



E 03 - GGNB Extended Methods Course 2015

Electrophysiology

ELECTRAIN 2015
(planned for 4 - 15 May 2015)

**European Neuroscience Institute Göttingen
ENI-G**

**supported by
FEDERATION OF EUROPEAN NEUROSCIENCE
SOCIETIES (FENS)**

Faculty:

Michael FERBER, xlab, Göttingen

Michael HÖRNER, European Neuroscience Institute (ENI-G), Göttingen

Ira MILOSEVIC, European Neuroscience Institute (ENI-G), Göttingen

Annette NICKE, Walther Straub Institute for Pharmacology and Toxicology, Munich

Luis PARDO, Max-Planck-Institute for Experimental Medicine, Göttingen

Reiner POLDER, npi electronic, Tamm

Ralph SCHLIEPHACKE, Max-Planck-Institute for Experimental Medicine, Göttingen

Oliver SCHLÜTER, European Neuroscience Institute (ENI-G), Göttingen

Joachim SCHMIDT, University of Cologne, Inst. Zoology, Cologne

Annett SPORNING, Max-Planck-Institute for Experimental Medicine, Göttingen

Walter STÜHMER, Max-Planck-Institute for Experimental Medicine, Göttingen

Erhard WISCHMEYER, University Clinic Würzburg

E 03 - GGNB Extended Methods Course 2015

ENI Electrophysiology Training (ENI-ELECTRAIN)

Date: (preliminary) 4 – 15 May 2015
Location: European Neuroscience Institute (ENI-G), Grisebachstr. 5, 37077 Göttingen
Participants: 8 for practical course (lectures are open for all PhD students)
(2 groups A+B of 4 participants each, groups switch topics after 1st week, participation for both weeks mandatory, topics will be assigned during the course)

TOPIC 1: *In vitro* Electrophysiology of Expressed Ion Channels
in *Xenopus laevis* oocytes (STÜHMER + PARDO)
(4 participants)

TOPIC 2: *In vivo* Electrophysiology of Identified Neurons
in *Hirudo medicinalis* (HÖRNER + FERBER)
(4 participants)

TOPIC 3: Measurement of synaptic parameters in mouse hippocampal
organotypic slices (SCHLÜTER + NN)
(4 participants)

TOPIC 4: Calcium measurements in cultured neurons and mouse brain slices
(MILOSEVIC + NN)
(4 participants)

Week 1/2 (4 – 8 May 2015 and 11 – 15 May 2015) ENI Lecture Hall, ENI Teaching Labs

Topic: Expression and electrophysiological characterization of different ion-channels in the *Xenopus* oocyte expression system

Techniques: cDNA expression techniques in *Xenopus* oocytes, Two-electrode voltage clamp configuration and measurements, Quantitative evaluation and statistical analysis of different ion channels/conductances

Lectures: see separate schedule from 9-11h, ENI Lecture Hall (open to all GGNB students)

Practical Training: Monday through Friday from 13-18h, ENI Teaching Labs

Presentation of results: Friday, ENI Lecture Hall, Friday afternoon: Cleaning-up

Week 1/2 (4 – 8 May 2015 and 11 – 15 May 2015) ENI Lecture Hall, ENI Teaching Labs

Topic: In-vivo electrophysiology of identified neurons in *Hirudo medicinalis*

Techniques: Single and double intracellular recording techniques, single cell fluorescent labeling and 3d-imaging, Characterization of spontaneous and stimulus-evoked electrical activity patterns in identified neurons, Analysis of synaptic connectivity and network properties, Pharmacological characterization of different electrical conductances.

Lectures: see separate schedule, ENI Lecture Hall (open to all GGNB students)

Practical Training: Monday through Friday from 13-18h, ENI Teaching Labs

Presentation of results: Friday afternoon, ENI Lecture Hall, Friday afternoon: Cleaning-up

Week 1/2 (4 – 8 May 2015 and 11 – 15 May 2015) ENI Lecture Hall, ENI Teaching Labs

Topic: Measurement of synaptic parameters in mouse hippocampal organotypic slices

Techniques: Miniature EPSC recording of CA1 pyramidal cells, evoked AMPA receptor and NMDA receptor mediated synaptic transmission of Schaffer collateral CA1 pyramidal cell synapses, lentiviral-mediated molecular manipulation of CA1 pyramidal cells

Lectures: see separate schedule, ENI Lecture Hall (open to all GGNB students)

Practical Training: Monday through Thursday from 13-18h, ENI Teaching Labs

Presentation of results: Friday afternoon, ENI Lecture Hall, Friday afternoon: Cleaning-up

Week 2 (11-15 May 2015) ENI Lecture Hall, ENI Teaching Labs

Topic: Calcium measurements in cultured cells (HeLa, primary cortical and hippocampal neurons) and mouse brain slices

