Lausanne & Geneva
FENS-IBRO Imaging Training Center
IMAGING NEURAL FUNCTION

August 22nd - September 10th 2010

Sponsored by

Organized by

Organizers

Alan Carleton

Department of Basic Neuroscience, CMU, University of Geneva.

Jean-Yves Chatton

Department of Cellular and Molecular Biology (DBCM), University of Lausanne.

Anthony Holtmaat

Department of Basic Neuroscience, CMU, University of Geneva.

Carl Petersen

Brain Mind Institute, EPFL, Lausanne.

Ralf Schneggenburger

Brain Mind Institute, EPFL, Lausanne.

Egbert Welker

Department of Cellular and Molecular Biology (DBCM), University of Lausanne.

Coordination

Sonia Bolea

Lemanic Neuroscience Doctoral School, University of Lausanne

Overview

We propose a PENS Training Center covering high-resolution optical imaging techniques to study neural function *in-vivo,* and in tissue preparations. This 3-weeks course for PhD students and post-docs in neuroscience will consist of morning lectures by international and local faculty, progressing from principles of imaging techniques to important recent advanced applications like voltage-sensitive dye imaging, intrinsic optical signalling, *in-vivo* 2-photon imaging, light-sensitive ion-channel imaging and others. Following the morning lectures the students will work on a scientific project (in small groups of 2-3) in the laboratories of the participating faculty, allowing them to gain hands-on experience with most of the aforementioned imaging techniques. The evenings will be reserved for poster presentations, round table discussions on ethics and professional skills, and social activities.

Invited Speakers: Bureau I., Cossart R., Dodt H.-U., Garaschuk O., Griesbeck O., Helmchen F., Hübener M., Klingauf J., Llano I., Nägerl V., Oertner T., Spring K. and Yasuda R.

Local Faculty: Bezzi P., Broillet M.C., Brown, M., Carleton A., Chatton J.Y., Hill S., Holtmaat A., Hornung J.P., Kiss J., Knott G., Lebrand C., Lüthi A., Magistretti P., Marquet P., Muller D., Nikonenko I., Petersen C., Schneggenburger R., Seitz A., Volterra A. and Welker E.

Program

The program starts on Sunday with a **welcome aperitif**. On the very first day of the PENS Training Center, a general **introductory meeting** will be held in Lausanne. Literature and readings will be provided during this introduction. On the first two evenings, Monday and Tuesday, each student will shortly **present his/her work and research interests**. Students will also present a **poster**, that will be on display in the lecture room for the rest of the week. Every week on Friday the PENS students present what they have done during the week in the PENS labs. This is a good opportunity to exchange ideas about the techniques learned.

Students are encouraged to discuss the lectures and their research interests with the speakers during the scheduled discussion sessions in the mornings. The discussions will have an informal format, facilitating interaction between faculty and students. The lecture series introduces imaging techniques and research applications, starting with the basics in week 1, progressing towards applied techniques in week 2 and 3. Material in form of methods papers or review papers will be provided ahead of time. The morning lectures will take place at the host institution of the corresponding week (Week 1 - UNIL, Week 2 - EPFL, Week 3 - UNIGE). Invited speakers, but also faculty, postdocs and PhD students from the host institution, will participate in these discussions. Thus, the morning sessions will provide opportunities for the students to informally discuss, with faculty and lab members, the presented techniques, experimental results of their projects, as well as ideas for new experiments.

Following the morning lectures, the students will start experimental work in their host labs in small groups (2 - 3 students). Each experimental project will last for a week. The PI of the host lab will introduce the students to the lab as a whole, and will make a schedule for the experimental work together with the students. The supervisor will provide readings on the specific imaging technique. The PI of the lab and faculty members will conduct the experiments under the guidance of an experienced post-doc from the lab. Questions about the experiments and analysis approaches will be discussed on a daily basis. This intense mentoring will guarantee an optimal access of the PENS students to the imaging techniques in the host lab.

The evenings will be reserved for poster presentations, round table discussions and presentations by the students about the experimental projects.

Daily schedule

SUNDAY, August 22nd 2010, in Lausanne, at the restaurant "Le Chalet Suisse"

17:30 – 19:00 Welcome drink
19:00 – 21:00 Welcome dinner

WEEK 1 at the DBCM, University of Lausanne
Room "Petit Auditoire", first floor

Principles of optical microscopy

MONDAY, August 23rd 2010

08:30 – 09:00	General Introduction to the course
09:00 – 10:00	*Lecture: Principles of light microscopy I* - **Jean-Yves Chatton** (UNIL; Head of Cellular Imaging Facility)
10:00 - 10:15	Discussion
10:15 – 10:30	Coffee break
10:30 – 11:15	Assignment of the experimental lab projects to the students, with participation of the project leaders for the first week.
11:15 - 19:30	**PENS labs: experimental work** Students can have lunch and dinner together with members of the host lab
19:30 – 21:00	**Slide/Poster presentations I by students** (First half of students).

TUESDAY, August 24th 2010

08:30 – 09:30	*Lecture: Principles of light microscopy II* - **Hans-Ulrich Dodt** (Vienna, Austria)
09:30 – 09:45	Discussion
09:45 – 10:00	Coffee break
10:00 – 11:00	*Lecture: Principles of fluorescence and confocal laser scanning microscopy* - **Kenneth Spring** (Bethesda, Maryland, USA)
11:00 – 11:15	Discussion
11:15 - 19:30	**PENS labs: experimental work** Students can have lunch and dinner together with members of the host lab
19:30 – 21:00	**Slide/Poster presentations II by students**. (Second half of students).

WEDNESDAY, August 25th 2010

08:30 – 09:30	*Lecture: Advanced confocal microscopy/multi-colour excitation* - **Arne Seitz** (EPFL; Head of Biological Imaging platform)
09:30 – 09:45	Discussion
09:45 – 10:00	Coffee break
10:00 – 11:00	*Lecture: Reconstruction and retrospective analysis of neurons* - **Egbert Welker** (UNIL)
11:00 – 11:15	Discussion
11:15 - 19:30	**PENS labs: experimental work** Students can have lunch and dinner together with members of the host lab
19:30 – 21:00	**Round table discussions** – Ethics in Animal Research. **Marcel Gyger,** EPFL **Alexandre Mauron,** Unige **Hugues Poltier,** Unil

THURSDAY, August 26th 1010

08:30 – 09:30	*Lecture: Time-lapse confocal microscopy* - **Cecile Lebrand** (UNIL)
09:30 – 09:45	Discussion
09:45 – 10:00	Coffee break
10:00 – 11:00	*Lecture: Principles of multi-photon excitation microscopy* - **Fritjof Helmchen** (Zürich)
11:00 – 11:15	Discussion
11:15 – 19:00	**PENS labs: experimental work** Students can have lunch and dinner together with members of the host lab

FRIDAY, August 27th 1010

08:30 – 09:30	*Lecture: Correlative light- and electron microscopic analysis of neurons* - **Graham Knott** (EPFL; Head of Life-Science EM facility)
09:30 – 09:45	Discussion
09:45 – 10:00	Coffee break
10:00 – 15:00	**PENS labs**: experimental work Students can have lunch together with members of the host lab
15:00 – 17:00	**PENS labs**: Preparation of presentations by students
17:15 – 19:00	**Project presentations.** All projects of the week will be presented by students in 10-15 minutes of informal presentation discussing the results, open questions, and possible future applications of the techniques learned in the first week of the course.
19:30 –	Dinner

PENS labs - Week 1 (University of Lausanne, UNIL):

1.1. Paola Bezzi and Jean-Yves Chatton
Total internal reflection (TIRF) microscopy

1.2. Marie-Christine Broillet
Calcium & sodium imaging in slices and cell cultures

1.3. Jean-Pierre Hornung
Time-lapse fluorescence microscopy

1.4. Cecile Lebrand
Time-lapse confocal microscopy

1.5. Anita Lüthi
Imaging of intracellular calcium oscillations

1.6. Andrea Volterra
Calcium imaging in glia (in vivo)

1.7. Egbert Welker
Neuronal (EM) reconstructions

WEEK 2 at the BMI, EPFL
Room SV 1717A, behind the SV restaurant

Applications of imaging techniques to probe neural function

MONDAY, August 30th 2010

08:30 – 09:30	*Lecture: Presynaptic Ca^{2+} imaging of cerebellar neurons* - **Isabel Llano** (Paris, France)
09:30 – 09:45	Discussion
09:45 – 10:00	Coffee break
10:00 – 11:00	*Lecture: Two-photon Ca^{2+} imaging in developing neuronal networks* - **Rosa Cossart** (Marseille, France)
11:00 – 11:15	Discussion
11:15 – 19:00	**PENS labs: experimental work** Students can have lunch and dinner together with members of the host lab

TUESDAY, August 31st 2010

08:30 – 09:30	*Lecture: In-vivo Ca^{2+} imaging of neurons with 2-photon laser-scanning microscopy* - **Olga Garaschuk** (Tübingen, Germany)
09:30 – 09:45	Discussion
09:45 – 10:00	Coffee break
10:00 – 11:00	*Lecture: Fluorescent Ca^{2+} indicators and quantitative Ca^{2+} imaging in neurons* - **Ralf Schneggenburger** (EPFL)
11:00 – 11:15	Discussion
11:15 - 18:30	**PENS labs: experimental work** Students can have lunch together with members of the host lab
18:30 – 20:00	Dinner by the lake Leman, at the Restaurant “Au petit port”, St. Sulpice

