Roscoff (France), 30 juin - 4 juillet 2010

Imagerie des circuits neuronaux en conditions physiologiques et pathologiques

Imaging brain circuits in health and disease

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Rapport sur la Conférence

Conference Report

RESUME DU RAPPORT

Conférence Jacques Monod intitulée : Imagerie des circuits neuronaux en conditions physiologiques et pathologiques

Roscoff, 30 juin – 4 juillet 2010

Les techniques d'imagerie de l'activité cérébrale avec une résolution cellulaire permettent d'obtenir des informations cruciales sur l'anatomie, le fonctionnement et les pathologies du cerveau. Depuis les travaux pionniers de Golgi et Ramón y Cajal il y a plus d'un siècle, de nombreuses méthodes ont été développées pour identifier et visualiser les cellules nerveuses. Le but de la conférence "Imagerie des circuits neuronaux en conditions physiologiques et pathologiques" a été de montrer comment ces nouvelles avancées technologiques permettent aux neurobiologistes d'étudier le fonctionnement du cerveau à l'échelle de réseaux de neurones, de neurones individuels et même de synapses dans des conditions physiologiques et pathologiques.

La conférence "Imagerie des circuits neuronaux en conditions physiologiques et pathologiques" a eu lieu à Roscoff du 30 juin au 4 juillet 2010. Elle a réuni 95 chercheurs dont 29 conférenciers invités et 66 participants (26 doctorants, 21 post-doctorants et 19 professeurs ou chercheurs avec un poste fixe), provenant de 12 pays différents (Europe, Israel, Amérique du Nord et Japon).

La conférence était structurée en six sessions comprenant les présentations des conférenciers invités (~20-25 min et 5-10 min de discussion) ainsi que huit présentations de participants sélectionnés sur la base de la qualité de leur recherche (10-15 min). De plus, quatre sessions de posters ont eu lieu. La première moitié de la conférence était dédiée aux études *in vitro* tandis que la seconde partie était consacrée aux approches *in vivo*. La qualité des présentations a été remarquable, certains travaux non publiés étant présentés pour la première fois. Les sessions de posters ont connu un grand succès et ont permis aux participants de discuter leurs travaux de recherche avec des chercheurs reconnus dans leur domaine d'expertise.

Les organisateurs ont le sentiment que cette conférence a rencontré un succès indéniable. De nombreux participants nous ont fait part de leur satisfaction et ont particulièrement apprécié le format de cette conférence. Le thème de cette conférence a permis de réunir des scientifiques de différentes disciplines à la pointe des recherches actuelles en neurosciences. Les organisateurs espèrent vivement que cette série de conférence se poursuivra dans l'avenir.

CONFERENCE REPORT

Final report from the Jacques-Monod Conference entitled: Imaging brain circuits in health and disease

Roscoff, June 30 – July 4, 2010

1 – General description of the meeting

The conference "Imaging brain circuits in health and disease" took place in Roscoff from June 30 to July 4, 2010. In total, 95 scientists participated. 29 were invited speakers and 66 were selected applicants. Among the 66 selected participants, 19 were professors or researchers with tenure positions, 21 were post-docs, and 26 were PhD students. Thus, all levels of scientific careers were represented at the conference.

The participants came from 12 different countries, as indicated:

Country	Invited speakers	Postdoc/Researchers applications	PhD students applications
France	12	21	11
Germany	6	3	9
USA	4	3	2
UK	3	3	0
Switzerland	2	2	3
Japan	1	1	0
Canada	1	1	0
Israel	0	2	0
Austria	0	2	0
Belgium	0	1	0
China	0	1	0
Hungary	0	0	1
Total	**29**	**40**	**26**

A total of 28 invited speakers attended the conference. The invited speakers, mostly high profile representatives of their respective fields, were selected to cover recent advances in imaging techniques from single molecule to *in vivo* signaling in physiological and pathological conditions. The main criterion for the selection was scientific excellence. The female representation was 14 % among the speakers and 25 % among the other participants.

The meeting was composed of six sessions with scientific talks (~20-25 minutes duration each and 5-10 min discussion), eight additional short talks (~10 minutes duration each and 5 min discussion), and four poster sessions. The talks of the invited speakers were of extremely high quality. In most cases, the scientific material presented was unpublished, and in many cases, the data had not been shown at prior scientific conferences. The first half of the meeting (first three sessions and poster session I) was dedicated to *in vitro* studies while the second half (last three sessions and poster session II) focused on *in vivo* approaches in

anesthetized and awake animals. The chairpersons were selected to enhance scientific interactions between different approaches. Based on the quality and the topic of the submitted abstracts, 8 were selected for short oral presentations. The sessions of short talks (10 minutes duration) mainly focused on new technical developments in brain imaging and gave the opportunity to young researchers to present their work. The poster sessions were also highly successful. We decided to dedicate more time to poster sessions compared to previous conferences, in order to give more opportunities for interactions between the younger participants and the invited speakers. Four poster sessions were scheduled with half of the posters presented during the first two sessions and the second half during the third and four sessions. Posters were of consistently high quality. The poster sessions were strongly visited and intense scientific discussions took place during and after these sessions.