Techniques: preparation of samples (cultured cells and mouse brain slices); ratiometric calcium imaging in cultured HeLa cells; calcium imaging in cultured cortical and hippocampal neurons; calcium imaging in mouse brain slices; quantitative evaluation of data

Lecture: Monday May 12 11-12.30 Calcium measurements in cultured neurons and brain slices
ENI Lecture Hall (open to all GGNB students)

Practical trainings: Mon-Fri from 13-18h, ENI Teaching Labs

Presentation of results: Friday afternoon, ENI Lecture Hall, Friday afternoon: Cleaning-up

SELECTED LITERATURE:

TOPIC 1: *In vitro* Electrophysiology of Expressed Ion Channels in *Xenopus laevis* oocytes

Stühmer, W. (1998) Electrophysiological recordings from *Xenopus* oocytes.
Methods in Enzymol. 293, 280-300.

TOPIC 2: *In vivo* Electrophysiology of Identified Neurons in *Hirudo medicinalis*

Carretta, M. (1988) The Retzius Cells in the Leech: A Review of their Properties and Synaptic Connections.
Comp. Biochem. Physiol. 91A, 3: 405-413

Gaudry, Q., Kristan, W.B. (2009) Behavioral choice by presynaptic inhibition of tactile sensory terminals.
Nature Neuroscience. 2009;12(11): 1450-57; doi:10.1038/nn.2400

Nicholls, J.G., van Essen, D. (1974): The nervous system of the leech. *Sci. American*, 230: 38-48

Rose, T, Gras, H, Hörner, M (2006) Activity-dependent suppression of spontaneous spike generation in the Retzius neurons of the leech, *Hirudo medicinalis* L..
Invertebrate Neuroscience 6: 169-176 (DOI 10.1007/s10158-006-0030-2)

TOPIC 3: Measurement of synaptic parameters in mouse hippocampal organotypic slices

Stein, V., House, D.R.C., Bredt, D.S., Nicoll, R.A. (2003): Postsynaptic Density-95 Mimics and Occludes Hippocampal Long-Term Potentiation and Enhances Long-Term Depression.
J. Neuroscience, July 2, 2003 • 23(13):5503–5506 • 5503

TOPIC 4: Calcium measurements in cultured neurons and mouse brain slices

Dawitz J, Kroon T, Hjorth J, Meredith RM (2011) Functional Calcium imaging in developing cortical networks.
J Vis Exp 56, e3550, doi:10.3791/3550

Grienberger C, Konnerth A. (2012) Imaging calcium in neurons. *Neuron* 73(5):862-85.

Participants 'ELECTRAIN 2015' 04. – 15. May, 2015

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2			FENS		
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SCHEDULE for Electrophysiology Course 'ELECTRAIN 2015'

W 1	Mon 04 May	Tue 05 May	Wed 06 May	Thu 07 May	Fri 08 May
09:15-10:45	Lecture: HÖRNER Identified neurons in invertebrates: The leech nervous system	Lecture: POLDER Instrumentation for Electrophysiology	Lecture: STÜHMER Voltage-gated ion channels	Lecture: SCHMIDT Rythmogenesis in the leech CNS	Lecture: NICKE Ligand-gated ion channels
11:00-12:30	Introduction Lecture: STÜHMER Heterologous ion channel expression in <i>Xenopus</i> oocytes	Lecture: STÜHMER Electrophysiological Methods		Data analysis/ Work on presentation	Data analysis/ Work on presentation
13:30-18:00	Introduction Lab Work Group assignment (Group: Oocyte) (Group: Leech) (Group: Hippoc.)	Lab Work	Lab Work	Lab Work	Short presentation Groups: Leech/ Oocyte/ Hippocampus

W 2	Mon 11 May	Tue 12 May	Wed 13 May	Thu 14 May	Fri 15 May
09:15-10:45	Lecture: SCHLÜTER Field potential measurements in hippocampal slices	Lecture: SCHLÜTER Synaptic potential measurements in hippocampal slices	Lecture: PARDO Structure/function relationship of ion channels / Analysis of current kinetics	Lecture: PARDO Non-neuronal channels	Data analysis/ Work on presentation
11:00-12:30	Lecture: MILOSEVIC Ca measurements in cultured neurons and brain slices	Lect.: WISCHMEYER Ion channels constitute the basis of perception		Data analysis/ Work on presentation	
13:30-18:00	Lab Work (Group: Oocyte) (Group: Leech) (Group: Hippoc.) (Group: Ca imaging)	Lab Work	Lab Work	Lab Work	Presentation Groups: Leech/ Oocyte/ Hippocampus/Ca- imaging Cleaning up

LECTURE / COURSE OUTLINE (LEECH)

LECTURE:

IDENTIFIED NEURONS IN INVERTEBRATES/ THE LEECH NERVOUS SYSTEM (M. Hörner)

Topics:

The lecture gives an overview on chemical transmitter systems in vertebrates and invertebrates. Some invertebrate animal models will be introduced to provide an overview on anatomical and functional properties of different sets of identified neurons. The leech, *Hirudo medicinalis*, will be introduced in detail.