WEDNESDAY, September 1st 2010

08:30 – 09:30	*Lecture: Geneticaly encoded Ca^{2+} indicators* - **Oliver Griesbeck** (München, Germany)
09:30 – 09:45	Discussion
09:45 – 10:00	Coffee break
10:00 – 11:00	*Lecture: FRET based imaging of 2nd messenger and kinase activation in neurons* - **Ryohei Yasuda** (Durham, North-Carolina, USA)
11:00 – 11:15	Discussion
11:15 – 19:30	**PENS labs: experimental work** Students can have lunch and dinner together with members of the host lab
19:30 – 21:00	**Round table discussions** – Outreach events / Media training **Beatrice Roth**, from EDAB / Brain Awareness Week **Mona Spiridon,** from the Geneva Neuroscience Center **Laura Spinney,** freelance science journalist

THURSDAY, September 2nd 1010

08:30 – 09:30	*Lecture: Principles of Total Internal Reflection microscopy (TIRF)*- **Jürgen Klingauf** (Göttingen, Germany)
09:30 – 09:45	Discussion
09:45 – 10:00	Coffee break
10:00 – 11:00	*Lecture: Total Internal Reflection microscopy (TIRF) for studying vesicle dynamics in glia cells* - **Paola Bezzi** (UNIL)
11:00 – 11:15	Discussion
11:15 – 19:00	**PENS labs: experimental work** Students can have lunch and dinner together with members of the host lab

FRIDAY, September 3rd 1010

08:30 – 09:30	*Lecture: Stimulated Emission Depletion microscopy (STED) microscopy for live imaging of neurons* - **Valentin Nägerl** (Bordeaux, France)
09:30 – 09:45	Discussion
09:45 – 10:00	Coffee break
10:00 - 15:00	**PENS labs: experimental work** Students can have lunch together with members of the host lab
15:00 – 19:30	**PENS labs: Preparation of presentations by students** Students can have dinner together with members of the host lab
19:30 – 21:00	**Project presentations.** All projects of the week will be presented by students in 10-15 minutes of informal presentation discussing the results, open questions, and possible future applications of the techniques learned in this second week of the course.

PENS labs - Week 2 (Ecole Polytechnique Fédérale de Lausanne, EPFL):

2.1. Sean Hill (1 student)
"In-silico" imaging of network activity using the Blue Brain Simulation Platform

2.2. Graham Knott (2-3 students)
Correlative light- and electron microscopic analysis of neuronal structure

2.3. Pierre Magistretti and Pierre Marquet (2-3 students)
Digital Holographic Microscopy (DHM)

2.4. Carl Petersen & Yves Kremer (2 students)
In vivo two photon calcium imaging of neuronal network activity with single cell resolution

2.5. Carl Petersen & Alexandros Kyriakatos (2 students)
Voltage-sensitive dye imaging of the cortical spatiotemporal dynamics evoked by a whisker deflection

2.6. Carl Petersen, Shovan Naskar & Michael Avermann (2 students)
In vitro whole-cell recordings targeted to GFP-expressing GABAergic neurons in neocortical mouse brain slices combined with optogenetic stimulation

2.7. Ralf Schneggenburger (2-3 students)
Quantitative Ca^{2+} imaging and Ca^{2+} uncaging

2.8. Ralf Schneggenburger & Arne Seitz (2-3 students)
Spinning disk confocal time-lapse imaging / FRET imaging of PK-C activity

WEEK 3 at the CMU, University of Geneva
Room 7001, Seventh floor

Applications of imaging techniques to probe neural structure and function, and optical activation of neurons

MONDAY, September 6th 2010

08:30 – 09:30	*Lecture: Voltage-sensitive dye imaging: Principles and in-vivo application* - **Carl Petersen** (EPFL)
09:30 – 09:45	Discussion
09:45 – 10:00	Coffee break
10:00 – 11:00	*Lecture: Intrinsic signal optical imaging combined with in vivo 2-photon microscopy* - **Mark Hübener** (Martinsried, Germany)
11:00 – 11:15	Discussion
11:15 – 19:00	**PENS labs: experimental work** Students can have lunch together with members of the host
19:00 – 21:00	Dinner at the pizzeria “Pasta d’oro”

TUESDAY, September 7th 2010

08:30 – 09:30	*Lecture: Laser scanning glutamate-uncaging-based mapping of synaptic connectivity* - **Ingrid Bureau** (Marseille, France)
09:30 – 09:45	Discussion
09:45 – 10:00	Coffee break
10:00 – 11:00	*Lecture: Light-activated ion channels to stimulate neurons in-vitro and in-vivo* - **Thomas Oertner** (Basel, Switzerland)
11:00 – 11:15	Discussion
11:15 – 19:00	**PENS labs: experimental work** Students can have lunch and dinner together with members of the host lab

WEDNESDAY, September 8th 2010

08:30 – 09:30	*Lecture: Ultra-structural analysis of genetically modified neurons and synapses*- **Irina Nikonenko** (UNIGE)
09:30 – 09:45	Discussion
09:45 – 10:00	Coffee break
10:00 – 11:00	*Lecture: Long-term in vivo 2-photon microscopy of neuronal structure* - **Anthony Holtmaat** (UNIGE)
11:00 – 11:15	Discussion
11:15 – 19:30	**PENS labs: experimental work** Students can have lunch and dinner together with members of the host lab
19:30 – 21:00	**Round table discussions** – Career opportunities in industry The Lemanic area, with more than 200 medical technology and biotechnology companies, and many sciences parks, is a European leader in technology transfer. Representatives from different biotech companies will discuss with the students about career opportunities in industry: **Martin Kussmann**, Group Leader Functional Genomics, Nestlé Research Center **Arthur Roach**, Merck Serono

THURSDAY, September 9th 1010

Public Holiday in Geneva. Social activities will be proposed.

FRIDAY, September 10th 1010

08:30 – 09:30	*Lecture: Light-activated ion channel control of the reward system* – **Matthew Brown** (UNIGE)
09:30 – 09:45	Discussion
09:45 – 10:00	Coffee break
10:00 – 11:00	*Lecture: to be confirmed*
11:00 – 11:15	Discussion
11:15 - 15:00	**PENS labs: experimental work** Students can have lunch together with members of the host lab
15:00 – 18:00	**PENS labs: Preparation of presentations by students**
18:00 – 19:30	**Project presentations --** All projects of the week will be presented by students in 10-15 minutes of informal presentation discussing the results, open questions, and possible future applications of the techniques learned in this third week of the course.
19:30 – 21:00	**Farewell Dinner in Geneva, at the "Restaurant Alhambar"**

PENS labs - Week 3 (University of Geneva):

3.1. Alan Carleton (3 students)
Intrinsic signal and voltage-sensitive dye imaging / Multi-beam 2-photon imaging

3.2. Anthony Holtmaat (3 students)
2-photon imaging of neuronal structure and synaptic proteins in vivo / Intrinsic signal optical imaging

3.3. Jozsef Kiss (3 students)
Imaging of migrating transplanted cells

3.4. Christian Lüscher (3 students)
Channelrhodopsin-mediated neuronal stimulation

3.5. Dominique Muller (3 students)
Confocal and two-photon imaging of neurons in organotypic slices

The ITC labs

UNIL
The UNIL labs are located in the Department of Cell Biology and Morphology of the University of Lausanne (rue du Bugnon 9, across the street from the hospital), whose research is centered on multidisciplinary studies in neuroscience. It heavily relies on imaging and electrophysiological approaches. Currently, the imaging instruments of the department include 2 two-photon microscopes (in vivo and in vitro), 2 confocal microscopes (one equipped for long-term time-lapse), 2 TIRF microscopes, 5 wide-field fluorescence microscopes for brain slice studies, 1 time-lapse fluorescence microscope. Also available in the department are standard molecular biology equipment, tissue culture and surgery facilities (e.g. for in utero electroporation) and a virus production lab.
The building also hosts a branch of Cellular Imaging Facility (CIF, http://www.unil.ch/cif), a core facility of the Faculty and the University Hospital (CHUV). In this building, the core facility offers 2 confocal microscopes, 1 fluorescence stereomicroscope, 1 time-lapse fluorescence microscope, and a wide-field fluorescence microscope. In the other two branches of the facility, other types of instrument are also available, such as a 2-photon microscope.
Finally, ultrastructural analysis can be performed at the electron microscopy facility of UNIL (Electron Microscopy Platorm, www.unil.ch/cme) present in the direct vicinity (300m from the department).

EPFL
The EPFL laboratories are in the Brain Mind Institute of the Ecole Polytechnique Fédérale de Lausanne (EPFL). In the Brain Mind Institute, inter-disciplinary neuroscience research is done on various levels spanning from functional genomics approaches to cognitive and behavioral neurosciences, with a strong commitment to cellular neurosciences. The three host labs together have several Set-Ups with conventional CCD imaging coupled with flash-photolysis-based uncaging, a two-photon microscope-based Set-Up coupled with *in-vivo* electrophysiology, and a digital holographic microscope (DHM). In addition, the Brain Mind Institute has another two-photon microscope for *in-vitro* (brain slice) imaging recordings. The Brain Mind Institute also has full access to the Bioimaging and optics platform (BIOp) of the EPFL School of Life Sciences. The BIOp hosts state-of the art light microscopes like point scanning confocal microscopes, spinning disk confocal microscopes (e.g. used for time lapse imaging) as well as another two-photon microscope, and these instruments will be made available for the training of the students of the PENS training center.
There will be a lecture room for the morning lectures, which can be used during the day as a reading room by the students.