The practical organization of the meeting, mainly performed by Dominique Lidoreau, was perfect, as it was the case for the prior meetings. The lecture hall near the coast provided an excellent environment for scientific discussions. The technical support was good. The hotels were appreciated by all participants as well as the excellent quality of the lunches and dinners prepared at the restaurant of the Gulf Stream hotel.

We are very grateful to the CNRS that provided most of the funding for this conference. Additional support came from the Institute for Advanced Studies (Technical University Munich, Germany) and allowed to pay the travel costs of 12 speakers (from Germany and non-european countries).

On Sunday morning, at the end of the general discussion, Daniel Choquet, Bordeaux, France, was confirmed as the president of the next meeting. Furthermore, Angus Silver, London, UK, was elected as the new vice-president.

In summary, the organizers feel that the meeting was a great success. We received very positive feedback from many participants, who strongly appreciated the format of the meeting. Many participants confirmed that it was one of the best meetings they attended.

2 - Report on scientific aspects: summaries of the lectures and discussions

The conference focused on imaging approaches to study brain circuits, from single molecules to *in vivo* neuronal networks, in both physiological and pathological conditions. New imaging methods were presented as well as their applications to investigate neuronal activity in different brain areas: sensory areas (somatosensory, visual, olfactory and auditory cortex), the cerebellum and the hippocampus. In addition, several talks described recent imaging studies on animal models of brain diseases, such as Alzheimer's disease, epilepsy and stroke. The conference was highly interdisciplinary, with neurobiologists, chemists, physicists and physicians joining their expertise to investigate the role of single neurons in the function and dysfunction of living neuronal networks. The first half of the meeting (first three sessions and poster session I) was dedicated to *in vitro* studies while the second half (last three sessions and poster session II) focused on *in vivo* approaches in anesthetized and awake animals.

The first session was devoted to novel methods investigating the 3D-morphology of brain circuits. Winfried Denk (MPI Heidelberg) presented his current research using serial block-face electron microscopy, a technique that involves the acquisition of tomographic images of brain samples using electron-microscopy. This technique helps to reveal the 3D-structure of neurons with nm-resolution. Next, Jean Livet (Institut de la vision, Paris) presented the 'brainbow-mouse' approach. This technique enables the labeling of a large

number of neurons with different fluorescent colors, and is thus a promising approach for the study of the connectivity in neuronal networks. The application of this method in the medial nucleus of the trapezoid body was described in this presentation. Then, Stéphane Dieudonné (ENS Paris) presented a cutting edge imaging technique using random-access two-photon microscopy, allowing sampling rates of several kHz. This technique can reveal synaptic inputs at a high temporal precision. Finally, Masanobu Kano (Tokyo University) presented *in vivo* investigations using both calcium imaging and electrophysiological recordings to reveal the interactions of climbing and parallel fiber inputs into the Purkinje cells during the development of the cerebellum.

During the second session, Angus Silver (London, United Kingdom) presented a two-photon acousto-optic lens microscope for imaging neural activity in three dimensions at kHz resolution. After that, Isabel Llano (Paris, France) talked about new findings on the function of cerebellar interneurons. Interestingly, her group characterized the firing pattern and subcellular channel distribution in these cell types. The third speaker was Alain Marty (Paris, France), who introduced the details and properties of presynaptic miniature GABAergic currents in developing interneuron. After that, David Digregorio (Paris, France) reported the optical recording of membrane voltage in subcompartments of neurons in brain slices using a two component voltage sensor. Finally, Martin Oheim (Paris, France) presented his work on lysosomes, which are one of the major secretory organelles in astrocytes. The results helped identify the lysosomes as major vesicular compartment undergoing Ca-regulated exocytosis from cortical astrocytes.