Reading assignments:

Briggman K:L., Abarbanel H.D.I., Kristan W.B. (2005) Optical imaging of neuronal populations during decision-making. *Science* 307, 896-901

Carretta, M. (1988) The Retzius Cells in the Leech: A Review of their Properties and Synaptic Connections. *Comp. Biochem. Physiol.* 91A, 3:405-413

Kravitz, E. A. (2000): Serotonin and aggression: insights gained from a lobster model system and speculations on the role of amine neurons in a complex behavior. *J. Comp Physiol. A*, 186, 221-238

Nicholls, J. G., van Essen, D. (1974): The nervous system of the leech. *Sci. American*, 230, 38-48

Stent, G.S. et al. (1978) Neuronal Generation of the Leech Swimming Movement. *Science* 200:1348-1357 (copies of the original papers available upon request)

Recommendable general textbooks of neurobiology:

M.J. Zigmond et al.: *Fundamental Neuroscience*, ISBN 978-0-12-374019-09, 3rd edition, Academic Press 2008

F. Delcomyn: *Foundations of Neurobiology*, ISBN 0-7167-2627-0, Freeman 1998

G.G. Matthews: *Neurobiology Molecules, Cells, and Systems*. ISBN 0-634-04496-9, 2nd edition, Blackwell 2001

Study questions

General:

- 1.) Which classes of chemical transmitters do you know?
- 2.) Name some chemical transmitters in vertebrates and in invertebrates.
- 3.) What are the principles and mechanisms of neuromodulation?
- 4.) How can an identified neuron be defined?

Structure and function of nerve cells:

- 5.) How can nerve cells and sensory cells be distinguished?
- 6.) Which forms of secretion are used by nerve cells?
- 7.) Which forms of membrane potentials of excitable cells do you know?
- 8.) Allocate types of electrical potentials to the different functional sites of a neuron.

COURSEWORK:

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF IDENTIFIED NEURONS IN THE LEECH NERVOUS SYSTEM (M. Hörner/ M. Ferber)

Topics:

The basic instrumentation used for electrophysiological recordings from identified neurons will be demonstrated. From fabrication of glass electrodes to the operation of simple current-clamp amplifiers the equipment used for intracellular recordings will be demonstrated.

'Hands-on' intracellular recording from identified leech neurons; design of simple electrophysiological experiments; staining of single identified cells by iontophoretic dye application through microelectrodes; visualization of single neurons by fluorescence microscopy

Tasks:

Prep. / Anatomy

- Dissection of the medicinal leech, *Hirudo medicinalis* (gross anatomy, internal organization and situs of the major inner organs)
- Preparation and dissection of single ganglia for inspection of single neurons
- Localization and identification of single cells in the dissecting microscope

Electrophysiology

- Fabrication of glass electrodes, measurement of Ohmic and capacitive resistances
- Demonstration and 'hands on' training of intracellular recordings from leech
- Analysis of biophysical properties of different identified cell types (current injections, spiking threshold, I/V curves etc.)
- Demonstration of staining of single cells by iontophoretic injection of the fluorescent dye Lucifer Yellow

Microscopy / Imaging

- Microscopic inspection of Lucifer-Yellow injected cells under UV-light-induced fluorescence
- Identification of different cell compartments of single Lucifer-Yellow injected cell

Methods:

Solutions

- Leech saline (mM): NaCl=58,44; KCl=74,55; Tris-HCl=203,30; CaCl₂=147,03; D-Glucose(H₂O)=198,17 (pH=7,4)
- Phosphate buffer: 13,60g KH₂PO₄/1000ml aqua dest. (buffer I); 17,80g Na₂HPO₄/1000ml (buffer II); mix 160ml buffer I and 840ml buffer II (pH=7,4)
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Single Cell Staining with Lucifer Yellow

- Iontophoretic intracellular injection of 3-5% Lucifer Yellow in 0.1M LiCl by applying hyperpolarizing currents for 10-20min
- Diffusion for 30-60min in saline

Tissue preparation

- Fixation of tissue in 4% Paraformaldehyde in 0.1M Phosphate buffer (pH=7,4) 1h
- Rinse in 0.1M Phosphate buffer 2x 10min
- Dehydrate in ethanol: 1x 10min in 50%, 70%, 96% and 2x 10min in 100% EtOH
- Clear in 100% Methylbenzoate or Methylsalicylate 2x 10min