-dependencies will be plotted from your IV-recordings or dose-response plots will be created from your dose-response recordings, utilizing the compound information generated in the Roboocyte program for the specific recording. The curve-fitting feature lets you easily calculate EC_{50} or IC_{50} values which can be exported as ASCII text for further processing. The figure shows a current-voltage curve of a voltage step series recorded from a test model cell.



Scripting Features

Roboocyte2 scripts are written in a JavaScript-like language, which allows for full automation of all imaginable experimental designs.

You can apply essentially unlimited numbers of compounds to an oocyte that shows correct responses to control stimuli.

You can set up response-dependent recording sequences and control routines with user-specified limits for various parameters. Typical control routines include electrode resistance checks, membrane potential and leak current checks, expression tests with a reference compound, reversibility checks, run-down or run-up tests, and so on. The Roboocyte2 skips cells that failed viability testing, thus saving compounds and preventing further delay to the test sequence. You can flexibly define the recording sequence for each oocyte separately and integrate control routines of your choice to be time- and cost-efficient.

Script templates for typical applications are included, and a highly-qualified support team can assist you in writing custom scripts specifically for your applications.

Free Updates and Support

Development of the Roboocyte2 software is constantly on-going, because Multi Channel Systems is eager to improve its products and to meet customers' needs. MCS will be glad to hear your suggestions and to add new features. Free software updates will be available to download from the MCS website.

A comprehensive manual, interactive HTML help, and a friendly and highly-qualified support team are provided to aid your progress.

Technical Specifications

Roboocyte2 Robot

- Dimensions: 320 mm x 320 mm x 310 mm (W x D x H)
- Weight: 23.2 kg
- Power supply:
  Voltage range: 100 to 240 VAC
  Frequency: 47 to 63 Hz
- Supply pressure:
  4 to 8 bar @ pressure regulator input
  3.2 bar @ Roboocyte2 input

ClampAmpC

General:

- Newly designed integrated digital TEVC amplifier
- Headstages included
- Operates fully-automatically and computer-controlled
- Active bath clamp with two independent reference electrodes
- Sampling rate: 1 Hz - 20 kHz
- Data resolution: 16 bit
- Recommended electrode resistance range: 100 kΩ to 1 MΩ

Current electrode output:

- Output range: -107 µA to +107 µA
- Effective current resolution: 1 nA
- Compliance voltage range: -100 V to +100 V

Voltage electrode input:

- Input range: -500 mV to +500 mV
- Voltage resolution: 0.125 mV
- Clamp voltage setpoint range: -500 mV to +500 mV
- Clamp voltage setpoint resolution: 1 mV

Amplifier gain settings:

- Proportional gain: 0 - 6700 nA/mV
- Integrator gain: 0 - 8000 1/s
- Typical rise time in voltage clamp mode: <1 ms

Performance and Throughput

- Operates with disposable standard 96-well plates
- Positioning accuracy: 20 µm in x/y and z-direction

Perfusion System

- 12-channel pinch valve Roboflow-System with two peristaltic pumps, adjustable flow rate 0.1 to 10 ml/min
- Full integration of an external liquid handler Gilson GX-271
- Either system is fully controlled and automated by the Roboocyte2 software
- Number of compounds is limited only by oocyte performance

Software

- Full automation and control of all devices and features including perfusion via scripting
- Connection to the Roboocyte2 via USB 2.0
- Data export in ASCII file format
- Linkage to Microsoft Access 2010 database (Microsoft Access not included)

Accessories

- Fully-installed computer and LCD monitor
- Stereo microscope
- Ready to use TEVC probes

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Applications

$GABA_A$ Receptors

Introduction

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system. GABA type A receptors are pentameric chloride channels assembled from a range of subunit isoforms, which influence the pharmacological properties of the receptor subtype.

GABAA receptors are targets for many clinically important drugs like anxiolytics, anticonvulsants, anesthetics, sedatives, muscle relaxants, barbiturates, and benzodiazepines.

Aim

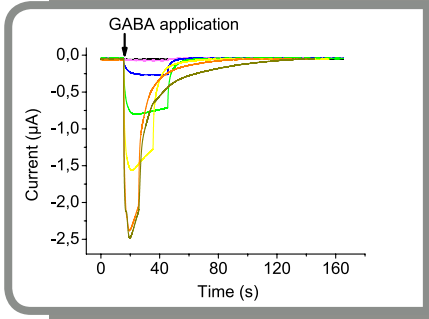
α_1 and β_2 subunits are expressed for 2-7 days after co-injection of both cDNAs in *Xenopus* oocytes, where they form functional chloride ion channel complexes in the oocyte membrane.

The aim is to analyze the pharmacological properties of this ion channel, for example, the dose-dependent activation by GABA or the dose-dependent inhibition of GABA induced currents with the Two-Electrode Voltage-Clamp method.