The third session was devoted to synaptic mechanisms and neuronal network function. Antoine Triller (Paris, France) presented his results on single-particle tracking for the study of the dynamic regulation of GABA receptors during plastic modifications of inhibitory synaptic transmission. In particular, he discussed the role of neuronal activity and integrins in the regulation of synaptic inhibition. Valentin Nägerl (Bordeaux, France) focused on synaptic plasticity and superresolution microscopy. He presented in detail his work on nanoscale imaging of living synapses using STED microscopy. Dirk Trauner (München, Germany) informed the audience about his work dealing with the development of new photochemical tools for controlling neuronal activity. In particular, he described the properties of hybrid photoreceptors and optical control of biological function, i.e. vision. Christoph Mulle (Bordeaux, France) talked about NMDA-receptor-dependent forms of synaptic plasticity. He presented interesting new evidence for NMDA-dependent LTP at hippocampal mossy fiber. Finally, Rosa Cossart (Marseille, France) presented data on the functional organization of developing hippocampal circuits. An important focus of here presentation was the discussion of the role of GABAergic hub neurons in organizing neuronal early network oscillations in the developing hippocampus.

The fourth session showcased several novel techniques for monitoring neuronal and dendritic activity in vivo. Arthur Konnerth (Munich, Germany) presented his lab's method for imaging calcium activity of individual putative synapses through a combination of whole-cell patch and two-photon microscopy. Matthew Larkum (Bern, Switzerland) discussed his lab's "periscope" method for examining dendritic activity of layer 5 neurons in both the anesthetized and awake mouse. David Tank (Princeton, USA) presented a system for recording whole-cell and fluorescent signals from awake-behaving animals. His lab built a virtual reality environment for mice where animals can explore an environment while head-fixed on a floating ball. Michael Brecht (Berlin, Germany) discussed a method for labeling cells that are recorded in awake and unrestrained mice. His lab used this approach to identify what cell types comprise grid-cells in medial entorhinal cortex. Tiago Branco (London, UK) spoke for his advisor Michael Häusser about their approach to recording dendritic activity by

using two-photon microscopy to uncage neurotransmitter at different positions within a neuron's dendritic tree. Finally, Serge Charpak (Paris, France) talked about a technique that uses a fluorescent oxygen sensor in order to measure oxygen dynamics in the olfactory bulb. Such data is important for relating neural activity to oxygen consumption and blood flow.

The fifth session focused on the physiology and patho-physiology of different sensory systems. In the first talk, Marla Feller (Berkeley, USA) described the origin of retinal waves which can be observed prior to the maturation of the retina. Then, David Fitzpatrick (Duke University, USA) presented his work on the development of direction-selectivity in the visual cortex of ferrets, including experiments using a bidirectional training paradigm to strengthen neuronal direction-selective responses. Mark Hübener (Munich, Germany) in the next talk discussed results obtained after stripe rearing of juvenile mice: after this training neurons were responding more the experienced orientation, providing evidence for visual cortical plasticity on the functional level. Nathalie Rochefort (Munich, Germany) in her presentation revealed new insights on the development of orientation and direction-selectivity in mice, stressing the fact that in contrast to the ferret orientation and direction-selectivity are present already at eye opening in the mouse. In the second part, she discussed results showing an impairment of sensory-evoked responses in a mouse model of Alzheimer's disease. Fritjof Helmchen (Zurich, Switzerland) showed experiments using the viral expression of a genetically-encoded calcium indicator in the barrel cortex of mice. Finally, Tim Murphy described different experimental models for stroke in the mouse focusing on the difference between acute and chronic changes after reduction of blood flow.

Finally, for the last session, Karl Deisseroth (Stanford, USA) presented his groundbreaking work on the development of a versatile and very efficient optogenetic toolbox. He gave an overview about the microbial opsins which are available for optical control of neuronal and non-neuronal cells. He showed that subcellular trafficking strategies allow for optical regulation in the far-red, green and blue spectrum, and that opsins, which do not generate sufficient photocurrents to enable neuronal excitation in their native form, are now specifically transported to the cellular membrane. By this local aggregation, multiple microbial opsins can now be employed for optogenetics, achieving multiple independent channels of control. Additionally, he presented data on biophysical modifications of already well known opsins (vChR2) to increase its temporal accuracy, enabling reliable optogenetic control of fast spiking cells. As a recent application of optogenetics, he presented a study combining fMRI with optogenetics. By optically stimulating CamK2 positive neurons in the motor cortex of the rat, a direct correlation between fMRI (BOLD) signal and neuronal activity was demonstrated. Next, Daniel Choquet (Bordeaux, France) introduced the concept of single molecule high resolution imaging to monitor AMPA glutamate receptor trafficking. Single molecule imaging techniques allow for the direct and unperturbed monitoring of single molecule dynamics. He presented data on the imaging of the fast diffusion of AMPAR in live neurons. Repetitive synaptic stimulation is known to reduce synaptic transmission. He demonstrated that fast lateral diffusion of AMPAR can replenish desensitized receptors with functional AMPAR in tens of millisecond time range. Also, he shed light on the role of CaMKII in trapping of AMPAR. Finally, Gero Miesenboeck shared data on an alternative approach to stimulate neurons with light in Drosophila. Co-expression of the Drosophila photoreceptor genes encoding arrestin-2, rhodopsin and the alpha subunit of the cognate heterotrimeric G protein sensitizes vertebrate neurons to light. By optically controlling genetically circumscribed subsets of dopaminergic neurons in the behaving fly, he mapped the origin of aversive reinforcement signals. Also, he presented a model on the role and benefits of physiological noise in the sensory systems of Drosophila, which enables weak external stimuli to rise above the detection threshold.