UNIGE
The University of Geneva (UNIGe) labs are all located in the department of Basic Neuroscience, on the 8th floor of the University Medical School (CMU, 1 rue Michel Servet) of the University of Geneva. The building houses several outstanding basic science departments. The 5 host labs are equipped with 2 in-vivo 2-photon microscopes, 2 in-vitro 2-photon microscopes, 1 multi-beam 2-photon microscope, 3 laser scanning confocal microscopes, many epi-fluorescence

electrophysiology setups, 3 intrinsic imaging setups, 1 fast voltage-sensitive dye and/or Ca^{2+} imaging setup, automated neurolucida reconstruction setup.
Within the department, molecular biology (e.g. PCR, gel-electrophoresis, virus production) and surgery facilities (e.g. cranial windows, virus injections, stereotactic manipulations) are available.
The faculty imaging facility platform, which will be accessible to students and faculty, is equipped with state-of-the-art imaging setups.
There are general lecture and coffee rooms that can be used by the students. Each lab will provide some desk space and a computer for the students to process their data and prepare presentations.

FENS-IBRO Imaging TC lab 1.1

Imaging synaptic vesicles: functional analysis.

Faculty: Paola Bezzi and Jean-Yves Chatton
PhD Students: Julie Marchaland, Corrado Cali

Description:
In this project we will combine two dynamic imaging techniques (total internal reflection fluorescence - TIRF- and confocal microscopy) to correlate functional and structural analysis of the "vesicular machinery" present at the level of a single synapse, as well as in cultured astrocytes. Dynamics of active vesicles stained with synapto-pHluorin, a fluorescent marker of exo-endocytosis, will be followed in real-time and analyzed before, during and after fusion events (exo- endocytosis) with TIRF and confocal imaging. In addition, near-plasma membrane mitochondrial ion dynamics will be followed during these processes. Experiments of live cell imaging will be accompanied with immunofluorescence imaging of the cells using relevant markers.

Techniques:
- total internal reflection fluorescence (TIRF) microscopy
- confocal microscopy
- electrical field stimulation
- immunocytochemistry
- image processing

Location:
- Imaging labs in the Dept. Cell biology and Morphology, DBCM, rue du Bugnon 9, Lausanne
- Cellular Imaging Facility, rue du Bugnon 9, Lausanne

Phone number: 021 692 5284

FENS-IBRO Imaging TC lab 1.2

Calcium/sodium imaging of olfactory neurons

Faculty: Marie-Christine Broillet

Postdocs: Vincent Bize, Julien Brechbühl

PhD Students: Nicolas Hurni, David Bovard

Master Students : Céline Biollay, Fabian Moine

Description:

Students will get hands-on experience by preparing olfactory tissue from transgenic fluorescently labeled mice. Calcium and sodium imaging will be used to follow the neuronal activity of Grueneberg or vomeronasal neurons in the presence of odorant or pheromone stimulations. Subsequently, the proteins involved in the pheromone transduction cascade will be identified by immunohistochemistry and visualized by confocal imaging.

Techniques:

- fine dissection of mice sensory tissue
- calcium/sodium imaging on tissue slices or cell culture
- electrophysiology (patch-clamp, intracellular)
- confocal imaging (immunocyto-histochemistry)
- cell cultures
- electron microscopy

Location:

DPT, 3rd floor, Broillet Lab

Phone number:

+41 21 692 53 69

FENS-IBRO Imaging TC lab 1.3

Imaging the serotonergic modulation of hippocampal CA1 dendritic growth

Faculty: Jean-Pierre Hornung

Postdocs: Julien Ackermann, Peggy Mittaud

Research associate : Mary-Aude Rochat

Description:

Students will participate to the preparation of organotypic slices and electroporation of hippocampal neurons which will be imaged with time-lapse confocal microscopy to image the growth of dendrites and its regulation by stimulation with serotonin and downstream signaling pathways. Image will be collected and analyzed using Imaris and Metamorph

Techniques:
- In utero electroporation
- Organotypic culture preparation
- Time lapse confocal microscopy
- Image analysis with Imaris and Metamorph software
- Dendritic growth analysis

Location:
DBCM, Rue du Bugnon 9, 1005 Lausanne, Hornung lab

Phone number:

+41 21 692 5121

FENS-IBRO Imaging TC lab 1.4

Dynamics of corpus callosum guidepost-neurons and cortical axons

Faculty: Dr. Cécile Lebrand

Postdocs:

PhD Students: M. Niquille and D. Valloton

Description:

Our results unravelled that, during embryonic development, the corpus callosum (CC) is populated in addition to astroglia by numerous glutamatergic and GABAergic guidepost neurons that are essential for the correct midline crossing of callosal axons (Niquille et al, PLoS Biology, 2009). To gain insight into the motility of the neurons within the white matter of the CC, and on the dynamic interactions between CC neurons and callosal axons during development, we have started confocal time-lapse video microscopy studies on embryonic GAD67-GFP brain slices.
During the FENS-IBRO training, the students will have to define how growth cones of callosal axons respond to neurons of the CC along their track by monitoring the dynamic behaviours of individual callosal growth cones while they encounter the guidepost neurons. They will learn to label cortical axons by performing *in utero* electroporation of a pCAG-tomato plasmid in dorsal pallium precursors of embryos at day 14.5. Next, they will have to prepare organotypic slices and monitor the growth dynamics of cortical axons by using confocal time-lapse video microscopy. Neuronal and axonal 3D reconstructions and dynamics will be analyzed using Imaris and Metamorph software.

Techniques:
- *in utero* electroporation
- organotypic slices
- tissue transplantation on slices
- confocal time-lapse video microscopy
- Imaris and metamorph analyses

Location:
DBCM, Rue de Bugnon 9, 1005 Lausanne, Switzerland

Phone number: office +41-21-6925268

FENS-IBRO Imaging TC lab 1.5

Calcium signalling through T-type calcium channels: role in sleep

Faculty: Anita Luthi

Postdocs: Simone Astori

PhD Students: Ralf Wimmer, Martina Perin

Description:

The imaging-related project of the lab focusses on the role of calcium-dependent cellular signalling in thalamic neurons and their role in determining the intensity of deep sleep. Specifically, we study the signalling capacity of T-type calcium channels in oscillating thalamic neurons. We have several mouse mutants deficient in a T-channel-dependent signalling complex and we create mechanistic links between their cellular and network alterations and aspects of the sleep EEG and sleep behaviour.

Techniques:
- Mouse Brain Slice Preparation (generating sleep-related oscillatory network activity)
- Cellular Electro-Physiology (Patch-Clamp, Extracellular)
- Calcium Imaging (EM-CCD)
- Monitoring of mouse Resting-Activity Cycles
- EEG recordings

Location:
DBCM, UNIL, Rue du Bugnon 9, 1005 Lausanne

Phone number: 021 692 5294

FENS-IBRO Imaging TC lab 1.6

In vivo 2-photon imaging of astrocytes

Faculty: Andrea Volterra

Postdocs: Julien Chuquet

Engineer: Nicolas Liaudet

Description:

We will present an initiation to the surgical procedure of the closed cranial window in mice aimed at studying the astrocytic network and its relationship with the brain vasculature. The combination of various calcium sensitive and insensitive dyes will be used to image in real time the calcium activity of astrocytes. The cannulation of the femoral vein to load the vascular compartment and image brain vessels (arterioles vs. venules, capillaries), measures of the variation of their calibers or the red blood cell flux will also be shown. Data analysis of the astrocyte Ca2+ events will be performed by use of software developed in the lab.

Techniques:

- Cranial window surgery
- Two-photon laser scanning microscopy
- Brain vascular imaging
- Astrocytes imaging
- Calcium imaging and data analysis

Location:

DBCM. Rue du Bugnon 9 1005 Lauusanne
1st floor, Volterra's Lab

Phone number:

+41 021-692-5256 (secretary)

FENS-IBRO Imaging TC lab 1.7

Ultra-structure of barrel cortex

Faculty: Egbert Welker

Postdocs: Aouatef Abaza

Description:

Students will be guided through the subsequent steps for the preparation and analysis of ultra-thin sections through layer IV of the barrel cortex. They will get familiar with the ultrastructure of the cerebral cortex and introduce to quantitative analysis of neuronal connections in our search for activity-dependent neuronal plasticity in adult cerebral cortex.

Techniques:
- Plastic embedding
- Ultramicrotomy
- Transmission electron microscopy
- Analysis of neurphil; synapse identification
- 3D-reconstruction of spines and dendrites

Location:

DBCM, UNIL.

Phone number:

+41 21 692 5125

FENS-IBRO Imaging TC lab 2.1

In silico imaging of a model neocortical column

Faculty: Sean Hill and Felix Schürmann

Description:
This project will introduce the student to large-scale biologically realistic computer simulations of the neocortical column and the numerical tools used to analyze its structure and activity across multiple levels. The Blue Brain Project employs a simulation-based research environment that allows researchers to approach the model neocortical column as a simulated piece of brain tissue which can be imaged in a similar manner as real tissue. Further, it can also be examined using entirely new techniques that allow the model circuitry and activity to be analyzed across multiple spatial and temporal scales from the level of ion channels and synapses to neurons and networks. Using the Blue Brain Project imaging and analysis framework, students will learn about a broad array of new in silico imaging techniques used to visualize static network structure (neurites, somas and synapses) as well as dynamic network activity, including calcium and voltage imaging, synaptic states, identifying subcircuits and large-scale population activity.

Techniques:
- Neuron simulator (running BlueGene/P parallel supercomputer)
- BlueBrain SDK (C++ based microcircuit analysis framework)
- BlueHub (Blue Brain visualization tool)
- Osirix (third-party medical imaging tool)
- Vistrails (scientific imaging workflow management and provenance tracker)
- Matlab (numerical analysis software)

Location:
Blue Brain Project, SV-BMI, AAB 2 01, EPFL, Lausanne.
http://bluebrain.epfl.ch/

Phone number: 021 693 9678

FENS-IBRO Imaging TC lab 2.2

Electron microscopy for neurobiologists - a practical introduction

Faculty: Graham Knott, Life Science / Basic Science

Postdocs: 2
Technical Staff: 2
PhD Students: 0

Description:

This week long project will introduce students to transmission and scanning electron microscopy for neurobiologists. It will focus on a range of methods that can be used to prepare tissues and cells for imaging at high resolution, with an emphasis on hands-on experience. Students will learn how to fix, embed and cut samples, as well as image them in transmission and scanning electron microscopes. During the course the participants will also be introduced to techniques for carrying out correlated light and electron microscopy, as well as practical ways of analysing their data with the latest imaging software.

