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Optical imaging of brain connectivity, from synapses to networks in action

Imagerie optique de la connectivité neuronale, des synapses aux réseaux en action

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Rapport sur la Conférence
Conference Report

General topic of the meeting

Discovering how neural circuits process information requires measurement of neural activity on many different scales, ranging from single synapses to large assemblies of neurons, best in the brain of behaving animals. The study of brain function at the microscopic and mesoscopic scale has been revolutionized by novel approaches combining the development of molecular tools and gene transfer methods with newly designed instruments that use light to visualize and manipulate the activity of synapses, neural cells and neural ensembles.

This international symposium has brought together world experts to present their latest discoveries and technological developments regarding brain connectivity, focusing on studies of synaptic, neuronal and network structure and function using microscopic imaging, connectomics, and optogenetics. This event has fostered scientific exchanges between neurobiologists specialized in synaptic mechanisms, synaptic integration, structure and function of neuronal connectivity, information processing in networks, and behavioural analysis, as well as developers of new optical methods, new probes, connectomics approaches and new ways to manipulate neurons and brain circuits with opto- or pharmacogenetic tools. This multidisciplinary interaction helps advance our understanding of brain connectivity and facilitate new approaches for investigating brain structure and function, both *in vitro* and *in vivo*, from its individual molecular components to complex networks in the working brain.

Indeed, advances in understanding connectivity critically depend on our capacity to improve the tools available for studying brain function. Improved technologies in the field of neuroscience are particularly important to tackle the great challenges in mapping the connections and interactions within and between highly complex neuronal networks. These multidisciplinary challenges and many others were addressed at this meeting which was a great opportunity to foster new collaborations to undertake novel challenges that will push further our ability to detect, measure, manipulate and follow the intricate components of neuronal and network function. This is key to understanding brain function in health and disease and thus to increase our ability to design novel treatments for neurological and psychiatric disorders.

Meeting overview

This international symposium brought together world experts to present their latest discoveries and technological developments on connectomics, imaging and optogenetic methods for studying synaptic, neuronal and network structure and function. This enabled scientific exchange between neurobiologists specialized in synaptic mechanisms, molecular biology, synaptic integration, network structure and information processing in networks with those actively developing new optical methods and optogenetic tools.

The meeting consisted of 104 participants, of which 27 were invited and 77 were applicants. The applicants were made up of 23 PhD, 23 post-docs, 3 research Engineers, 28 staff scientists (18 PIs).

Invited speakers were rather balanced in gender (12 women/15 men) and were from wide geographic origin (9 French, 10 from Europe, 6 from the US, 1 from Japan, 1 from South Corea). A majority of the invited speakers were not speakers at the previous CJM conference in 2014.

Sessions

The meeting has started with two evening lectures from major players in the field, at two ends of the spectrum of brain organization, from the nanoscale organization of synapses (Daniel Choquet, Bordeaux) to the deficient neural networks in mouse models of Alzheimer's disease (Arthur Konnerth, Munich).

The conference was organized in four sessions, which covered the range of scale analysis of the brain from synapses to networks in behaving conditions, including a session on the novel developments of probes, viral tools and optical instruments.

In a the first session "**Novel instruments and methods**" speakers have reviewed technological progresses which comprises novel molecular tools as well as new types of microscopes and optical approaches, which are particularly aimed at expanding methods to study mesoscale neural dynamics. Scott Sternson provided the development of pharmacologically selective chimeric ion channels for flexibly manipulating a diverse range of ion conductances in defined cell types linked to food intake. Ehud Isacoff presented new appraoches for the analysis of synaptic transmission through light-activated ionotropic receptor channels. Haruhiko Bito discussed about new Calcium probes for live imaging of ensemble of neurons. These advancements provide the basis for a growing repertoire of novel tools to address the role of active neuronal networks in cognitive behaviors.

Angus Silver and Katrin Willig described novel microscopes based on sophisticated laser scanning schemes to improve the speed and 3D applicability of functional network imaging in vivo. Valentina Emiliani presented techniques for simultaneous imaging and optogenetic control of neuronal populations *in vivo* with single-cell precision. At a microscale level, Dmitri Rusakov proposed approaches using time-resolved fluorescence imaging to monitor nanomolar ion concentrations and quasi-instantaneous molecular diffusion in live glial cells and neurons in situ. Two short talks were selected from the applications from Zsolt Lenkei (Paris) who talked about methods combining ultrasound focussing and optical imaging for the study of brain function, and from Adam Packer (Mike Haüsser's lab, London), who presented Technologies for all-optical interrogation of neural circuits in behaving animals.