Signals

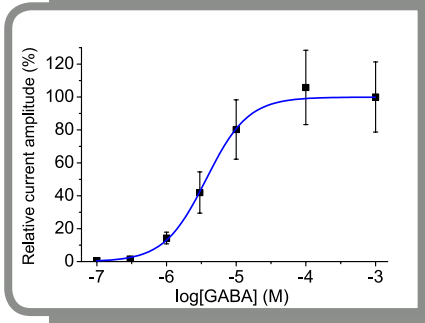
GABA-induced currents were recorded at a holding potential of –60 mV. In general, GABA was applied for 10–30 s to minimize desensitization but also to ensure saturating responses at lower concentrations. The maximum GABA-induced current was reached after 2.5 s, and the baseline current was reached again within 20 s after GABA wash-out. A successful recording of GABA-induced currents (with a minimum amplitude of 500 nA) was obtained in about 40 % of oocytes.

The figure shows an overlay plot of recordings with different concentrations of GABA (0.1 µM, 0.3 µM, 1 µM, 3 µM, 10 µM, 100 µM, 1 mM).



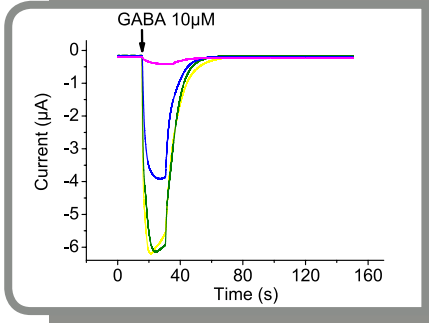
Dose Response Analysis

Currents were normalized to the maximal current obtained with 1 mM GABA. The $EC5_0$ value for the rat $GABA_AR$ subtype $\alpha_1\beta_2$ determined with automated TEVC was 3.7 µM, which is comparable to EC_{50} values obtained with conventional TEVC recording.



Inhibition of the $GABA_A$ Receptors by Bicuculline

The figure shows the dose-dependent inhibition of GABA-induced currents by bicuculline. Bicuculline was applied for 2 min prior to the application of 10 µM GABA in the continued presence of the drug. All concentrations were tested consecutively and automatically on the same oocyte. Each drug application step was followed by a wash step of 2-5 min prior to the application of the next test concentration.



(Pehl, U., Leisgen, C., Gampe, K. and Günther, E. (2004). „Automated Higher-Throughput Compound Screening on Ion Channel Targets Based on the *Xenopus* laevis Oocyte Expression System." Assay Dru Dev Technol 2(5): 515-524.)

Applications

hERG Current

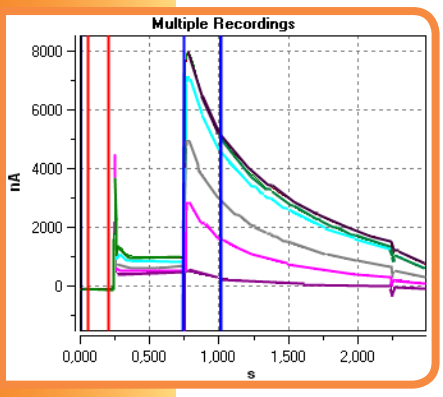
Introduction

Potassium channels critically contribute to cardiac repolarization, that is, to the final phase of the action potential that returns the cell to its resting state. The human ether-à-go-go related gene (hERG) encodes the pore forming subunits of the potassium channel that mediates rapidly activating delayed rectifier K+ currents (IKr). Drugs that block potassium channels can lead to a prolonging of the action potential.

Aim

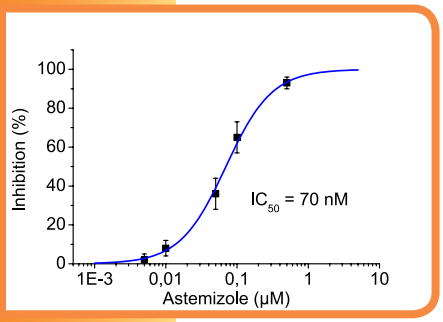
Drug-induced Long QT-Syndrome (LQTS) and Torsade de Pointes arrhythmia are a pressing public health issue. Inhibition of hERG is considered a significant risk factor for cardiac safety. In the last few years, a number of drugs have been withdrawn from the market due to adverse cardiac side effects leading to LQTS.

As a consequence, the pharmaceutical industry tends to screen for unwanted side effects of drug candidates on the cardiac action potential in the early drug profiling stage. An automated electrophysiological screening with the Roboocyte can be used to characterize the effects of pharmaceutical compounds on hERG ionic currents.



Recording of hERG Currents

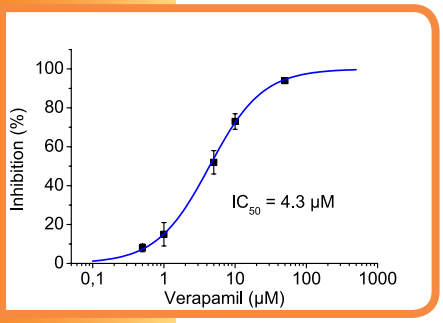
The screenshot from the Roboocyte Analysis window shows an overlay of hERG induced currents. The heterologously expressed hERG channel was activated by a 500 ms depolarizing step to 0 mV from a holding potential of -90 mV, and a steady state current was observed. Since the rate constant for recovery from inactivation is faster than the deactivation rate constant, a step back to -85 mV elicits a large tail current, as there are many channels that have not proceeded from the opened to the closed state. The hERG channels were blocked by increasing concentrations of Astemizole at 5, 10, 50, 100, and 500 nM.