Techniques:
- transmission electron microscopy
- scanning electron microscopy
- immuno electron microscopy
- correlated light and electron microscopy
- serial section electron microscopy
- block face scanning electron microscopy

Location:
Bio Electron Microscopy Facility - Life Science Faculty, EPFL
Centre of Interdisciplinary Electron Microscopy - Basic Sciences, EPFL

Phone number: +41 21 693 1862

FENS-IBRO Imaging TC Lab 2.3

Multimodality microscopy to investigate the neuron-glial dynamics

Faculty: Pierre Marquet, Pierre Magistretti

Postdoc : Pascal Jourdain

Description:
The goal of this project is to investigate the dynamics of the neuron-glial network with an integrated imaging technique involving digital holographic microscopy (DHM), a new non-invasive 3D optical imaging technique with a nanoscale resolution, video fluorescence microscopy and electrophysiology. A special attention will be given to study how neuronal activity and different metabolic states can modulate, in relation with specific intracellular ions concentration changes (Na^+, Ca^{2+} etc.), neuron-glial morphology in specific cellular micro-domains. Specifically, minute cellular morphological changes will be assessed by DHM measurements and ion intracellular concentration by the utilization of fluorescence probes.

Techniques:
- Digital holographic microscopy (DHM),
- Video fluorescence microscopy
- Electrophysiology

Location:
Laboratory of Neuroenergetics and Cellular Dynamics, SV-BMI, Lab AI3217, EPFL, Lausanne.

Phone numbers: 0216931899
0216935182

FENS-IBRO Imaging TC lab 2.4

In vivo two photon calcium imaging of neuronal network activity with single cell resolution

Faculty: Carl Petersen

Postdocs: Yves Kremer

PhD Students: -

Description:

In order to understand how neocortical neuronal networks function in vivo, it is critical to measure simultaneously from many neurons. This can be achieved by introducing calcium sensitive fluorescent indicators into the population of neurons and measuring the changes in fluorescence over time with single cell resolution using two photon microscopy. In this project we will label neurons in the mouse primary somatosensory barrel cortex with fluorescent calcium indicators and we will measure the spontaneous and evoked activity upon tactile whisker stimulation.

Techniques:
- In vivo two photon calcium imaging

Location:
EPFL – AI3110

Phone number: 079 327 4525 (Carl)

FENS-IBRO Imaging TC lab 2.5

Voltage-sensitive dye imaging of the cortical spatiotemporal dynamics evoked by a whisker deflection

Faculty: Carl Petersen

Postdocs: Alexandros Kyriakatos

PhD Students: -

Description:

Tactile information is relayed from the whiskers on the snout of a mouse to the primary somatosensory barrel cortex. A single whisker deflection evokes an early localized response, which later spreads across a large part of sensorimotor cortex. The spatiotemporal dynamics of this sensory response can be imaged by applying voltage-sensitive dye to the cortical surface and measuring changes in fluorescence with a fast and sensitive camera.

During this project you will learn how to perform in vivo VSD imaging in mice. Specifically, you will learn:

1) how to perform large craniotomies,
2) staining protocols of the cortex with blue voltage sensitive dyes,
3) how to identify and measure the evoked signals following sensory stimulation of the whiskers in awake mice under different experimental conditions.
4) to map the different parts of the forelimb onto the sensory cortex.

Techniques:
- In vivo voltage-sensitive dye imaging

Location:
EPFL – AI3110

Phone number: 079 327 4525 (Carl)

FENS-IBRO Imaging TC lab 2.6

In vitro whole-cell recordings targeted to GFP-expressing GABAergic neurons in neocortical mouse brain slices combined with optogenetic stimulation.

Faculty: Carl Petersen

Postdocs: -

PhD Students: Shovan Naskar & Michael Avermann

Description:

The neocortex contains a large number of different types of neurons. These can in part be distinguished on the basis of different patterns of gene expression, which has driven the effort to characterise genetically engineered mouse lines differentially expressing GFP in specific classes of neurons. In this project, we will study the types of GABAergic neurons that are GFP-labeled in one mouse line. In the project, brain slices will be prepared and whole-cell recordings will be targeted to GFP-expressing neurons. The intrinsic electrical properties of the neuron will be studied together with the dendritic and axonal morphology. In addition, we will investigate the synaptic inputs onto these neurons through optical stimulation of neurons expressing Channelrhodopsin 2.

Techniques:
- In vitro whole-cell recordings
- Two photon microscopy
- Channelrhodopsin 2

Location:
EPFL – AI3110

Phone number: 079 327 4525 (Carl)

FENS-IBRO Imaging TC lab 2.7

Quantitative Ca^{2+} imaging and Ca^{2+} uncaging

Faculty: Ralf Schneggenburger, EPFL

Postdocs: Dr. Olexiy Kochubey

PhD Students: Yunyun Han, Ozgur Genc

Description:

In this one week of practical work, students will be introduced to quantitative Ca^{2+}-imaging approaches with various fura-2 indicator dyes (fura-2, fura-4f, fura-6F), using an upright microscope with a high-resolution, fast CCD imaging system. This will be combined with photolytic manipulation of the intracellular free Ca^{2+}-concentration by the use of a light-sensitive Ca^{2+}-chelator (DM-nitrophen) and a flash lamp. Hands-on experiments can be done in neurons in brain slices.

Techniques:
- CCD - based microscopic imaging
- Calibration of ratiometric Ca^{2+} indicators (fura-2, fura2FF)
- Photolyzable Ca^{2+} chelators (DM-nitrophen, NP-EGTA)
- Ca^{2+} uncaging with a flash lamp
- dendritic and nerve-terminal $[Ca^{2+}]_i$ transients
- brain slice preparation (brainstem, hippocampus)

Location:
Laboratory of Synaptic Mechanisms, Brain-Mind Institute, EPFL, Lausanne.
Building AI, 2nd floor, room 110

Phone number: 021 69 31608

FENS-IBRO Imaging TC lab 2.8

Time-lapse imaging of nerve terminals / FRET imaging of PKC activity in neurons

Faculty: Ralf Schneggenburger (EPFL), Arne Seitz (Bioimaging and Optics Platform BIOP, EPFL)

Postdocs: Nicolas Michalski

PhD Students: Ozgur Genc, Elin Falk

Description:
Time-lapse imaging of large nerve terminals (calyx of Held) in a brain slices from transgenic "Brainbow" mice expressing CFP / YFP /RFP will be performed. These experiments will be done on a scanning confocal microscope (Zeiss LSM 700) and on a Spinning disk confocal (Perkin Elmer Ultraview), and the advantages of each imaging approach for time-lapse imaging will be evaluated. Techniques for studying the intrinsic "Brainbow" labeling of neurons in fixed tissue, combined with immunohistochemical labeling of a specific protein will also be demonstrated. In a second project, FRET (fluorescence resonance energy transfer) will be done to image the activation of protein kinase-C (PKC) in cultured neurons. These experiments will be done on a CCD imaging system by measuring the CFP/YFP emission ratio to study the time-course of PKC activation. Complementary experiments will be done on a confocal microscope to measure FRET with acceptor photobleaching. This course is for a group of 2 - 3 students, and will last from Monday 30.08 to Friday 03.09.2010.

Techniques:
- time-lapse imaging with a confocal microscope (ZEISS LSM 700)
- time-lapse imaging with a spinning disk confocal (Perkin Elmer Ultraview)
- FRET imaging using wide-field fluorescence excitation and emission ratioing
- FRET imaging with acceptor bleaching in a confocal microscope
- Combined immunohistochemistry with fluorescently labeled secondary antibodies and endogenous "Brainbow" (CFP / YPF / RFP) imaging in fixed neuronal tissue

Location:
Bioimaging and Optics Platform BIOP, EPFL, Lausanne
Building AI, ground floor, room AI0130-AI0135.

Laboratory of Synaptic Mechanisms, Brain-Mind Institute, EPFL, Lausanne.
Building AI, 2nd floor, room 110

Phone number: 021 69 31608

FENS-IBRO Imaging TC lab 3.1

In vivo imaging of odor representation in the mouse olfactory bulb

Faculty: Prof. Alan Carleton

PhD Students: Roberto Vincis

Description:

The sense of smell is central to animal's life as it involves feeding, mating, predator avoidance, social interactions or navigation. Odors are sensed by a family of olfactory receptor proteins expressed by sensory neurons. These neurons select one receptor out of a large possible repertoire, and their axons converge in a receptor specific manner onto segregated anatomical structures called glomeruli in the vertebrate olfactory bulb (OB). Odorants evoke spatio-temporal patterns of activated glomeruli, being a first basis for olfactory coding. In this project, we will use *in vivo* imaging techniques allowing us to visualize the glomeruli patterns induced by different odorants in the olfactory bulb of anesthetized or awake mice. We will study how these representations are changing with concentration and mixtures.

Techniques:
- Intrinsic signal imaging
- SynaptopHluorin imaging

Location: Centre medical universitaire
University of Geneva
1 rue Michel Servet
1211 Geneva 4

Building A-B; 8th floor
Room 8218

Phone number: 022 379 54 62 (lab) or 022 379 54 26 (PI office)

FENS-IBRO Imaging TC lab 3.2

Optical imaging of barrel cortex in vivo

Faculty: Anthony Holtmaat

Postdocs: Fred Gambino, Vanessa Schubert

PhD Students: Michele Cane, Daniel Lebrecht, Jerome Randall

Description:

Students will get hands-on experience with installing a cranial window in transgenic, fluorescently labeled, mice. Intrinsic signal optical imaging will be used to map whisker-related activity in the barrel cortex through the cranial window. Subsequently, in the same mice, fluorescent dendritic and axonal structures will be imaged over two or three days in defined regions in the barrel cortex. Dendritic and axonal structure will be analyzed in neurolucida. Spine and axonal bouton dynamics will be analyzed using custom software.

