NEuroscience Workshop Saclay 2014: Emerging Imaging Technologies in Neuroscience

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Poster session

Plenary session

Tracing visual processing streams in the zebrafish brain

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Retinal images are transmitted to the brain via the axons of retinal ganglion cells (RGCs). These projections build representations of the identity and location of visual objects. RGCs project to about a dozen brain regions. In a systematic attempt to delineate the visual processing pathways in the larval zebrafish, we constructed a comprehensive map of the connectivity between RGCs and retinorecipient areas. By unbiased sparse genetic labeling and in vivo imaging, we identified > 70 RGC types based on the combination of axonal targets and dendrite stratification patterns. This number far exceeds current estimates of RGC diversity derived from work in other vertebrates. We found that a dot moving horizontally across an LED screen evoked prey-capture maneuvers in immobilized fish. This response is selectively tuned to size and speed of the stimulus. By two-photon GCaMP6 imaging, we identified a pretectal area that responded robustly to the optimal prey stimulus. Laser ablations showed that this area is necessary for prey-catching behavior. Interestingly, the RGCs linked to this area fall into just two morphological classes. Thus, a specific retinofugal pathway, dedicated to prey detection, may provide input to an elementary object recognition circuit in the fish brain.

Keywords: zebrafish brain, visual processing pathways

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Advances in multiphoton imaging of developing tissues

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Modern issues in systems biology require tissue-scale measurements of multiple cell parameters. Multiphoton fluorescence microscopy has proven invaluable for tissue studies with its ability to provide subcellular resolution in thick/live samples. However established methods are still limited in terms of speed, depth, innocuity, and ability to simultaneously probe multiple parameters.
We will discuss some recent advances, such as efficient combination of fluorescence with coherent contrasts (THG, SHG) [1,2], multicolor two-photon excitation using wavelength mixing and its application to brainbow tissue imaging [3,4], and high-throughput two-photon imaging using light-sheet excitation [5,6]. We will illustrate the benefit of these strategies for high-information content imaging of developing tissues and embryos.
Refs: [1] Olivier, Science (2010). [2] Zimmerley, Phys Rev X (2013). [3] Mahou, Nat Methods (2012). [4] Loulier, Neuron (2014). [5] Truong, Nat Methods (2011). [6] Mahou, Nat Methods (2014).

Keywords: multiphoton imaging

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A sensory motor circuit for binocular motion integration in larval zebrafish

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Zebrafish process whole field visual motion, extract the net direction of such stimuli and use this information to guide their swimming behavior to match the direction and speed of these external cues. This innate behavior, called the optomotor reflex (OMR) is ubiquitous in the animal kingdom and presumably serves to stabilize an animal's position in the world when it is being moved by external forces.
Here we use closed loop behavioral assays in freely swimming fish that allows specific and independent stimulation of the two eyes – with coherent as well as conflicting motion signals - and allows us to answer questions of how the two eyes interact to combine, suppress and filter the various permutations of motion stimuli.

We use whole brain imaging in tethered larvae to identify the complete neural circuitry underlying these various sensory motor transformations. Specifically we provide a working model of the complete circuit, that quantitatively captures the complete behavioral output as well as the response characteristics of the majority of the active neurons identified by independent cluster analysis.
This rate based computational model makes very specific predictions about connectivity and synaptic polarity of the functionally identified neurons, is easy to test and falsify and serves as an ideal platform and hypothesis generator for a whole range of future experiment.

Keywords: zebrafish, neural circuitry

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Optogenetics and Wave front shaping

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The combination of light microscopy and optogenetics offers the possibility to control activation and inhibition of neuronal activity enabling the analysis of well-defined neuronal population within intact neuronal circuits and systems. Interestingly, optogenetics has already permitted to address key biological questions with relatively simple illumination methods using widefield visible light illumination. However, some limitations in the specificity of genetic targeting and the intricate morphology of the brain make it challenging to, for example, individuate subsets of genetically identical interconnected cells, or to establish the role of specific spatiotemporal excitatory patterns in guiding animal behavior. To reach such degree of specificity, more sophisticated illumination methods are required.
Here I will present a series of new methods recently developed in my group for highresolution single and two photon optogenetics based on the temporal control of ultrafast pulses for axial localization of the illumination volume and on either digital holography or the generalized phase contrast method for lateral light patterning. Exemplary experiment showing two-photon activation of ChR2 in brain slices will be showed.
Finally I will present some recent results demonstrating optogenetics activation with near single cell resolution in freely behaving animals by performing holographic light patterning trough a recently developed fiberscope.

Keywords: optogenetics, neuronal activity

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Behavioral correlates of apical dendrite activity

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Of the six layers of the neocortex, layer 1 (L1) remains the most mysterious. L1 has been physiologically inaccessible for most of neuroscience's history, being comprised almost entirely of apical dendrites from pyramidal neurons. The advent of *in vivo* imaging technologies has recently opened up investigation of this major component of cortical circuitry. Even in primary sensory cortex, L1 apical dendrites receive long-range synaptic connections from higher-order cortical and thalamic areas. These connections have the potential to influence sensory processing in the context of self-generated motion, higher-order stimulus features, behavioral state, and the behavioral relevance of particular stimuli. The feedback nature of L1 connections suggests that their main effects on apical dendrites transpire when animals are engaged in a task. My talk will focus on our efforts to image apical dendrite activity during behavior. We train transgenic mice on various head-fixed behavioral tasks involving whisker-mediated sensation. Genetically encoded calcium indicators are selectively expressed in the apical tufts of L2/3 or L5 pyramidal neurons, and mice are imaged by two-photon microscopy during task performance. I will discuss how the dendrites respond to various sensory, motor, and behavioral events. I will also present a swept light sheet technique for acquiring volumes at relatively high-speed using a single microscope objective.