Inhibition of hERG Tail Currents by Astemizole

Astemizole is an antihistamine that provides relief from allergy symptoms. The drug has been withdrawn from the U.S. market due to cardiac safety problems.

Astemizole blocks the IKr current by inhibition of the hERG K^+ channels. The measured IC_{50} value of 0.069 µM is virtually identical to published data.



Inhibition of hERG Tail Currents by Verapamil

The phenylalkylamine verapamil is used in the treatment of cardiovascular diseases such as angina pectoris, hypertension, and supraventricular tachyarrhythmias.

The IC_{50} value of 4.3 µM measured with the Roboocyte is comparable to published data (3.8 µM) measured with a conventional setup.

(Data kindly provided by IonGate Bioscience GmbH, Frankfurt / Main, Germany, www.iongate.de)

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Applications

Na/K-ATPase Transporter

Introduction

The Na/K-ATPase is a ubiquitous and critically important membrane protein that transports 2 K^+ ions into and 3 Na^+ ions out of the cell against the electrochemical gradient by using the energy of the hydrolysis of 1 ATP molecule per transport cycle.

The transporter serves many functions, including creating and maintaining the transmembrane Na^+ and K^+ gradients that contribute to membrane potential and excitability, driving secondary active transport systems coupled to Na^+ fluxes, and determining a significant fraction of the cellular metabolic rate via ATP hydrolysis. 30-70 % of the cell's ATP is used for this transporter.

Moreover, Na/K-ATPase is the pharmacological receptor for cardiac glycosides, which are widely used in the treatment of heart failure because of their positive ionotropic effect, and is possibly also the physiological receptor for endogenous ouabain-like compounds.

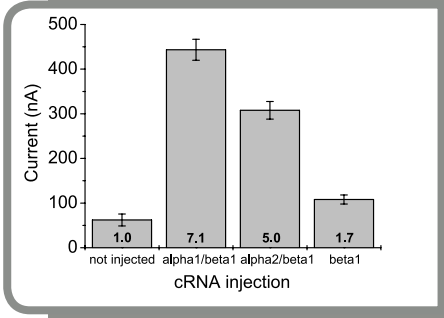
Aim

Four different Na/K-ATPase isozymes were expressed in *Xenopus* laevis oocytes and investigated with the Roboocyte. *Xenopus* laevis oocytes also express an endogenous Na/K-ATPase. To distinguish between the endogenous and heterogeneous forms, the Na-pump current of injected oocytes was compared with that of non-injected cells.

Compounds can then be tested on oocytes to reveal potential effects on Na-pump transport activity. In this case, the effect of the inhibitor ouabain on endogenous and human α_2/β_1 Na/K-ATPase was analyzed.

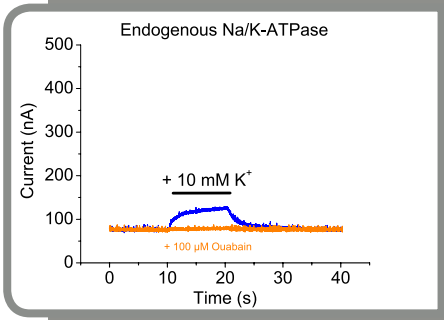
Oocyte Expression

Oocytes injected with cRNA encoding human Na/K-ATPase subunits and preloaded with Na+ showed pump currents that were 1.7-fold (*Xenopus* laevis α/human β_1 Na/K-ATPase) to 7.1-fold (human α_1/β_1 Na/K-ATPase) higher than those measured in non-injected oocytes (endogenous *Xenopus* laevis Na/K-ATPase), which is in good agreement with published data. Thus, it is possible to discriminate between *Xenopus* laevis and human Na/K-ATPase.



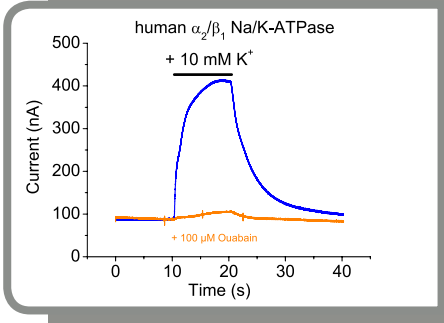
Inhibition of the Endogenous Na/K-ATPase by Ouabain

The effect of the inhibitor ouabain (100 µM, exposure time 2 min) on endogenous Na/K-ATPase is shown. Note that even the small endogenous currents of only 50 nA can be perfectly resolved with the Roboocyte's digital TEVC amplifier.



Inhibition of a Human Na/K-ATPase by Ouabain

This graph shows the effect of ouabain (100 µM, exposure time 2 min) on the human α_2/β_1 Na/K-ATPase.



(Data kindly provided by IonGate Bioscience GmbH, Frankfurt / Main, Germany, www.iongate.de)

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