Techniques:
- Cranial window surgery
- Intrinsic signal optical imaging
- Two-photon laser scanning microscopy
- Neurolucida reconstruction of imaged dendrites/axons
- Spine and bouton counting

Location:

CMU, 8th floor, Holtmaat lab

Phone number:

+41 22 3795428

FENS-IBRO Imaging TC lab 3.3

Cellular dynamic of cortical precursors cells during early postnatal development: analysis of cell migration and neurite outgrowth.

Faculty: Jozsef Kiss

Postdocs: Michael Boitard, Gael Potter, Patrick Salmon and Charles Quairiaux

Description:
Perinatal damaging insults are the most important cause of brain injury in the preterm infant. A critical feature of this pathology is that it impacts on the mechanisms that govern cortical network formation including neuronal precursor migration and positioning. Therefore, understanding the mechanisms and regulation of migratory events in the perinatal developmental context is crucial to understand how alterations in this process might contribute to neurodevelopmental disorders. The goal of this project is to examine the migration pattern and dynamics of a pool of postmitotic neuronal precursors in the subventricular zone that migrate radially into the medial limbic cortex (cingulate and retrosplenial cortex) during the first 4 postnatal days. These precursor cells give rise to layer 2 pyramidal neurons that form typical dendritic bundles and project to the contralateral cortex through the corpus callosum. This model permits to follow the development of an identified cell type over several developmental stages from the premigratory cell pool, through the radially migrating neuroblast stage to the positioning of layer 2 pyramidal cells with the progressive elaboration of dendritic bundles and axonal projection. To explore cortically directed migration of neurons from the subventricular zone, we use lentiviral based approches to label progenitor cells and overexpress or knockdown genes in these cells. Since lentiviral vectors carries a fluorescent reporter gene (e.g. GFP) as well as a gene of interest, this approach allows the transformation of neural progenitors in situ and offers direct experimental access to the process of recruitment of SVZ-derived neurons into the postnatal cortex. The migratory pattern cells and axons will be analyzed in ex in vivo slices. Using time-lapse videomicroscopy, we track the individual pathways of progenitor cells, measure speed and directionality of their migration and investigate the relationship between migrating progenitors and the extracellular matrix.

Techniques:
- Time-lapse video analysis
- Imaging of lentivector labeled cells in tissue
- Fluorescent and confocal optics
- Electron microscopy

Location:
Department of Neurosciences, CMU, Rue Michel-Servet 1, 1211 Geneva.
E-mail:Jozsef.kiss@unige.ch

Phone number (lab): 022 379 5240

FENS-IBRO Imaging TC lab 3.4

Channelrhodopsin-mediated neuronal stimulation

Faculty: Christian Lüscher

Postdocs: Camilla Bellone, Mathew Brown, Kelly Tan, Vincent Pascoli, Marc Turiault

PhD Students: Christina Bocklish, Arnaud Lalive, Ti-fei Yuan

Description:

Students will get hands-on experience with injecting channelrhodopsin in transgenic mice and implanting canula for direct stimulation. Simultaneous *in vivo* electrophysiological recordings and light stimulation will be used then to study the proprieties of Dopaminergic neurons in the mesolimbic system. Students will also get experience with two-photon glutamate uncaging (2-PLU) at isolated sections of DA neuron dendrites in order to selectively stimulate single synapses. At the same time neurons will be filled with fluorescent dye and dendritic and axonal structure will be analyzed.

Techniques:
- Injection Channelrhodopsin
- Canula implantation for in vivo chennelrhodopsin-mediated neuronal stimulation
- Simultaneous *in vivo* electrophysiological recordings and light stimulation.
- Two-photon laser scanning microscopy

Location:

CMU, 8th floor, Lüscher lab
Contact person: Camilla Bellone

Phone number:

+41 22 3795437

FENS-IBRO Imaging TC lab 3.5

Imaging of spine dynamics in hippocampal slice cultures

Faculty: Dominique Muller

Postdocs: Mathias De Roo, Irina Nikonenko, Bernadett Boda, Yann Bernardinelli, Carmen Flores

PhD Students: Sylvain Steen

Description:

Students will get hands-on experience with confocal imaging and electron microscopic reconstruction of synapses in organotypic hippocampal slice cultures. They will transfect slice cultures using the gene gun technology and then proceed to confocal analysis of dendritic spine turnover by imaging the same cells over several days. Additionally students will have the opportunity to work with a multibeam 2-photon system and analyse calcium signals in populations of hippocampal neurons loaded with a fluorescent dyes applied by bolus injection. Finally they will have also the opportunity to follow the procedures used for 3D electron microscopic reconstruction of transfected neurons.

Techniques:
- Organotypic slice cultures
- Gene gun transfection
- Single, two-photon and multibeam laser scanning microscopy
- Spine turnover analysis
- 3D electron microscopic reconstruction of transfected neurons

Location:

CMU, 8th floor, Muller lab

Phone number:

+41 22 3795434

Facilities

Accommodation for the first two weeks will be in the Maison de Rhodanie, a students' housing located 10 minutes by bus from the Lausanne train station, and 5 min by foot from the lake. The building offers wireless internet connection. Students can reach the UNIL and EPFL venues by bus and metro in 15-20 minutes. Passes for local public transport will be provided.

For the last week, the students will move to the Hotel Carmen in Geneva, located at walking distance (10 minutes) from the UNIGE host labs and the old town of Geneva.

A lecture room, exclusively reserved for the students of the PENS Training Center, is available at all three teaching locations. Coffee will be served in these rooms in between the lectures. The lecture rooms are located in the same building as the host labs. Thus, students can easily reach the host labs after the morning sessions. The lecture rooms will also be used for the evening sessions.

Lunch and dinner services are available on site or nearby, on campus. Students are encouraged to have lunch and dinner with members of the host lab, faculty and other students.

Free passes for public transport, that are needed to commute between the housing facilities and teaching venues, will be provided for faculty and students.

Students' abstracts

Dialog between intrinsic and synaptic properties shapes activity in the molecular layer of the cerebellum
Pepe Alcami and Alain Marty
CNRS and University Paris Descartes

The cerebellar cortex is a quasicristalline structure crucial for sensori-motor integration and motor learning. Interneurons in the molecular layer (MLIs) of the cerebellum constitute a feedforward inhibition circuit which is coplanar to the sole output of the cerebellar cortex, Purkinje cells. This allows the isolation of the network in sagittal slices, preserving local GABAergic transmission and the local interneuron network connectivity while eliminating the big majority of excitatory glutamatergic inputs, orthogonal to the interneurons plane. The spontaneous pattern of activity found in the isolated network corresponds to an auto-organisation of activity according to its intrinsic properties, from channels to the network. Here, we study how activity emerges, from channels to neurons in this preparation in order to gain insights into how interneuron networks organise their activity in the central nervous system to predict how local cortical microcircuits formed by interneurons might modulate activity at the output stage of a cortical structure.

Axonal plasticity after laser nanosurgery
A. L. ALLEGRA MASCARO(1), P. CESARE(2,3), L. SACCONI(1), G. GRASSELLI(2), P. STRATA(2,3), F. S. PAVONE(1);
(1) Univ. Florence, Sesto Fiorentino (FI), Italy; (2) EBRI-Santa Lucia Fndn. (IRCCS), Rome, Italy; (3)Dept. of Neurosci. and Natl. Inst. of Neurosci., Univ. of Turin, Turin, Italy

In the adult nervous system, different population of neurons corresponds to different regenerative behavior. Although previous works show that olivocerebellar fibers are capable of axonal regeneration in a suitable environment as a response to injury (Rossi et al., 2001), we have hitherto no details about the real dynamics of fiber regeneration. Recently, two photon imaging has been coupled to laser-induced lesions to perform in vivo multiphoton nanosurgery in the CNS of living mice expressing fluorescent proteins.We exploited this innovative technique to investigate the reparative properties of Climbing Fibers (CFs) in the adult CNS, following the time evolution of this plastic process in vivo. In this work, olivocerebellar axons were labeled through an injection in the inferior olive of a lentivirus expressing EGFP; after a survival period of 2-3 weeks, a craniotomy is performed on the vermis and lateral emispheres of the cerebellum.
Two photon microscopy is used to image labeled CFs in the cerebellar molecular layer of the adult anesthetized mouse. The nanosurgery technique is applied to the distal portion of a CF; 3D reconstructions of the imaged fiber through the days after this point disruption are then used to detect morphological changes in time.
Time lapse imaging of the same region over the following days shows that there may be a structural rearrangement of the dissected neuron itself or of the surrounding fibers. According to our preliminary results, the regeneration comes not from the damaged end but from adjacent portions, suggesting that some molecules may be released and recall a reorganization of the surroundings in

order to compensate for the lost afference. Furthermore this unique model could allow, through manipulation of the viral vector, to explore in detail the biochemical mechanisms underlying the reparative process. The great potential of long-term two-photon imaging, coupled to genetic manipulation, opens great opportunities to further investigate the dynamic properties of neurons and their rearrangement following an injury.