A second session dealt with "**Network dynamics and behaviour"** (moderated by Claire Wyart). Major advances have been made in connecting the cellular level to the systems level by applying optical methods in awake, behaving animals, a topic, which has been covered in this session. Experts in in vivo two-photon calcium imaging have presented their latest results on neuronal population dynamics as measured in various brain regions during specific behaviors. For example, genetically-encoded calcium indicators expressed in the neocortex of head-fixed mice that are presented with various sensory stimuli while moving or acting in a virtual environment, are applied to study behavior-related neural circuit dynamics in visual cortex (Sonja Hofer), somatosensory cortex (Fritjof Helmchen, Ingrid Bureau), or hippocampus (Rosa Cossart). Two-photon targeted patch clamp recordings enable to record *in vivo* from identified populations of cortical neurons to evaluate their role in synaptic plasticity and in sensory information processing (Troy Margrie, Anthony Holmaat). Besides the mouse, the transparent zebrafish larva is a particularly suitable organism for analyzing network dynamics during behaviour. The roles of specific neuronal populations in zebrafish larvae forebrain, midbrain, and spinal cord were discussed (Claire Wyart). Coming from the field to neurdevelopment, Laure Bally-Cuif has used these techniques to image the dynamics of neural

progenitor cells over time zebrafish. All presentations have made our understanding progress towards the analysis of the high-dimensional multivariate time series of network dynamics - representing the interplay of internal dynamic representations and externally driven activity - and its correlation with specific behavioral aspects, such as body movements or decisions made by the animal. In this session, two short talks were selected from the abstracts (Ingrid Bureau and Guillaume Sandoz).

The use and development of novel tools and instruments also was a common theme for the third session on **"Imaging synapses at nano/micro scale".** Synapses are central to neuronal connectivity in the brain and understanding the mechanisms underlying their precise structure and function and the mechanisms by which they are subject to plasticity remains a major focus of neuroscience research. In this session we have brought together world experts on synaptic signalling and receptor function. Latest methods and results for imaging the location of key proteins within the synapses and for monitoring the function and dynamic organization of receptors and interacting proteins were presented for both excitatory and inhibitory synapses (Julie Perroy, Daniel Choquet, Elly Nedivi). For instance, Valentin Nägerl presented the latest advances in STED microscopy, which break the classical diffraction limit of optical microscopy, providing unprecedented details on the nanostructure of synapses and dendritic spines in live neurons. Thomas Oertner convincigly showed how combining imaging and optogenetic approches enable the measurement of single synapses function over time. This session comprised 4 short talks selected from the applications, dealing with short term plasticity at a single bouton level (Sandrine Pouvreau), with plasticity of GABAergic connectivity in relation to addiction (Manuel Mamelli), mechanisms underlying the plasticity dendritic calcium signals (Friedrich Johenning), and sensorimotor mismatches in mouse models of Alzhiemer's disease (Sabine Liebscher). This session has highlighted the huge potential imaging methods have for analyzing synaptic function and the new concepts that are emerging in the field of synaptic function and plasticity.

There is great excitement about the prospect of mapping the intricate synaptic connectivity in the brain, and to unravel the diversity of the neuronal cell types that compose neural circuits. The fourth session **“Neuronal connectivity - connectomics”** examined the structure of brain circuits and presented the latest developments in connectomic approaches at the mesoscale level. The considerable technical challenges faced in large scale connectomics are being rapidly addressed. The whole brain of a rodent can be rendered transparent for imaging, without damaging cells and proteins. Brain clearing methods allow researchers to see connectivity within the brain. The compatibility of these methods with endogenous fluorescence imaging, immunohistochemistry, RNA single-molecule FISH, long-term storage, and microscopy with cellular and subcellular resolution was presented (Viviana Gradinaru). Brain clearing followed by light-sheet microscopy was applied to the 3D reconstruction of circuits during postnatal development of the cerebellum (Alain Chédotal). Major technological advances for 3D brain mapping and analysis were also presented by Nicolas Rénier (short talk selected from abstracts). Results obtained by rabies-based viral methods through expression florescence markers in discrete connected neuronal populations described cortical circuits underlying memory in physiological conditions and in mouse models of intellectual disability (Andreas Frick). An alternative method, the mGRASP system based on the split-GFP reassembly technique, indicated with high spatial resolution the locations of synapses within a specified circuit (Jinny Kim). Finally, large scale approaches to derive a comprehensive taxonomy of cell types in a neocortical circuit was addressed combining transcriptomic, morphological, connectional and

electrophysiological properties of different kinds of neurons, and correlate these properties with circuit functions (Hongkui Zeng).

In summary, this international conference has brought together world leading scientists studying brain function on multiple different scales with physicists and neuroscientists that develop and use new tools to study brain function. This conference has embraced the question of brain connectivity at a variety of scales from synapses and neurons, to networks in conditions of behavior. We have also taken good care of renewing considerably the speakers. In this renewal we have favoured junior group leaders and paid a particular attention to gender balance. Diversity was also favoured by selecting 8 short talks from junior registered participants.

The general atmosphere was excellent. The participants to the conference have benefitted from a very open minded setting, as evidenced also during the poster sessions which were very well attended. This made the conference attractive to a good fraction of labs that study the neuroscience of synapses and circuits both in France and the rest of the world.

The attendants have unanimously plebiscited a continuation of this conference series, and have chosen **Fritjhof Helmchen** and **Valentina Emilliani** as Chair and co-chair to propose a new event in 2018. We believe that the visibility of the conference, notable for being an excellent place for interactions between researchers developing tools and those applying them for functional questions, will again attract motivated PhD students and post-docs from all over the world, and be a major forum for fruitful international interactions.