Keywords: two, photon microscopy, cortical layers

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Ultrafast Doppler and fUltrasound Imaging

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In the last fifteen years, the concept of plane wave transmissions rather than line by line scanning beams broke the conventional limits of ultrasound imaging. By using such large field of view transmissions, the frame rate reaches the theoretical limit of physics dictated by the ultrasound speed and an ultrasonic map can be provided typically in tens of micro-seconds ($>$ 1000 frames per second). Interestingly, this leap in frame rate is not only a technological breakthrough offering completely new ultrasound imaging modes and open new application, but at such frame rates, it becomes possible to track in real time transient vibrations – known as shear waves – propagating through organs and provides quantitative maps of tissue stiffness whose added value for diagnosis has been recently demonstrated in many fields of radiology.
For blood flow imaging, ultrafast Doppler permits high-precision characterization of complex vascular and cardiac flows. It also gives ultrasound the ability to detect very subtle blood flow in very small vessels. In the brain, such ultrasensitive Doppler paves the way for fUltrasound (functional ultrasound imaging) of brain activity with unprecedented spatial and temporal resolution compared to fMRI. Examples will emphasize the potential of this new imaging modality. fUS technology could open new avenues in neuroscience. For therapy, localizing the epileptic focus using fUS during surgery could be a major application. Functional imaging on newborns will also be of major interest in order to increase our knowledge in cognitive science. Beyond clinical application, it will be a fantastic tool for people in neuroscience working on small animals. This technology should help them answer unsolved questions.

Keywords: Ultrafast Doppler, fUltrasound, epilectic, newborn, small animals

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Fetal brain development study using MRI

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The development of ultrafast 2D acquisition sequences has led to significant improvements in the clinical utility of fetal MRI. However, the slice acquisition time is still very critical and has to be as short as possible to reduce the impact of fetal and maternal motion on the exam. Since 2005 there has been a series of techniques coming from computer vision that have been developed to address these limitations in fetal MRI. These motion-correction methods provide a new window into early human brain growth study. To this end, new algorithms have also been investigated to deal with tissue segmentation and the construction of spatiotemporal atlases. I will present in this talk an overview of the current research into fetal brain MRI processing for early brain development studies. I will focus on the three main steps for fetal MR data processing: image reconstruction, feature extraction (segmentation and tractography) and temporal analysis (cortical folding).

Keywords: fetal MRI

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Towards a dynamic map of neuronal circuits

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Knowledge on structural connectivity in neuronal circuits is necessary for understanding information representation and processing in local circuits. However, as some examples of well-characterized neuronal architectures illustrate, structural connectivity alone it is not sufficient to predict how input stimuli are mapped onto activity patterns of neuronal populations and how the collective dynamics of all neurons in the network leads to behavior. Addressing this challenge has been hampered by lack of appropriate tools and methods that allow parallel and spatiotemporally specific application of excitation patterns onto neuronal populations while capturing the dynamic activity of the entire network at high spatial and temporal resolution. The combination of new optical excitation techniques, optogenetics and high speed functional imaging are providing new opportunities to address this question and move towards a dynamic map of neuronal circuits.
I will address a number advances in that respect that we have recently implemented in our lab using two different technologies. One approach relies on "sculpting" the excitation volumes in biological samples using non linear optics and the other relies on light field imaging, a tomography type approach for simultaneous readout of neuronal activity in 3D. Using these techniques we have recently shown brain-wide functional imaging of entire nervous systems at single cell resolution [1]. Further, we demonstrate intrinsically simultaneous volumetric Ca-imaging in the entire brain of larval zebrafish during sensory stimulation [2]. We are able to track the activity of 5000 neurons distributed throughout the brain at 20Hz volume rate. The simplicity of this technique and the possibility of the integration into conventional microscopes make it an attractive tool for high-speed volumetric functional-imaging. These tools combined with high speed optogenetic control of neuronal circuits [3, 4], advanced statistics tools and mathematical modeling and will be crucial to move from an anatomical wiring map towards a dynamic map of neuronal circuits.

References:

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2. Prevedel, R., et al., Simultaneuos whole-animal 3D-imaging of neuronal activity using light-field microscopy. Nature Methods, 2014 (in press)

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4. Losonczy, A., et al., Network mechanisms of theta related neuronal activity in hippocampal CA1 pyramidal neurons. Nature Neuroscience, 2010. 13(8): p. 967-72.

Keywords: neuronal circuits, non linear optics, light field imaging, optogenetics

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Oral session

Tefor core facility: an electronic atlas of standardized Zebrafish neuroanatomy

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With the growing interest on Zebrafish as a model organism the need for precise anatomic data arose. The evolution of microscopic techniques enabled already precious atlases of the Zebrafish. However, while these atlases are valuable to learn the general neuroanatomy, their image data only has limited use during the analysis of phenotypes in new specimens. With ViBE-Z (Ronneberger et al. (2012) an electronic atlas exists of 3days old Zebrafish, however for research in the juvenile or adult Zebrafish these data are not relevant.
Our approach tries to overcome this shortcoming by imaging complete brains in high resolution 2-photon-microscopy together with their fluorescent expression patterns. Pivotal parts are the CLARTIY protocol (Deisseroth et al., 2013) and the VibMic (Tefor Core Facility and Leica Microsystems).
The CLARITY protocol facilitates good antibody penetration and enhances accessibility of deep structures while the VibMic enables imaging of structures outside the reach of a normal microscope objective in high resolution by the combination of a conventional vibratome with an IR equipped confocal microscope. Mechanic sectioning and successive block face imaging under the surface of the specimen allows us to image specimens of very large z-dimension as wholemount fish.
The high density of the image data results in big file sizes. Since we are aiming to provide data in highest resolution we are currently developing a data management system for 3D and serial 2D display with subcellular resolution.

The goal of the Tefor core facility is to generate neuroanatomic data under standardized conditions for the expression patterns of all intensively used Zebrafish lines and register them into an electronic atlas of the Zebrafish neuroanatomy. Since this atlas is foreseen to be shared with and annotated by its users we hope to provide a backbone for the discussion and distribution of neuroanatomic data to the Zebrafish community.

Keywords: zebrafish, neuroanatomy, vibmic, clarity, data management, high resolution

*Speaker

Representation of global motion in the mouse barrel cortex, a voltage sensitive dye (VSD) imaging study

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Sensory whiskers in the mystacial pad of rodents are mapped onto layer IV of the primary somato-sensory cortex (S1) as discrete units named "barrels". Each barrel-related vertical column processes information coming primarily from its corresponding whisker in the snout of the animal. Previous experiments in our lab done with extracellular recordings showed that neurons in the rat S1 and thalamus not only show a direction preference for local stimulation of the principal whisker but also for the direction of a global motion across the whisker pad (Jacob et al., 2008; Ego-Stengel et al., 2012).
To further understand how the cortical network processes global tactile scenes, we performed VSD imaging of the mouse barrel cortex under anesthesia while applying global tactile stimuli using a 24-multi-whisker stimulator (Jacob et al., 2010).
Global motion was obtained by presenting multiwhisker stimuli that were locally invariant but globally coherent, resulting in 8 directions of apparent motion. Our results show that different directions of stimulation produce responses in the barrels of different magnitude and that there is a spatial organization of global motion direction selectivity with ventral motions preferentially activating barrels related to caudal and dorsal whiskers, and caudal motions preferentially activating barrels related to rostral and ventral whiskers.
When compared to responses evoked by single arcs/rows of whiskers, responses to global motions appear highly sublinear. Preliminary analyses indicate that these sublinearities, as well as the propagation of the responses across the barrel field, are likely to vary depending on the direction of the apparent motion.