In addition to the invited speakers, eight additional speakers were selected from the submitted abstracts to give short talks of 10 minutes each. These talks were mostly focused on the development and/or application of novel imaging technologies and methods. David Ogden (Paris, France) presented a method of precise photolysis of caged Ca^{2+} for studying the vesicular release parameters of GABA at synapses of molecular layer interneurons of the cerebellar cortex. Jean-Francois Nicoud (Strasbourg, France) introduced a new family of chemically synthesized fluorophores and caging platforms that was optimized for two-photon excitation and potentially useful for *in vivo* imaging studies. Kazuo Kitamura (Tokyo, Japan) demonstrated example recordings of visually guided patch-clamp ("shadow patching"), which can be readily combined with calcium imaging. Benjamin Grewe (Zurich, Switzerland) presented a recently constructed high-speed two-photon microscope based on patterned point sampling achieved by Acousto-Optical-Deflectors. The system was proven to be able to record from up to 50 neurons of layer 2/3 at about 300 Hz sampling rate. Simon Rumpel (Vienna, Austria) demonstrated the use of photo-activable fluorescent proteins to label specific neurons during *in vivo* recordings in order to identify them post-hoc for *in vitro* labeling and recordings. German Sumbre (Paris, France) presented data recorded from the zebrafish visual system and showed that the rhythmic activity of neuronal ensemble preserve information about repetitive stimuli for a long duration up to 20 s. José Tiago Goncalves (Los Angeles, USA) presented a new technique of spatial temporal multiplexing for multifocal two-photon calcium imaging. Example data showed that this technique enables simultaneous recording from 4 focal planes at different depths, which had not been possible using standard single-beam excitation-emission detection techniques. Valentina Emiliani (Paris, France) introduced a method of holographic photolysis for stimulating multiple cells in mouse hippocampus slices.

In summary, the conference successfully brought together scientists working on the function of the brain at different levels. Photo-chemists, molecular biologists, physicists, physicians and neuroscientists presented new tools to study brain circuits at the level of single synapses, neurons and neuronal networks. The technological aspect of the conference as well as its partial focus on disease attracted researchers and students from most laboratories that use imaging in France and abroad. Thus, the meeting made an important contribution to current attempts to merge bottom-up and top-down approaches, leading to a more unified understanding of higher brain functions. We found that this interdisciplinary approach was extremely stimulating, and hope that this outstanding series of conferences will continue.

3 - Concluding remarks and recommendations for future meetings

We feel that the conference was a big success at all levels. The main purpose of the meeting, to foster interactions between scientists working at the molecular, the cellular, and the network level, was achieved. The participants came from 12 different countries, showing the international interest for this meeting. Intense scientific discussions took place and future collaborations were discussed during the conference. In addition, the PhD students had many opportunities to interact with renowned scientists in their field of research.

As emphasized by many speakers, the format of the Roscoff conferences is an excellent one. No major change is needed and the support of Dominique Lidoreau was, as for the previous conferences, excellent. For possible future conferences, the organizers would like to make the following comments:

- This field of research covered in this meeting, namely the high-resolution analysis of neuronal circuits in the living brain, is just emerging and it is expected to provide many more exciting results in the coming years. The quality of the invited speakers as well as the international attendance testify the high interest for this field. The new available techniques now allow to link *in vivo* recordings and behavioral tasks. This is a topic of highest interest for a future conference.

- Short talks were well appreciated and gave the opportunity to young researchers to present their work. We selected eight short talks and think that this is an appropriate number.

- In addition to the short talks, we dedicated more time to poster sessions compared to previous conferences, in order to give more opportunities for interactions between the participants and the invited speakers. Based on the extremely positive resonance, it would seem appropriate to keep or even extend the time dedicated to poster sessions.

September 18, 2010

President	Vice-President
Arthur Konnerth	Daniel Choquet