Tuning the activity of the enteric nervous system: a role for both endogenous and exogenous mediators

Werend Boesmans, Jan Tack, Pieter Vanden Berghe
Center for Gastroenterological Research, KULeuven, Leuven, Belgium

To accurately coordinate gastrointestinal (GI) functions more or less independent of the brain, the gut has a nervous system of its own. This so called enteric nervous system (ENS) comprises different types of neurons that are organized in specialized networks to control the mixing and propulsive movements of the bowel. ENS research has mostly focused on the initiation of the peristaltic reflex, as if it were an on/off phenomenon that once started always leads to the same outcome. We hypothesize that modulation of ongoing activity and synaptic communication by intrinsic factors such as endocannabinoids and neurotrophins plays a more important role than generally accepted. It is also plausible that extrinsic factors, such as substances present in our diet, influence ENS signaling and the peristaltic reflex. Tastants such as menthol indeed have prominent effects on GI motility, but the underlying mechanisms are poorly understood. Spontaneous network activity, monitored by Fluo-4 imaging in primary cultures of guinea-pig myenteric neurons, was significantly increased by cannabinoid receptor 1 (CB1) antagonists and abolished by CB1 activation. Using inhibitors of the endocannabinoids degrading enzyme, we were furthermore able to show that there is endogenous production of a CB ligand in the ENS. Brain-derived neurotrophic factor (BDNF) exposure also resulted in an amplification of network activity which was reversed by the tropomyosine-related kinase (Trk) receptor blocker K-252a. The transient receptor potential melastatin 8 (Trpm8) channel agonist menthol dampened spontaneous Ca^{2+} spiking and abolished the Ca^{2+} waves propagating spontaneously in gut smooth muscle cells. FM1-43 imaging revealed that CB1 antagonists reduce the number of synaptic vesicles that were recycled during an electrical stimulus whereas BDNF treatment facilitated FM1-43-labelled vesicle destaining in enteric terminals. Given the opposite effects of agonists and antagonists, we suggest that ENS signaling is under permanent control of CB1 receptor activity. The promotion of motility by BDNF seems to result from its potent modulating role on neuronal activity and synaptic communication. The fact that menthol dampens activity in enteric nerves and muscle could well be the underlying mechanism via which menthol-containing herbal drugs exert their effect on GI motility.

Differential affinity for hippocalcin in dendrites of hippocampal neurons

Cherkas V.P.1, Dovgan A.V.1, Kononenko N.I.1, Haynes L.P.2, Tepikin A.V.2, Burgoyne R.D.2 & Belan P.V.1
1 Bogomoletz Ins. Physiol., Kyiv, Ukraine; 2 Physiol., Univ. of Liverpool, Liverpool, United Kingdom

Hippocalcin is a Ca2+-binding protein which is a key mediator of many cellular functions including

synaptic plasticity and learning. Hippocalcin possesses a Ca2+-dependent conformation transition that allows hippocalcin to translocate to membranes upon Ca2+ binding. We have earlier shown that hippocalcin may decode neuronal activity into a site specific hippocalcin translocation in dendrites of hippocampal neurons. In this work we have examined some biophysical mechanisms leading to hippocalcin translocation in dendrites. The neuronal plasma membrane can have suboptical invaginations, such as caveolae, that effectively increase plasma membrane area. Thus, even during uniform hippocalcin insertion in the plasma membrane such sites would demonstrate increased amount of translocated hippocalcin compared to plain membrane loci. Co-expressing hippocalcin-ECFP and pEYFP-Mem, a fusion protein that targets EYFP to plasma membranes we tested whether the translocation was spatially correlated with plasma membrane enriched sites. No significant correlation was observed implying higher hippocalcin affinity for translocation sites. We also checked whether translocation sites are associated with sites of higher [Ca2+]i. Creating an uniform in a dendritic segment [Ca2+]i transients, we have shown that hippocalcin translocation was significantly different in neighbouring sites having equal [Ca2+]i transients. Producing long-lasting elevations of [Ca2+]i by activation of different Ca2+ mobilizing mechanisms we have also demonstrated that hippocalcin translocation was observed in the same set of site independently on Ca2+ source. These results indicate that [Ca2+]i is not the only determinant of hippocalcin translocation. We have concluded that the neuronal plasma membrane probably reveals differential affinity for hippocalcin forming in this way permanent sites participating in hippocalcin signalling.

Pharmacological Properties of Backpropagating Action Potential Evoked Ca2+ Transients in Hippocampal Fast Spiking Interneurons

Balázs Chiovini(1), Gergely F. Turi(1), Gergely Katona(1), Attila Kaszás(1), Anikó Vágvölgyi(1), E. Sylvester Vizi(1), Balázs Rózsa(1)
(1)Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary

Fast spiking (FS) GABAergic interneurons are essential for the regulation of network oscillations in the hippocampus. However, the composition of dendritic voltage gated calcium channels (VGCCs) and their effects on dendritic integration are less examined.
In this study, dendritic two-photon measurements were combined with patch clamp recordings and pharmacological approaches in order to reveal the possible subtypes of voltage gated Ca2+ channels taking part in Ca2+ signaling induced by backpropagating action potentials (bAPs) during sharp-wave activity. The distribution of the bAP evoked Ca2+ transients was examined in the dendritic arbor at different distances from the soma. Compared with the data in the literature obtained in pyramidal cells, our results show that Ca2+ signals in the FS interneurons are more attenuated as a function of distance. To examine the role of the VGCCs in this process, low concentrations of Ni2+ and nimodipine were applied in the bath under the blockade of voltage gated sodium channels. Low concentrations of Ni2+ slightly decreased the amplitude of the bAP evoked Ca2+ transients, while nimodipine totally abolished the Ca2+ influx. Our results suggest that L-type Ca2+ channels are present in dendritic compartments of FS interneurons, and play an important role in the generation of the Ca2+ influx during bAPs.

Intrinsic activation/inactivation properties of CaV2.1 calcium channels are shifting by Pregabalin modulating the neurotransmitter release.
Mariano N. Di Guilmi, Francisco J. Urbano, Carlota Gonzalez- Inchauspe, Osvaldo D. Uchitel. Inst. Fisiología, Biología Molecular y Neurosciencieas. UBA. CONICET. Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina.

Mechanisms by which Pregabalin (PGB) might act as an anticonvulsant are unknown. The α2-δ type 1 auxiliary subunit of voltage-gated calcium channels is the primary high-affinity binding site for PGB. Here we studied PGB effects on excitatory transmitter release of principal neurons of mice Medial Nucleus of the Trapezoid Body as well as we recorded presynaptic calcium currents (IpCa) present on the afferent called Calyx of Held in brainstem slices using whole cell patch clamp recordings.

We found that a dose-response relationship showed a maximum drug effect at 500 μM. At this concentration, PGB reduced the amplitude of EPSCs by a 30%. No differences were observed in the depression rate from high frequency trains but a faster rate of recovery from synaptic depression at 100 Hz was observed in the presence of PGB (p=0.043). We found no differences in the mean amplitude of miniature EPSCs while observing greater minis frequencies -PGB versus +PGB conditions (1.71±0.35 Hz and 0.49±0.06 Hz, respectively (p=0.0044)). On the other hand, multiple effects on IpCa by PGB were observed. PGB blocked CaV2.1 channels-mediated currents and decrease their facilitation during 100Hz train, without changing their voltage-sensitive activation. However, two pulses inactivation protocol showed a larger rescue of the inactivation. Additionally, the inactivation curve observed with PGB showed a clear change on the kinetic but not on the half-activation voltage using a long conditioning pulse protocol.

The results presented in this work could help to elucidate the possible PGB actions on cortical areas and its potential pharmacological action on neuropathotogies.

Supported by: UBACYT X-223; FONCYT (ANPCyT) PICT 2005-32113; 2006-199, Wellcome Trust (to ODU); FONCYT (ANPCyT) PICT 2007-01009, PIDRI-PRH 2007 (to FU).

A cortical stroke prevents visual plasticity in mice: imaging of cortical activity, behavioural analyses of vision and therapeutic approaches.
Franziska Greifzu1, Silvio Schmidt2, Karl-Friedrich Schmidt1, Otto W. Witte2, Siegrid Löwel1 1 Institute of General Zoology and Animal Physiology, Friedrich-Schiller-University Jena, Erbertstr. 1, 07745 Jena, Germany 2 Hans Berger Clinic of Neurology, University of Jena Medical School, Erlanger Allee 101, 07747 Jena, Germany

Stroke is a major cause of death and disability in the industrialized countries. It is an encouraging observation that most patients recover to some degree from the deficits. It is assumed that this is due to plasticity. Many in vitro studies indicated that there is an increased plasticity in the perilesional zone of infarcts. In this study we investigated in vivo the impact of a photothrombotically induced cortical stroke on the plasticity of the neighbouring visual cortex after 7 days of monocular deprivation (MD). Visual function was analyzed behaviourally with a virtual optomotor system. Visual cortical maps were recorded using intrinsic signal optical imaging. After MD, control animals

showed a significant enhancement of visual acuity and a significant ocular dominance (OD) shift in the optical imaging experiments. In contrast, in animals with a cortical stroke, there was neither a significant enhancement of visual acuity nor a significant OD-shift. In contrast, in the intact, contralateral hemisphere, OD-plasticity was unaffected thus showing that the vanished plasticity was not caused by global mechanisms affecting the entire brain. Since stroke is associated with inflammation, we next tested the therapeutic effect of the anti-inflammatory drug ibuprofen: Daily injections restored the enhancement of visual acuity, but not OD-plasticity in the lesioned hemisphere. When MD was induced 2 weeks after stroke, sensory improvement was also restored, but not OD-plasticity in the lesioned hemisphere. These data underline that the two forms of plasticity are brought about by totally different mechanisms. In addition, the reduced sensory learning is an acute effect sensitive to inflammation, while the reduced OD-plasticity is longer-lasting. Our results indicate that cortical plasticity cannot be conceptualized solely as a local process and that non-local influences might play a much more important role for learning phenomena than previously assumed. Supported by BMBF.