Keywords: VSD imaging, mouse, barrel cortex, direction selectivity

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Spontaneous neuronal network dynamics reveals circuit's functional adaptations for behavior

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In the absence of sensory stimulation sensory brain areas remain highly active. Notably, this spontaneous neuronal activity is spatiotemporally structured, according to the coarse functional and anatomical circuitry. Furthermore, structured spontaneous activity influences brain computations, since its structure is only mildly modulated by sensory inputs and it partially accounts for the variability of stimulus-evoked neuronal responses. Nevertheless, the neuronal interactions underlying these spontaneous activity patterns, and their biological relevance, remain elusive. Here, we addressed these open questions.
We used two-photon calcium imaging of intact non-anesthetized transgenic zebrafish larvae expressing pan-neuronally GCaMP3 to monitor the neuron-to-neuron spontaneous activity fine-structure in the optic tectum. The vertebrate optic tectum, analogous to the mammalian superior colliculus, contains functional sensory maps of the external world, and it is involved in spatial detection, attention and the generation of commands for orienting motor behaviors. In zebrafish, the tectum is the most complex visual region, and is essential for visually guided prey detection and capture.

We observed that spontaneous tectal activity was organized in topographically compact neuronal clusters. However, the latter were not a mere collection of neighboring neurons but represented true functional assemblies, specifically grouping functionally similar neurons. Collectively, they reflected the tectal retinotopic map, even in the absence of retinal inputs. Furthermore, we showed that assemblies represent all-or-none-like cooperative sub-networks shaped by competitive dynamics, a mechanism suited for their efficient and robust coordinated recruitment. Notably, the spontaneous assemblies were tuned to the same angular sizes and spatial positions as larva's prey-detection performance in behavioral assays, arguing for their behavioral relevance. Our results reveal that structured spontaneous activity represents sensory functional maps that emerge from the circuit's intrinsic non-linear dynamics. Spontaneous activity patterns reflect advantageous neuronal mechanisms that promote the extraction of biologically relevant visual features and assure robust circuit functioning in noisy natural environments.

Keywords: two, photon calcium imaging, in vivo, zebrafish, neuronal networks, spontaneous activity, neuronal assemblies

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Processing of odor-evoked neural activity in the olfactory cortex

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Olfactory behaviors require the identification of odors across a large range of different concentrations, yet are exquisitely sensitive to changes in odor concentrations. To accomplish this seemingly paradoxical task the olfactory system must generate odor representations that are, at once, both concentration-dependent and concentration invariant.
We have used in vivo two-photon microscopy to characterize odor-evoked activity in the olfactory bulb and piriform cortex of mice. We find that the density of odor-evoked activity in the olfactory bulb scales with odor concentration. In contrast, piriform odor representations are largely concentration invariant, indicating substantial normalization of olfactory bulb output by piriform microcircuits. We have identified parvalbumin-expressing interneurons, a subpopulation of piriform inhibitory neurons, as a candidate cell type to mediate piriform concentration invariance.

Our results provide important new insights into the computations performed by olfactory neural circuits.

Keywords: Olfaction, piriform cortex, odor representation, 2, photon

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Creating an atlas of children brain connectivity

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Diffusion imaging offers neuroscientists a unique opportunity for in vivo investigation of the white matter connectivity using 3D fiber reconstruction (tractography) techniques. It has a considerable potential for studying normal and abnormal brain development as diffusion parameters quantitatively reflect various maturation processes, while tractography enables studying regional maturation within distinct bundles. However, tractography datasets are extremely complex, containing millions of fibers of various shapes and lengths. Extracting individual white matter bundles from such datasets is a challenging task that has not been completely solved yet. Bundles are commonly extracted based on regions of interest (ROI) that are used to include or exclude certain fibers. These ROIs can be defined manually in individual subjects but this is very time-consuming and expert dependent. Alternatively, ROI atlases can be applied using affine or non-linear transformations, but results strongly depend on the transformation quality and do not take into account fiber shapes. Recently, fiber-clustering techniques have been proposed for automatic bundles identification (Guevara et al. 2012), based on an atlas of main bundles generated over a group of adult subjects. This approach has the advantage of taking into account fiber shapes and localization variabilities. Furthermore, it can be used to analyze white matter microstructural properties when it is not possible to perform reliable tractography (e.g. in case of white matter diseases, like demyelination) by projecting the atlas to the subject data. However, this atlas was generated for adults hindering its application to children as fiber shapes and lengths change during development. Thus, reliable bundle identification in children requires using dedicated atlases. In this work, we describe creation of such an atlas from a group of 17 children aged between 17 and 81 months and the way in which this atlas may help to extract new information about various pathologies of the white matter.

Keywords: Diffusion Imaging, white matter, tractography, atlas, clustering, children

*Speaker

Non invasive detection of action potentials in a bulk neural tissue from intrinsic mechanical properties of neurons.

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During action potentials, physical properties of neurons membrane change drastically since an important part of its components are charged molecules. Mechanical deformations triggered by neural activity are notably expected in the tissue. These changes can be monitored via neurons optical properties, using phase imaging. Their detection would be interesting in neuroimaging, since no chemical or genetic manipulation, (that becomes increasingly difficult with organism "complexity") would be needed to follow non-invasively single neuron activity.
Previous studies have demonstrated that optical properties of nerves and giant axons do change during activity, notably birefringence properties and optical path. Nevertheless, at our knowledge, such effects have never been observed in mammalian cortex neurons with cellular resolution in real time. Moreover, the biological origin of these optical changes are still being discussed.

To be able to detect action potentials from such intrinsic mechanical deformations, it is first important to better understand the spatial and temporal characteristics of such mechanical deformations associated with action potentials.

Towards this goal, we report here the elaboration of a multimodal setup combining Full Field Optical Coherence Tomography and Structured Illumination Microscopy. It enables to simultaneously perform phase imaging and calcium imaging with a submicron transverse resolution at several hundred frames per second. We should therefore be able to follow the entire dynamics of action potentials. The mechanical deformations can be inferred from the optical phase measurement in the axial direction, with a nanometer resolution. This setup can also perform optical sectioning, so, in principle, we will be able to investigate such electromechanical coupling inside tissues, as well as in cultures.
Today, we are characterizing the setup, and we are trying to perform some measurements in neuronal cultures. Depending on the advancement of the experiments, I would insist more on the setup, or on the results we might obtain before December.

Keywords: Optical Phase Imaging, Intrinsic detection of action potentials, Electromechanical Coupling

*Speaker

Genetically functionalized magnetosomes as MRI contrast agent for molecular imaging: in vitro proof of binding and in vivo proof of contrast

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Molecular imaging aims at detecting molecular markers in a preserved environment using non-invasive imaging modalities. Specific contrast agents are dedicated to one imaging modality and functionalized to target pathological biomarkers, in order to reveal preliminary stages of disease. Combining high magnetic field scanners and innovative contrast agents, Magnetic Resonance Imaging (MRI) might achieve the high sensitivity and specificity requested by molecular imaging applications.
Magnetosomes are iron-oxide nanoparticles of interest for MR-based molecular imaging. These regular crystals of magnetite embedded in a lipid bilayer are produced by magnetotactic bacteria and can be functionalized for biomarkers targeting using genetic tools [1]. Furthermore, they present promising MR contrasting properties [2].

Here, translational gene fusion was used to successfully produce from AMB1 strain magnetosomes expressing RGD peptides at their membrane. We obtained in vitro proof of specific binding of these RGD-magnetosomes with U87 cell line (human model of glioblastoma), known for expressing 3 integrins targeted by RGD. The genetic fusion of RGD peptide with Venus (Venus-RGD), a variant of GFP, enabled to demonstrate by fluorescence imaging both binding and internalization of Venus-RGD-functionalized magnetosomes, assessing the specificity. The MR contrasting properties of these nanoplatforms were then measured using relaxometry which demonstrates their great sensitivity for MR imaging.

As a first proof of contrast, we validated the ability to acquire mouse brain angiogram [3] combining intravenous injection of wild type magnetosomes with high field MRI on a mouse model of glioblastoma developed for future molecular imaging studies.

This work demonstrates the feasibility of producing functionalized magnetosomes along with the ability to use them in vivo as contrast agent for MRI, opening the way for their use in further molecular imaging studies.

Ginet et al, 2011, PLoS One

Faivre and Schüler, 2008, Chem Rev

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Maneissing and Neissen, 2005, Inf Process Med Imaging

Keywords: Molecular imaging, Contrast enhanced MRI, Magnetosomes, Glioblastoma

Patterned photostimulation of auditory cortical networks drives perceptual discrimination in mice

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Sensory cortical areas in mammals are often organized in maps, where different stimuli activate different groups of neurons within the larger network. New optical approaches make it possible not only to image the activity of multiple neurons simultaneously in anesthetized or behaving animals, but also to manipulate this activity at arbitrary, selected spatial locations, by adressing light to the location of the chosen cells, which express light-sensitive ion channels or pumps. It is thus in principle feasible to test causally the impact of neuronal groups representing different sensory stimuli on perception and behavior. To advance towards this goal, we built a patterned photostimulation rig using an LED-based videoprojector. The optical system projects an 1280x800 pixel screen onto an area of about 2-by-3 mm, with a maximum intensity of 40 mW/mm2 for the blue LED (455 nm). This approach allows the activation of arbitrary neuronal columns across the extent of cortical areas under a chronic cranial window in mice expressing Channelrhodopsin-2 in excitatory cortical neurons.

Using this tool, we show that for mice having learnt to discriminate between two auditory stimuli in a Go-No Go licking task, the detection of the rewarded sound can be perturbed by direct activation of subregions in the auditory cortex. We also show for the first time that naive mice can learn over the course of two weeks to discriminate between the direct activation of two different regions of auditory cortex. These results demonstrate that auditory cortex activation causally influences auditory sensation in mice, and that it is possible to engineer with light discriminable artificial stimuli within a single cortical area. We are currently exploring which parameters of the optogenetic stimuli influence general aspects of auditory perception, such as saliency. We also aim at understanding what exact auditory percepts may be generated during patterned optogenetic stimulation.

Keywords: optogenetics, audition, cortex, discrimination, perception, pattern, photostimulation, channelrhodopsin, excitatory neurons

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Poster session

Chronic assessment of cerebral hemodynamics during rat forepaw electrical stimulation using functional ultrasound imaging

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Functional ultrasound imaging is a method recently developed to assess brain activity via hemodynamics in
rodents. Doppler ultrasound signals allow the measurement of cerebral blood volume (CBV) and red blood

cells' (RBCs') velocity in small vessels. However, this technique originally requires performing a large craniotomy

that limits its use to acute experiments only. Moreover, a detailed description of the hemodynamic changes that

underlie functional ultrasound imaging has not been described but is essential for a better interpretation of neuroimaging

data.

To overcome the limitation of the craniotomy, we developed a dedicated thinned skull surgery for chronic imaging.

This procedure did not induce brain inflammation nor neuronal death as confirmed by immunostaining.We

successfully acquired both high-resolution images of the microvasculature and functional movies of the brain

hemodynamics on the same animal at 0, 2, and 7 days without loss of quality. Then, we investigated the spatiotemporal

evolution of the CBV hemodynamic response function (HRF) in response to sensory-evoked electrical

stimulus (1 mA) ranging from 1 (200 s) to 25 pulses (5 s). Our results indicate that CBV

*Speaker

HRF parameters such

as the peak amplitude, the time to peak, the full width at half-maximum and the spatial extent of the activated

area increase with stimulus duration. Functional ultrasound imaging was sensitive enough to detect hemodynamic

responses evoked by only a single pulse stimulus.We also observed that the RBC velocity during activation

could be separated in two distinct speed rangeswith the fastest velocities located in the upper part of the cortex

and slower velocities in deeper layers. For the first time, functional ultrasound imaging demonstrates its potential

to image brain activity chronically in small animals and offers new insights into the spatiotemporal evolution of
cerebral hemodynamics.

Keywords: Neurovascular coupling, Functional imaging, Cerebral blood volume (CBV), Doppler ultrasound

Coexpression of the homeogenes barhl2, otx2 and irx3 specifies the identity and properties of the caudal forebrain signaling center

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We investigated the gene regulatory network that governs formation of the Zona limitans intrathalamica (ZLI), a signaling center that secretes Sonic Hedgehog (Shh) to control the growth and regionalization of the caudal forebrain. Using loss- and gain-of-function, explants and grafting experiments in amphibians, we demonstrate that barhl2 acts downstream of otx2 and together with the iroquois (irx)-3 gene in establishment of the ZLI compartment initiated by Shh influence. We find that the presumptive (pre)-ZLI domain expresses barhl2, otx2 and irx3, whereas the thalamus territory caudally bordering the pre-ZLI expresses barhl2, otx2 and irx1/2 and early on irx3. We demonstrate that Barhl2 activity is required for determination of the ZLI and thalamus fates and that within the p2 alar plate the ratio of Irx3 to Irx1/2 contributes to ZLI specification and size determination. We show that when continuously exposed to Shh, neuroepithelial cells coexpressing barhl2, otx2 and irx3 acquire two characteristics of the ZLI compartment—the competence to express shh and the ability to segregate from anterior neural plate cells. In contrast, neuroepithelial cells expressing barhl2, otx2 and irx1/2, are not competent to express shh. Noteworthy in explants, under Shh influence, ZLI-like cells segregate from thalamic-like cells. Our study establishes that Barhl2 activity plays a key role in p2 alar plate patterning, specifically ZLI formation, and provides new insights on establishment of the signaling center of the caudal forebrain.

Keywords: development, forebrain, morphogenesis, Sonic Hedgehog, Signaling, Compartment

*Speaker

Visual cortical representations are enhanced by cross-modal auditory interactions in mice

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Perception relies importantly on multi-modal integration. In particular, the interaction between vision and audition is the source of multiple crossmodal illusions in humans (e.g. Mc Gurk effect, double flash illusion, ventriloquism), in which one modality influences perceptions in the other. The neural mechanisms of such cross-modal interactions remain elusive. To test how sounds can modulate representations in the primary visual cortex (V1) of awake mice, we used 2-photon microscopy to record the activity of large populations of neurons while playing uni- or bi-modal sequences, consisting of visual stimuli generating apparent motion towards or away from the animal, and auditory stimuli of increasing and decreasing amplitude or frequency. We observed that a small fraction of the neurons inside V1 responded to sounds and even displayed auditory tuning. An even larger fraction of neurons had their responses to a visual stimulus modified by the sounds played simultaneously. At the level of the whole neuronal population, the main effect was that responses to visual sequences appeared to be more reproducible when accompanied by auditory sequences than when played in silence. These observations suggest that an auditory input can improve the cortical responses to visual stimuli, and hence their perception. We will perform behavioral experiments to test this hypothesis.

Keywords: two photon microscopy, neuronal assembly, audio visual integration, awake imaging, population analysis

*Speaker

Evolution of appetite regulating neurons in the blind cavefish Astyanax mexicanus

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Astyanax mexicanus is a single species of teleost fish, composed of two morphs : the cave-dwelling blind morph and the river-dwelling sighted morph. While food is abundant in rivers, it is way scarcer in caves, which makes food finding an important challenge. Differences in behaviors and sensory capacities improving foraging in the caves have been studied, but neuroanatomical comparisons of brain centers that regulate appetite are lacking. We therefore aimed to establish a detailed mapping of the expression of nine appetite-related neuropeptides at five different stages of development for both the cavefish and the surface fish. By counting the neurons expressing each of these neuropeptides in the hypothalamic region, we were able to show that the cavefish larvae display more appetite-stimulating neurons and less appetite-inhibiting neurons than the surface fish larvae. We are now trying to understand the developmental differences that result in those shifts and the behavioral and metabolic effects that those induce.

Keywords: evolution, development, brain, appetite, hypothalamus, Astyanax mexicanus, neuropeptides

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2-spoke placement optimization under explicit SAR and power constraints in parallel transmission at ultra-high field

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To mitigate the B1+ inhomogeneity at ultra-high field, the spokes method [1] combined with parallel transmission is a promising technique. However, the spokes placement optimization [2-4] for the Magnitude Least Squares (MLS) pulse design problem [5] has never been done in direct conjunction with the explicit SAR and hardware constraints. In this work, the optimization of 2-spoke trajectories and RF-waveforms is performed under these constraints explicitly and for axial slices of the human brain at 7T. The problem is simplified by making the observation that only the distance kx,y between the 2 spokes is relevant for the MLS normalized root mean square error (NRMSE). The Active-Set (AS) algorithm [6] starts from a set of 121 initial k-space candidates distributed on a Cartesian grid and performs for all of them simultaneous optimizations of both the RF waveforms and the k-space locations, solving the MLS problem under strict SAR and power constraints. Fixing the first spoke (0,0), for each independent optimization, both the spokes-weights and the second spoke (kx,ky) coordinates were free to evolve simultaneously. For each subject, the 121 optimizations under strict SAR and power constraints could be performed in only 14 seconds. According to Fig.1, a NRMSE of less than 2 % was systematically returned for all 4 subjects at 7T over an axial slice in the 2-spoke configuration, providing evidence for certain robustness with respect to subject, slice placement and B0 shim. However, as shown here (Fig.1 and 2), 2-spoke seem to be enough to obtain excellent 2D excitation uniformity for human brain applications at 7T. References: [1] Saekho et al. MRM, 2006. [2] Ma et al. MRM, 2011. [3] Grissom et al. MRM, 2012. [4] Yoon et al. MRM, 2012. [5] Setsompop et al. MRM, 2008. [6] Hoyos-Idrobo et al. IEEE TMI, 2014.

Keywords: Parallel transmission, B1+ inhomogeneity mitigation, spokes, RF optimization, spoke placement.

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Pfkfb4 regulates dorsal ectoderm early patterning in vertebrate embryos, by a glycolysis-independent mechanism affecting the Akt pathway

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While metabolism of the developing embryo remains poorly understood, a growing interest is given to stem cells metabolism. Glycolysis and oxidative phosphorylation are the two main metabolic pathways that allow cells to produce energy, in normal and pathological conditions. Many enzymes and substrates are involved in these processes and PFKFB (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase) enzymes are among the most important glycolytic regulators. In cancer cells, PFKFB enzymes are upregulated and stimulate glycolysis, which contributes to tumor survival and spreading. We found that pfkfb4 is expressed in the dorsal ectodermal cells of Xenopus laevis embryos, including the prospective neural crest (NC) and neural plate. Loss of this enzyme affects neural patterning during gastrulation and NC and epidermal patterning at neurula stages. The affected cells remain in an early progenitor state and eventually undergo apoptosis. Interestingly, we found that pfkfb4 participates in these events by a novel, non-glycolytic role, which impairs Akt signaling. A constitutively active form of Akt rescues pfkfb4 knockdown phenotype on neural patterning. This study unravels three novel types of regulations in early embryonic patterning: a) the involvement of a key metabolic regulator in ectoderm early patterning; b) a new, non-glycolytic function of a metabolic enzyme; c) finally, a link between pfkfb4 and Akt signaling, suggesting a connection between patterning and cell homeostasis during embryogenesis. Together, these results highlight how cell progression towards patterning and differentiation involves unsuspected links with the cellular stress-sensing machinery.

Keywords: xenopus embryo / metabolism/ akt

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Ultrasonic matrix transducer design for 4D ultrafast Doppler neuroimaging: application for clinical fUltrasound and small animal brain imaging.

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Functional Ultrasound (fUS) was recently introduced as a new neuroimaging modality able to image deep brain activity via neurovascular coupling with unprecedented spatiotemporal resolutions. Thanks to ultrafast ultrasound acquisition, sensitive blood flow measurements enable the imaging of subtle haemodynamic changes in small brain vessels and thus brain activity through neurovascular coupling.
To date, the main limitation has been the identification of the activation regions with only a 2D "slice" of information of the whole brain.

Nevertheless, 4D Doppler imaging could be performed with a 2D fully populated matrix (M2D). However, today this technology seems inappropriate in the neuroscience domain for several technical reasons. M2D needs to be miniaturized and to reach a higher frequency for imaging small animals. Concerning clinical applications, M2D made up of several thousand transmit/receive elements is not compatible with commercial scanners relying only on a limited number of electronic channels.

Our first investigation has focused on the emerging concept of a row-column addressing matrix (RCA) [Fig a], which effectively reduces the number of elements from NxN to 2xN channels and thereby allows compatibility with commercial scanners. The figure shows a comparison between a M2D (1024 channels, 3.5Mhz, Vermon) and an emulated RCA.

These promising results [Fig b], have encouraged us to choose this technological solution to make a broader range of probes (6 to 15 Mhz) with small form factor that will soon be tested on an Aixplorer scanner (SuperSonic Imagine).
This new probe will open the door to new neuroscientific applications that would not have been possible with M2D, such as functional imaging experiments on freely moving awake rats, or the understanding of a baby's cognitive development using fUS through the fontanel window. The extension of the RCA matrix concept to fUltrasound imaging of the brain will be discussed in detail in this work.

Keywords: Functional Ultrasound, Row column addressing, Ultrasonic matrix transducer design

*Speaker

Adenosine-Squalene nanoparticules and neuroprotection: toward a theranostic tool

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Brain diseases represent a major health concern due to population aging. However, drug delivery with therapeutic efficiency remains the main challenge of central nervous system drug development. It has also been proposed that the use of MRI contrast agents could be helpfull in the earliest decisional steps of patients handling. Nanotechnologies may promote brain drug delivery together with the transport of a diagnostic agent, opening the way to a theranostic approach. Hence, we recently covalently linked the squalene and the adenosine ("squalenoylation"), in order to obtain stable nanoparticles able to provide dramatic neuroprotection in a stroke model and a spinal cord injury model. The encapsulation of USPIO in the nanoparticles allowed to obtain a theranostic nanoformulation with efficient MRI contrast properties as T2*-shortening contrast agent.

By nanoprecipitating the adenosine-squalene (AdSQ) we obtained stable nanoparticules, with a mean diameter of 120 nm. The pharmacological efficiency of the adenosine-squalene nanoparticles was studied in a model of cerebral ischemia in mice and a spinal cord injury model in rats, showing an impressive neuroprotective effect due to a primary vascular action of the nanoparticles. Using an in-vitro model of human blood-brain barrier and FRET nanoparticles, we were able to show that the nanoparticles were opening during the transcytosis and exocytosed in a molecular form in the brain parenchyma. The in vivo biodistribution of radiolabeled nanoparticles in mice confirmed that the prodrug was metabolized during transcytosis. Finally, by co-nanoprecipitating the AdSQ with USPIO, we were able to obtain magnetic nanoparticles and to characterize them as for their properties of relaxivity.

These results show for the first time that the "squalenoylation" technology is competent for the delivery of hydrophilic drugs to the brain sanctuary, and could became a new theranostic platform for neurological diseases.

Keywords: nanoparticles, squalene, stroke, spinal cord injury, USPIO

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Zero-order suppression for holographic photo-excitation

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Wavefront shaping via computer generated holograms encoded on liquid crystal Spatial Light Modulators (SLMs) has been introduced in neuroscience at the end of the last decade as a flexible way to photostimulate neurons. However, its use is frequently hindered by the remaining fraction of undiffracted light from the SLM, the so-called "zero-order". Here we propose to suppress the contribution of the zero-order by introducing aberrations in holographic systems based on non-linear excitation mechanisms. Aberrations are then corrected for the excitation spot by the SLM, except for the zero-order component that remains aberrated. A decrease by 4 orders of magnitude in zero-order-induced two-photon fluorescence intensity is demonstrated with a simple cylindrical lens as an aberrating optical element, at the moderate expense of a 12% decrease in diffraction efficiency of the SLM. Combination with temporal focusing is shown to further decrease zero-order fluorescence by a factor of 10.

Keywords: Nonlinear optical signal processing, Diffractive optics, Spatial light modulators

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When can temporally focused excitation be axially shifted by dispersion?

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Temporal focusing (TF) allows for axially conned wide-eld multi-photon excitation at the temporal focal plane. For temporally focused Gaussian beams, it was shown both theoretically and experimentally that the temporal focus plane can be shifted by applying a quadratic spectral phase to the incident beam. However, the case for more complex wave-fronts is quite different. Here we study the temporal focus plane shift (TFS) for a broader class of excitation proles, with particular emphasis on the case of temporally focused computer generated holography (CGH) which allows for generation of arbitrary, yet speckled, 2D patterns. We present an analytical, numerical and experimental study of this phenomenon. The TFS is found to depend mainly on the autocorrelation of the CGH pattern in the direction of the beam dispersion after the grating in the TF setup. This provides a pathway for 3D control of multi-photon excitation patterns.

Keywords: Three dimensional microscopy, Ultrafast nonlinear optics, Digital holography

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Improving brain tumor surgery using intra-operative shear wave elastography and micro Doppler

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Improving quality of brain tumor resection is a major concern for neurosurgeons. Shear Wave Elastography (SWE) and micro Doppler could be decisive this issue. By giving access to tissue stiffness SWE is an emerging technology that enable surgical procedure guiding. A clinical study was undertaken, including normal brain and tumors data collected from intra-operative SWE and micro Doppler data. The aim is to improve shear wave imaging for brain tissue investigation by correlating in vivo stiffness data and histology. At the same time micro blood flow was studied in vivo during surgery by using a new micro Doppler mode.
Shear waves were generated by using ultrasonic acoustic radiation force and imaged in real-time with a linear array driven by an ultrafast ultrasound scanner (Aixplorer, Supersonic Imagine) up to 20 000 frames/s. The study was performed intra-operatively on 62 adult patients presenting brain tumor. While stiffness measurements were systematically compared to histology, micro blood flow close to the tumor was also studied by using micro Doppler mode.

Histology allows classification of the different types of brain tumor into four main groups: metastases (N=15), meningiomas (N=15), low-grade gliomas (N=16) and high-grade gliomas (N=16). SWE was able to characterize each group of tumor by a mean elasticity value, respectively: 16.7 ± 8.3 kPa, 32.9 ± 9.4 kPa, 25.2 ± 5.3 kPa, and 10.5 ± 5.2 kPa. Statistical analysis using ROC curve analysis shows that SWE could help for diagnosis during tumor resection by distinguishing benign tumors from malignant tumors (AUROC: 0.77, $p < 0.0001$). Regarding the use of micro Doppler during surgery, it enhance blood volume visualization and thus reduce hemorrhage by improving small vessel localization.
The combination of these two techniques, by giving access to both mechanical properties and blood flow, can improve quality of brain tumor resection and consequently patients' quality of life.

Keywords: Ultrasound, brain, tumor, intraoperative, elastography, Doppler

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Behavioral repertoire of the larval zebrafish in virtual reality

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Zebrafish is an ideal animal model for the use of optical imaging techniques. At only 6 dpf, it displays a rich repertoire of visually guided motor behavior, like prey tracking, optomotor response or phototaxis.Its small brain and transparency enables to record simultaneously from large portion of the brain involved in sensorimotor processing at a single cell resolution.
In order to allow the study of visually guided behavior in a head fixed condition, we developped a virtual reality system. For this purpose, we generated a large library of freely swimming zebrafish larva behaviours, from which we extracted key kinematic features that distinguish each of the monitored behaviours (ie position, orientation, shape of the tail).

Using machine learning techniques, we inferred the changes in orientation and velocity from the shape of the tail. In head-restrained larvae we monitored the tail movements and digitised them in real time. This technique enabled us to infer the intended movement of the larva. These data were then used to feed and update a virtual visual environment displayed around the larva.

In contrast to current state of the art techniques, our method is capable of handling behaviors that rely on different categories of movement. Thus, in head fixed condition, the larva is able to produce optomotor response, as well as prey tracking, in which the larva produces fine changes in orientation and position required to capture virtual preys.

Keywords: Zebrafish, Virtual Reality, Optical Imaging, Behavior

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EEG evidence of statistical learning in preverbal infants

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Statistical learning is a powerful implicit learning mechanism. It has been shown that very young infants use adjacent transitional probabilities to segment continuous speech into its constituent words. In this study, using electroencephalography, we investigate infants' ability to compute statistical dependencies between more distant elements, and extract the underlying structure of a continuous speech stream. We also explore the interplay between experience and maturation, comparing 8-months-old full-term with preterm infants matched by maturational age or duration of exposure to speech. First, during a training session, infants were exposed to a 2 minutes continuous synthesized speech stream comprising AxC "words" separated by subliminal 25ms pauses. Then, during the subsequent test session, infants were presented with either "rule-words", that did not appear during training, but followed the training rule, or "part-words", that appeared in the stream, but violated the rule. Using frequency tagging to analyze the training, we found a significant power increase at the syllabic but also at the word frequencies suggesting that infants were indeed parsing the stream into words. Rule learning was confirmed by the significantly different responses to "rule-words" and "part-words" around 550ms and 1400ms after word onset. These results, observed in the 3 groups, suggest that long-distance dependencies are rapidly and easily used by infants to extract structural regularities in speech.

Keywords: Language development, electroencephalography, implicit learning

*Speaker

Multiparametric MRI to study brain development: Two novel promising approaches

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Magnetic Resonance Imaging (MRI) and Diffusion Tensor Imaging (DTI) are important tools in developmental neuroscience providing various parameters that reflect different microstructural properties of the maturing brain tissues. Although it is possible to make inferences on the brain maturation stage based on individual MRI/DTI parameters, multiparametric approaches merging complementary information from several parameters may provide additional knowledge that cannot be revealed with individual parameters. Here we present two examples of such approaches and their applications.

The first approach was used to describe maturation asynchrony across 18 white matter bundles during normal development. This approach compared infant (aged 3- to 21-week old) and adult groups using the Mahalanobis distance computed from four complementary MRI parameters: quantitative T1 and T2 relaxation times, longitudinal and transverse diffusivities from DTI. In agreement with post-mortem studies on the maturation progression across brain regions, this approach finely confirmed maturation asynchrony across the bundles and was more reliable than univariate approaches. Additionally, Mahalanobis distance allowed estimating the relative maturational delays between the bundles confirming that the motor and sensory pathways mature much earlier and faster than associative bundles in the first months of life.

The second approach focuses on quantification of the brain myelin content through an MRI index, named Myelin Water Fraction (MWF), which is derived from multicomponent analysis of T1 and T2 relaxation signals. MWF corresponds to the fraction of water trapped by the myelin sheaths relative to the total water volume within a voxel, and it is thought to better correlate with the myelin load than individual MRI/DTI parameters. Whereas existing MWF quantification strategies require long acquisition and/or post-processing times, we describe an original approach enabling fast and robust MWF mapping to be used both in research studies on normal or pathological development (e.g. in children with metachromatic leukodystrophy) and in clinical practice.

Keywords: Magnetic Resonance Imaging (MRI), Diffusion Tensor Imaging (DTI), development, white matter, Mahalanobis distance, Myelin Water Fraction (MWF)

*Speaker

Assessment of Macromolecular and Metabolic Alterations during Normal Brain Aging in the Dark Agouti Rat using 1H MRS at 17.2 Tesla

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Introduction

Normal brain aging is usually associated with a decline in brain function. Yet, the neural basis of age-related cognitive dysfunction in the healthy aging brain remains to be elucidated. Metabolic alterations have been observed during normal human aging in vivo using the technique of Magnetic Resonance Spectroscopy (MRS) on 1H and 13C nuclei previously1,2. In our study, we sought to explore these metabolic alterations in the healthy rat brain in vivo using 1H MRS at 17.2 T.

Methods

For this study, Dark Agouti rats were used because of their reduced weight at an advanced age. The following 3 cohorts were used: 6 "Young" rats (1 month old), 6 rats (8 months old) and 4 "Elderly" rats (16 months old). Animals were anesthetized using isoflurane and body temperature was maintained at 37° ± 0.5°. All experiments were performed on a 17.2 T/26 cm Bruker BioSpec MRI scanner. Anatomical images were used for positioning. 1H MR Spectra were acquired using LASER on a 50 μL volume of interest (figure 1). Absolute metabolite concentrations were derived using water as an internal reference of concentration and were corrected for relaxation effects. Statistically significant differences between the young and elder cohorts were established using a bilateral Welch's t-test.

Results and conclusions

Figure 1 shows typical 1HMR spectra from a young and an old rat. Figure 2 summarizes some of the metabolic changes observed with brain aging. Notably, significant increases of myo-inositol (+26%) and choline levels (+35%) were found as well as a higher content of NMR-visible macromolecules, consistent with glial activation and neuro-inflammation. Besides, decreases of GABA (-19%) and glutamate concentrations are consistent with a decline of neural function during aging.

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Keywords: Brain, Aging, NMR Spectroscopy, Rat, Ultra, High Magnetic Field, Quantification

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MRI guided ultrasound induced blood-brain barrier disruption for the safe and efficient drug delivery to the brain

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The blood brain barrier (BBB) protects the central nervous (CNS) system by regulating the exchange with the blood, but also prevents promising drugs from accessing the brain in sufficient quantity. Recently, a technique using short sonications of circulating microbubbles has shown its capability to disrupt the BBB locally, transiently and non-invasively, allowing large molecules to access the CNS, and facilitating the delivery of drugs to the brain. To do so, MRI guidance ensures a precise control of the disruption location and deposited acoustic intensity prior to BBB opening. MRI also enables to map and quantify the diffusion of MR contrast agents injected after disruption.
We developed a MR compatible motorized setup for rodent transcranial experiments which allows the displacement of the ultrasound transducer within a 7T MRI preclinical scanner. Coupled to the implementation of a MR Acoustic Radiation Force Imaging (MR-ARFI) sequence allowing the localization of ultrasound focal spot with no damages to the brain, we are able to choose precisely the location of the disruption site prior to the BBB opening, improving the reproducibility of our experiments. The motorization of the transducer also enables performing BBB disruption along arbitrary trajectories or over large regions of the brain, for example to deliver molecules to a whole hemisphere, keeping the other as a control. Finally, being able to move the transducer allows testing the influence of different acoustic conditions on one animal during the same session.
This system, together with quantitative MRI sequences enabled us to study the safety of ultrasound parameters used for BBB disruption. We also measured the dynamic of BBB closure after its disruption and estimated the maximum size of molecules which can cross the BBB thank to this technique.

Keywords: drug delivery, ultrasound, MR guided, Blood Brain Barrier, Blood Brain Barrier opening

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COMPREHENSIVE ANALYSIS OF CONNECTIVITY IN A CENTRAL NUCLEUS

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Fine-scale connectivity patterns of neural circuits are established during development through both formation and elimination of axonal branches and synapses. Understanding the rules governing this process requires synapse-level mapping of neural wiring in order to measure the degree of convergence and/or divergence within a circuit. While such mapping has been possible in the peripheral nervous system, it is challenging to undertake in the brain due to the problem of scale. Most neural tissue is densely packed with fine neural processes extending long distances in three dimensions and with small synaptic structures. One exception is the binaural circuit of the auditory brainstem. This circuit has unusually large synaptic structures called calices of Held, located in the medial nucleus of the trapezoid body (MNTB). In early postnatal life synapse elimination is thought to result in a strict 1:1 connectivity between pre- and post-synaptic neurons. However, using transgenic Brainbow mice harboring distinct color labels in individual neurons, we observe unambiguous instances of 2:1 axonal convergence in the MNTB at mature stages. To quantify and map these multi-innervations, we have developed methods to reconstruct and analyze all ~2500 caliceal synapses of the MNTB from serial brainstem sections imaged with confocal microscopy. At three postnatal stages examined (after sound-evoked activity onset), we observe a constant ~10% fraction of multi-innervated MNTB neurons. Thus, a subset of MNTB neurons appears to resist a transition to mono-innervation. Our analysis reveals that these unexpected 2:1 convergences appear in non-random clusters and

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follow a density gradient within the MNTB. To understand which factors help maintain these convergent binaural axons, we have established methods to retrace them to their origin in intact brainstem tissue, using novel multicolor two-photon microscopy approaches. These approaches should prove useful to analyze connectivity in the MNTB, and cytoarchitecture in other large tissue volumes.

Keywords: circuits and connectivity, whole, nucleus analysis, Brainbow, binaural circuit, circuit maturation

Prdm12 regulates placode and neural crest development in Xenopus laevis embryo

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The neural crest is a transient embryonic cell population that gives rise to a wide variety of derivatives in vertebrates. We are interested in the gene regulatory network orchestrating neural crest early induction at the neural border. We have recently shown that Pax3 is a key actor in this process (1). In a large-scale screening approach, we have identified Prdm12 as an immediate early target of Pax3 in the developing embryo.
The Prdm proteins modulate transcription of target genes and play critical roles in a variety of developmental processes. