

## Applications: nACh Receptors

### Introduction

Nicotinic acetylcholine receptors (nAChRs) are diverse, but homologous, members of the cys-loop superfamily of multi-subunit, ionotropic neurotransmitter receptors. In vertebrates, they are composed of five membrane spanning protein subunits, which assemble to form the central ion channel pore. Currently, 17 homologous subunits ( $\alpha$ 1– $\alpha$ 10,  $\beta$ 1– $\beta$ 4,  $\gamma$ ,  $\delta$ ,  $\epsilon$ ) have been identified.

nAChRs play a key role in the signal transduction between cells at the nerve/muscle synapses. The endogenous neurotransmitter is acetylcholine, which can be mimicked by the tobacco alkaloid, nicotine. nAChRs are largely distributed both in the peripheral and central nervous systems; growing evidence accumulated from animal and human studies demonstrating the involvement of nAChRs in high brain functions and in important neurodegenerative pathologies.

### Aim

The rat  $\alpha$ <sub>7</sub> rat nAChR subunit is expressed after cDNA injection in *Xenopus* oocytes, and the channel protein is incorporated into the oocyte membrane forming homomeric  $\alpha$ <sub>7</sub> nAChRs.

The aim is to analyze the pharmacological properties of this ion channel with the Two-Electrode Voltage-Clamp method. The oocytes are exposed to test compounds to show potential effects on the ion channel activity. As a positive control compound, acetylcholine (ACh) is applied in different concentrations with the 222 XL liquid handler from Gilson that operates under full control of the Roboocyte program.

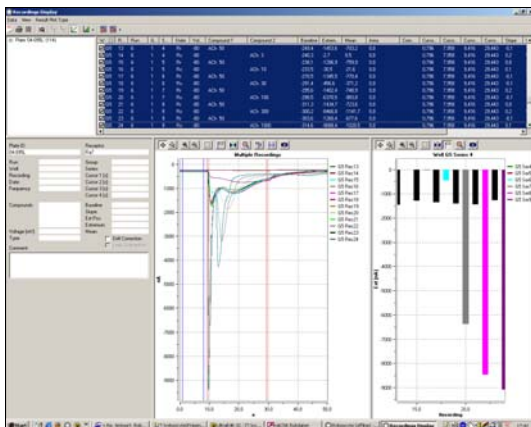


### System

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.

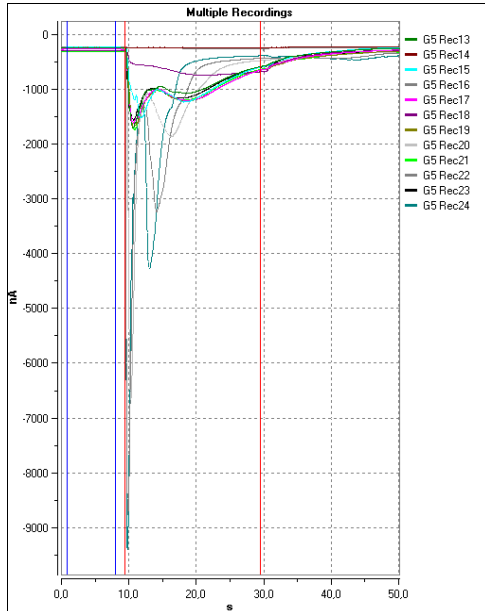
You can choose between a 16-channel perfusion system or a liquid handling station that holds up to 400 compounds. Recording protocols can be run fully automatically without supervision, even over night. Provided that oocytes are of good quality, hundreds of compounds can be tested on a single well plate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

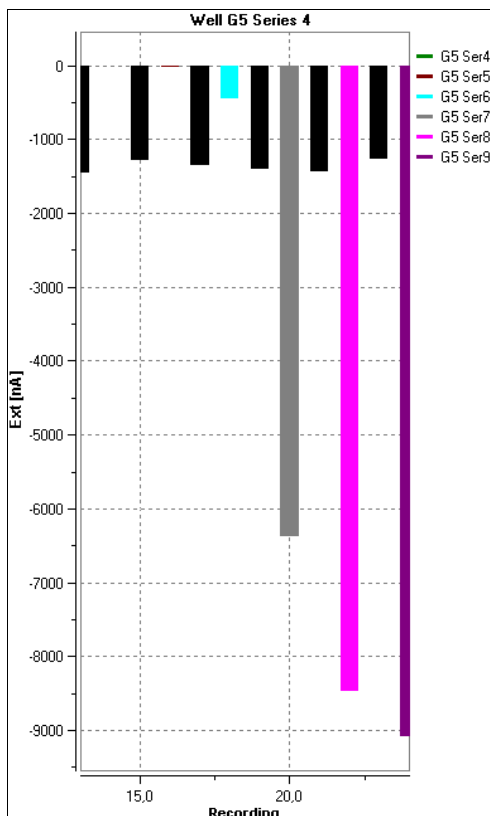
The extremum, the mean, and the region under the curve are extracted from a predefined region of interest with baseline subtraction, and current-voltage and dose-response curves are plotted fully automatically as well. All results are filed into a database. You can sort the results, print report sheets,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



## Signals

The  $\alpha_7$  subunit from the rat nACh-Receptor was expressed after cDNA injection in *Xenopus* oocytes. Acetylcholine (ACh) was applied in concentrations of 50  $\mu$ M (reference measurement), 50  $\mu$ M, 50  $\mu$ M, 3  $\mu$ M, 50  $\mu$ M, 30  $\mu$ M, 50  $\mu$ M, 300  $\mu$ M, and again 50  $\mu$ M for 20 s each. The recording was started 10 s prior to compound application. Receptor current desensitization occurs in the range of seconds. On the left you see a screenshot from the Roboocyte software. The two blue bars define the baseline, the red bars define the region of interest. The cursor position can be predefined in the recording protocol or in the Data Plot window used for online monitoring of the recording.

(Data kindly provided by Bayer AG, Leverkusen, Germany, [www.bayertechnology.com](http://www.bayertechnology.com))



## Dose-Response Relationship

This is a different presentation of the same data – a column plot, which is generated automatically after the completion of a concentration series with the Roboocyte. The response (extremum of the region of interest after the baseline subtraction) is plotted versus the recorded sweep. The dose response recordings are plotted in colors, the reference measurements are in black. You see that the six control responses to the 50  $\mu$ M dose are very stable in the range of 2  $\mu$ A. The response to the highest dose is greater than 9  $\mu$ A.

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