roboocyte®

multichannel systems

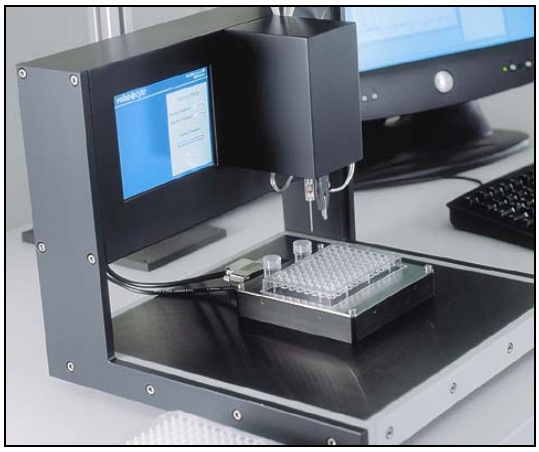
Applications: $GABA_A$ Receptors

Introduction

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system. The GABA type A receptors are pentameric chloride channels assembled from a range of subunit isoforms, which influence the pharmacological behaviour of the receptor subtype.
$GABA_A$ receptors are targets for many clinically important drugs like anxiolytics, anticonvulsants, anesthetics, sedatives, muscle relaxants, barbiturates, and benzodiazepines, for example valium.

Aim

The 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 modulation by GABA or the dose-dependent block of GABA induced currents with the Two-Electrode Voltage-Clamp method.

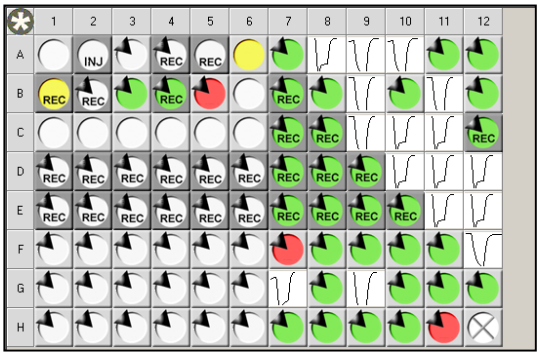


System

Oocytes are injected, recorded, transported, and stored conveniently in standard 96 well plates. mRNA or cDNA is injected fully automatically with the Roboocyte.

The novel digital amplifier has been optimized for TEVC (Two-Electrode Voltage-Clamp) experiments. Voltage steps can be freely designed to your needs. Resulting currents are recorded with the Roboocyte program.

You can choose between a 16-channel perfusion system or a liquid handling station that holds up to 400 compounds. Recording protocols can be run fully automatically without supervision, even over night. Provided that oocytes are of good quality, hundreds of compounds can be tested on a single well plate with 96 oocytes.



Software

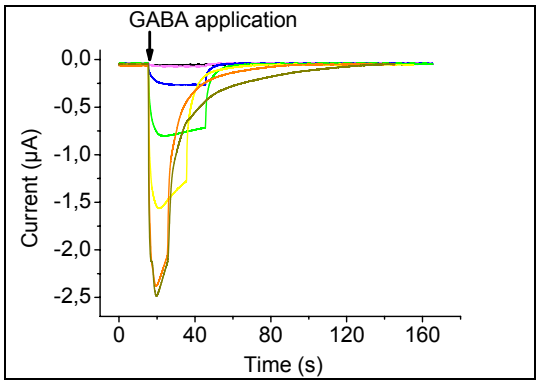
The Roboocyte system is fully software controlled. Amplifier and perfusion parameters, recording times, viability and stability checks, P/n leak subtraction, and your own custom checks are set up in separate recording protocols, one for each application. You load the appropriate protocol and start the session with a single mouse-click.

The extremum, the mean, and the region under the curve are extracted from a predefined region of interest with baseline subtraction, and current-voltage and dose-response curves are plotted fully automatically as well. All results are filed into a database. You can sort the results, print report sheets, and export the extracted results, the graphs, or the raw data to your custom program.

roboocyte®

Applications: GABAA Receptors

Multi Channel Systems
MCS GmbH
Aspenhaustrasse 21
72770 Reutlingen
Germany
Fon +49 – 7121 – 90 92 50
Fax +49 – 7121 – 90 92 511

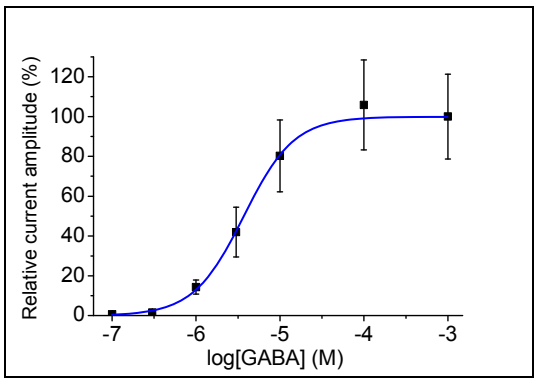


Signals

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 to ensure saturating responses also at lower concentrations. The maximum of the GABA-induced current was reached after 2.5 s, and the baseline current was reached again within 20 s after GABA washout. A successful recording of GABA-induced currents (with a minimum amplitude of -500 nA) was obtained in about 40 % of the 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).

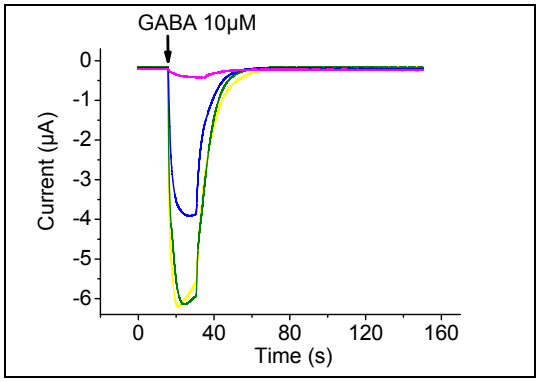
(Pehl, U., Leisgen, C., Gampe, K. and Günther, E. (2004). "Automated Higher-Throughput Compound Screening on Ion Channel Targets Based on the Xenopus laevis Oocyte Expression System." Assay Drug Dev Technol 2(5): 515-524.)



Dose Response Analysis

Currents were normalized to the maximal current obtained with 1 mM GABA. The EC_{50} 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 of 5.8 µM or 4.3 µM obtained with conventional TEVC recording.

(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 Drug Dev Technol 2(5): 515-524.)



Inhibition of the $GABA_A$ Receptors by Bicuculline

The figure shows the dose-dependent block 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 Drug Dev Technol 2(5): 515-524.)