Inhibition of synapse maturation by non-conventional NMDA receptors:measurements of spine turnover rates and activity-dependent spine growth

Laura A Kehoe, Isabel Perez-Otano
Centro de Investigacion en Medicina Aplicada (CIMA), Universidad de Navarra, Laboratorio de Neurobiologia Celular, Spain

A correct formation, maturation and stabilisation of synapses, the basic structures that connect neurons with each other, is central for the development and proper functioning of neuronal networks. Most synaptic rearrangement occurs during “sensitive periods” of postnatal development, with vast numbers of synapses formed and then strengthened/stabilised or eliminated. Rearrangement activity diminishes as adult patterns of connectivity are established, at least in part because of changes in the subunit composition of NMDA-type glutamate receptors (NMDARs).
Recent results from our lab suggest a key role for the juvenile NR3A subunit in switching the fate of a synapse between stabilisation and elimination. Whereas removal of NR3A speeds up synapse maturation, continued NR3A expression inhibits synapse maturation and stabilization, and promotes elimination both in transgenic animals (Roberts et al, 2009) and in neuronal cultures in vitro. We have shown that increasing the expression of NR3A in hippocampal cultures reduces the size and density of spines, and this effect is independent of the timing of NR3A overexpression. Reliance on activity was tested by suppressing synaptic activity with the sodium channel blocker tetrodotoxin (TTX), and specific NMDAR (D-AP5) or AMPAR antagonists (DNQX) during the period of exogenous NR3A expression. We have found that decreases in spine density resulting from increased NR3A require ongoing activity.
Our current work uses time-lapse imaging of NR3A-transfected hippocampal slices to explore the mechanisms involved in these newly-discovered roles of NR3A, by asking if changes in NR3A levels modulate spine formation, elimination and structural remodelling in basal conditions, during activity blockade, and/or in response to plasticity-inducing stimuli (De Roo et al., 2008).

Synaptic localization of seizure-induced matrix metalloproteinase-9 mRNA

F.A. Konopacki (a,b,c,e), M. Rylski (b), E. Wilczek (d), R. Amborska (b), D. Detka (b), L. Kaczmarek (b), G.M. Wilczynski (a,d)
(a) Department of Neurophysiology, Nencki Institute of Experimental Biology, 02-093 Warsaw, Poland (b) Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology, 02-093 Warsaw, Poland (c) Postgraduate School of Molecular Medicine, 02-093 Warsaw, Poland (d) Medical University of Warsaw, 02-091, Warsaw, Poland (e) Present affiliation: Dept of Anatomy, University of Bristol, Bristol, UK

The phenomenon of dendritic transport and local translation of mRNA is considered to be one of the most fundamental mechanisms underlying long-term synaptic plasticity. Matrix metalloproteinase 9 (gelatinase B) (MMP-9) is a matrix metalloproteinase implicated in synaptic long-term potentiation and hippocampus-dependent memory. It was recently shown to be prominently up-regulated in the hippocampal dentate gyrus (DG) upon kainate-mediated seizures. Here, using a high resolution nonradioactive in situ hybridization at the light- and electron-microscopic levels, as well as subcellular fractionation, we provide evidence that in the rat hippocampus, MMP-9 mRNA is associated with dendrites and dendritic spines bearing asymmetric (excitatory) synapses. Moreover we observe that after kainate treatment the number of dendrites and synapses containing MMP-9 mRNA increases markedly. Our results indicate that we are observing the phenomenon of dendritic transport of seizure-induced MMP-9 mRNA.

Trafficking of extrasynaptic AMPA receptors in tonicaly firing dorsal horn neurons is involved in the maintenance of inflammatory pain

O. Kopach1, J.-S. Park2, R.S. Petralia3, P. Belan1, Y.-X. Tao2 and N. Voitenko1
1Department of General Physiology of Nervous System, Bogomoletz Institute of Physiology, Kiev, 01024, Ukraine 2Department of Anesthesiology and Critical Care Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA 3Laboratory of Neurochemistry, National Institute of Deafness and Other Communication Disorders, National Institutes of Health, Bethesda, Maryland 20892, USA

Trafficking of synaptic AMPA receptors (AMPARs) in dorsal horn (DH) neurons is necessary for the maintenance of nociceptive hypersensitivity. Here, we analyzed if trafficking of extrasynaptic AMPARs in rat substantia gelatinosa (SG) neurons is also changed during inflammation and may contribute to the maintenance of inflammatory pain. Immunochemistry and patch-clamp recordings combined with Ca2+ imaging showed an increased Ca2+ permeability of the plasma membrane associated with an enhancement of transmembrane AMPA-induced current in SG neurons at 24 hour after Complete Freund's Adjuvant (CFA)-induced inflammation. These changes were accompanied by an increased sensitivity to selective inhibition of the Ca2+-permeable AMPARs and an inward rectification of AMPA-induced currents. We have concluded that CFA-induced inflammation markedly increased surface expression of extrasynaptic Ca2+-permeable AMPARs and their proportion in a total pool of functioning extrasynaptic AMPARs. The changes in AMPARs trafficking was observed only in SG neurons characterized by an intrinsic tonic firing properties, but not in ones exhibiting a strong adaptation. These results suggest that an altered trafficking of extrasynaptic AMPARs in tonically firing SG neurons might contribute to the maintenance of persistent pain.

Acknowledgements: NIH Grants (NS058886, NS057343), the JHU Blaustein Pain Research Fund (Y.X.T), INTAS 8061 (N.V.) and the Intramural Research Program of NIDCD (R.S.P.)

Spatiotemporal activation of the rat sensorimotor cortex by stimulation in the paws. A study with voltage-sensitive dye imaging in vivo.

M.L. Morales-Botello, J. Aguilar, G. Foffani
Neurosignals Group, Hospital Nacional de Parapléjicos, SESCAM, Toledo 45071, Spain

The present work studies the spatiotemporal activation patterns of the sensorimotor cortex in response to somatosensory stimuli in the paws in anesthetized rats. The cortical responses were recorded in one hemisphere using voltage-sensitive dye (VSD) imaging with high temporal (0.5ms) and spatial (50µm x 50µm) resolution. Somatosensory stimuli were separately delivered to the four paws by applying electrical pulses. We obtained the following main results. (1) The distance between the cortical points of initial activation (foci) in response to forepaw and hindpaw stimuli was approximately 2mm. (2) The response amplitude and the maximal cortical region activated was significantly bigger in response to forepaw stimuli compared to hindpaw stimuli. (3) The activation evoked by stimulation of the contralateral paws started within the somatosensory cortex and then propagated to the motor cortex. (4) The activation evoked by stimulation of the ipsilateral paws consisted of two separate foci that did not spatially match the activation evoked by stimulation of the contralateral paws. These results suggest that cortical activation measured with voltage-sensitive dye imaging in response to stimulation of the paws is consistent (1) with the known somatotopic map of the rat primary somatosensory cortex and (2) with the greater innervation density and larger cortical representation of the forepaw compared to the hindpaw. In addition, our results suggest (3) that the motor cortex is also involved in the processing of somatosensory stimuli, and (4) that the sensorimotor cortex possesses two different coexisting maps of the contralateral and ispilateral body.

Dendritic spine plasticity can stabilise synaptic weights

Cian O'Donnell(1), Matthew F Nolan(2) and Mark CW van Rossum(1)
Institute for Adaptive and Neural Computation (1), Centre for Integrative Phsiology (2), University of Edinburgh, UK.

Stabilisation of synaptic weights is important for long-term memory. Most existing attempts to explain synaptic weight stability assume elaborate molecular signalling mechanisms. We propose a simpler alternative model. Here, changes in dendritic spine size following plasticity stabilises synaptic strength simply by modifying local calcium dynamics.

Recent experimental studies demonstrate that the size of individual dendritic spines is increased or decreased following induction of synaptic potentiation or depression respectively (Matsuzaki et al, 2004; Harvey et al, 2008). However, the consequences of altered spine size for signaling events within the spine are not clear. Using a biophysical computer model of a dendritic spine and a common calcium-dependent plasticity rule, we find that different NMDAR conductance to spine-size relationships can result in stable, unstable or even bistable synaptic weight dynamics. When we use parameter estimates from the experimental literature, the model predicts that real spines fall into

the 'stable' category. Our model is sufficient to explain the experimental observations that weak synapses are most susceptible to plasticity protocols and that large spines are the most persistent.

Truncated Disrupted in Schizophrenia 1 impairs cortical perisomatic inhibition
Jonas-Frederic Sauer and Marlene Bartos
Institute of Medical Sciences, University of Aberdeen, Aberdeen AB25 2ZD, United Kingdom

Synaptic inhibition mediated by parvalbumin-expressing fast-spiking interneurons (FS-INs) is a key regulator of cortical rhythmic activity patterns which are assumed to underlie various cortical functions such as working memory. In schizophrenia, working memory is severely and consistently impaired, suggesting that deficits in GABAergic inhibition may contribute to defects in neuronal synchronization and thereby in the pathogenesis of this disease. Although post mortem studies imply that impaired inhibition by FS-INs may contribute to cortical deficits in schizophrenia, direct functional evidence is scarce. Here we use a schizophrenia mouse model that expresses truncated Disrupted in Schizophrenia 1 (Disc1tr; Shen et al., J. Neurosci 28:10893:10904, 2008), closely resembling a genetic alteration found in a Scottish family with extremely elevated prevalence of schizophrenia. By using paired whole-cell patch-clamp recordings from FS-INs and their postsynaptic target cells in slice preparations of the prefrontal cortex of Disc1tr mice, we find that strength and reliability of FS-IN-dependent perisomatic inhibition is drastically reduced. In contrast, kinetic and dynamic properties of perisomatic GABA release as well as intrinsic membrane properties of FS-INs are unchanged. Intracellular labeling of the recorded FS-INs in combination with confocal microscopy reveal no changes in their morphological characteristics and number of output synapses. Our current investigations indicate that presynaptic properties of FS-IN output synapses may be altered resulting in a reduced reliability of transmitter release. We believe that our study will provide new insights in the cellular mechanisms underlying cortical deficits in schizophrenia.

Information processing by lateral hypothalamic orexin neurons
Cornelia Schoene, Denis Burdakov
University of Cambridge, Cambridge, UK

Orexin neurons are of crucial importance for the regulation of both arousal and appetite, and recent studies also suggested an involvement of the orexin system in drug addiction and reward. As malfunction of orexin circuits is linked to several human diseases, understanding them is crucial to design future therapies. It was recently shown that orexin neurons can be divided into two populations, depending on their electrophysiological characteristics. These “H-type” (hyperpolarising) and “D-type” (depolarising) neurons also have overlapping but distinct distributions within the lateral hypothalamus and respond differently to changes in extracellular glucose levels. They therefore might play different information processing roles in these neural circuits. However, whether “H-” and “D-type” orexin neurons are truly distinct neuronal subtypes, or if they merely represent different physiological states of the same subtype remains to be investigated.
Here we report data indicating that the strength of excitatory and inhibitory synaptic transmission is different in these two orexin neuron populations. Miniature postsynaptic currents in GFP-labelled

orexin neurons were investigated by whole-cell patch-clamp recordings in acute mouse brain slice preparations. Preliminary experiments indicate that “H-type” neurons have stronger excitatory synaptic inputs compared to “D-type” neurons. Conversely, “D-type” neurons have stronger inhibitory inputs.
In order to further elucidate this “D-“ vs. “H-type” dichotomy we are starting to investigate the computational properties of these neurons. Since these depend strongly on the architecture of dendritic trees, we will perform three dimensional reconstructions from dye filled neurons. Future experiments will also address network connectivity, using retrograde tracers, and possible differences in ion channel expression and distribution between “H-” and “D-type” orexin neurons. Furthermore, whether “D-“ and “H-type” orexin neurons can be converted into each other will be investigated by inducing various plasticity mechanisms.
We hope that this line of investigation may provide the much-needed cellular correlates of the emerging functional dichotomy (sleep vs. appetite) of the orexin system, particularly in relation to a novel theory suggesting that both behaviours are regulated by different sets of orexin cells.

Direction selective responses in the dendrites of visual interneurons of the fly
Christian Spalthoff and Rafael Kurtz
Department of Neurobiology, Bielefeld University, Postbox 100131, 33501 Bielefeld / Germany

Various tasks of visual systems, including course control, collision avoidance and detection of small objects, require at the neuronal level the dendritic integration and subsequent processing of many spatially distributed visual motion inputs. While relatively much is known about the pooled output in these systems, the motion tuning of the elements that provide the input has yet received little attention.
To visualize the motion tuning of these inputs we examined the dendritic activation patterns of neurons that are selective for characteristic patterns of wide-field motion, the lobula-plate tangential cells (LPTCs) of the blowfly. These neurons are known to sample direction-selective motion information from large parts of the visual field and combine these signals into axonal and dendro-dendritic outputs, with different cells each tuned to a distinct motion pattern.

Fluorescence imaging of intracellular calcium concentration allowed us to take a direct look at local dendritic activity and the resulting local preferred directions in LPTC dendrites during activation by wide-field motion in different directions. These “calcium response fields” mostly resemble a retinotopic dendritic map of local preferred directions in the receptive field, the layout of which is a distinguishing feature of different LPTCs.

Local Faculty

Paola Bezzi

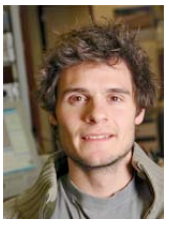
Department of Cellular and Molecular Biology (DBCM), University of Lausanne.

Marie-Christine Broillet

Department of Pharmacology and Toxicology (DPT), University of Lausanne.

Matthew Brown

Department of Basic Neuroscience, CMU, University of Geneva.



Alan Carleton

Department of Basic Neuroscience, CMU, University of Geneva.

Jean-Yves Chatton

Department of Cellular and Molecular Biology (DBCM), University of Lausanne.

Sean Hill

Blue Brain Project, EPFL, Lausanne

Anthony Holtmaat

Department of Basic Neuroscience, CMU, University of Geneva.

Jean-Pierre Hornung

Department of Cellular and Molecular Biology (DBCM), University of Lausanne.

Jozsef Kiss

Department of Basic Neuroscience, CMU, University of Geneva.

Graham Knott

Interdisciplinary Centre for Electron Microscopy, EPFL, Lausanne

Cécile Lebrand

Department of Cellular and Molecular Biology (DBCM), University of Lausanne.

Anita Lüthi

Department of Cellular and Molecular Biology (DBCM), University of Lausanne.

Pierre Magistretti

Brain Mind Institute, EPFL, Lausanne

Pierre Marquet

Brain Mind Institute, EPFL, Lausanne

Dominique Muller

Department of Basic Neuroscience, CMU, University of Geneva.

Carl Petersen

Brain Mind Institute, EPFL, Lausanne

Ralf Schneggenburger

Brain Mind Institute, EPFL, Lausanne

Arne Seitz

Bioimaging and Optics platform, EPFL, Lausanne

Andrea Volterra

Department of Cellular and Molecular Biology (DBCM), University of Lausanne.

Egbert Welker

Department of Cellular and Molecular Biology (DBCM), University of Lausanne

Invited Speakers

Ingrid Bureau
Maturation and plasticity of cortical maps
Institut de neurobiologie de la méditerranée (INMED), Marseille, France

Rosa Cossart
Maturation of functional GABAergic microcircuits in the developing cortex
Institut de neurobiologie de la méditerranée (INMED), Marseille, France

Hans-Ulrich Dodt
New imaging methods for brain research
Center for Brain Research, Medical University of Vienna, Austria

Olga Garaschuk
In vivo function of neuronal networks
Institute of Physiology, Tübingen University, Germany

Olivier Griesbeck
Cellular dynamics: Probing the brain with fluorescent proteins
Max Planck Institute of Neurobiology, Martinsried, Germany

F.Helmchen
In vivo 2-photon imaging of neural activity; 3D imaging of neural network activity; Imaging of glial cells
Brain Research Institute, University of Zurich, Switzerland

Mark Hübener
Visual System Development
Max Planck Institute of Neurobiology, Martinsried, Germany

Jurgen Klingauf
Microscopy of Synaptic Transmission
Göttingen Graduate School for Neurosciences and Molecular Biosciences (GGNB), Göttingen, Germany

Isabel Llano
Calcium Imaging of Central Neurones
Laboratory of Brain Physiology CNRS UMR8118, Paris, France

Valentin Nägerl
Synaptic transmission and excitability; Synaptic plasticity
Bordeaux Neurosciences Institute, Bordeaux, France

Thomas Oertner
Physiology and plasticity of individual synapses
Friedrich Miescher Institute (FMI), Basel, Switzerland

Kenneth Spring
Fluorescence and confocal laser scanning microscopy

Ryohei Yasuda
Molecular Mechanisms of Synaptic Plasticity
Dept. of Neurobiology, Duke University Medical Center, Durham, USA

Students

Pepe Alcami
CNRS and Paris Descartes University, Dept. of Neuroscience, Paris, France

Anna Letizia Allegra Mascaro
University of Florence, LENS (European Laboratory for Non-linear Spectroscopy), Sesto Fiorentino , Italy

Werend Boesmans
KULeuven, Center for Gastroenterological Research, Leuven, Belgium

Volodymyr Cherkas
Bogomoletz Institute of Physiology, General Physiology of the Nervous System, Kyiv, Ukraine

Balázs Chiovini
Institute of Experimental Medicine of the Hungarian Academy of Sciences, Dept. of Pharmacology, Budapest, Hungary

Mariano Di Guilmi
IFIBYNE, UBA-CONICET, Buenos Aires, Argentina

Franziska Greifzu
FSU Jena, General Zoology and Animal Physiology, Jena, Germany

Laura Ann Kehoe
Centro de Investigacion medica aplicada, Universidad de Navarra, Cellular Neurobiology, Neurosciences, Pamplona, Spain

Filip Konopacki
University of Bristol, Dept. of Anatomy, Bristol, UK

Olga Kopach
Bogomoletz Institute of Physiology, General Physiology of Nervous System, Kiev, Ukraine

Maria Luz Morales Botello
Hospital Nacional de Parapléjicos, Unidad de Investigación, Toledo, Spain

Cian O'Donnell
University of Edinburgh, Dept. of Informatics, Edinburgh, UK

Jonas-Frederic Sauer
University of Aberdeen, Institute of Medical Sciences, Department of Neuroscience, Aberdeen, UK

Cornelia Schoene
University of Cambridge, Dept. of Pharmacology, Cambridge, UK

Christian Spalthoff
Bielefeld University, Dept. of Neurobiology, Bielefeld, Germany

The Lemanic Neuroscience Academic Environment

Ecole Polytechnique Fédéral de Lausanne (EPFL)

BMI – Brain Mind Institute

University of Lausanne (UNIL)

DBCM – Département de Biologie Cellulaire et de Morphologie
DP – Département de Physiologie
CIF – Cellular Imaging Facility

University of Geneva (UNIGE)

GNC – Geneva Neuroscience Center

CHUV – Centre Hospitalier Universitaire Vaudois

CNP – Centre de Neurosciences Psychiatriques (Cery)
Division de Neuropsychologie
Service de Neurologie
Service de Neurochirurgie
Service de Radiodiagnostic

HUG – Hôpitaux Universitaires de Genève

Département de Neurosciences Cliniques
Département de Psychiatrie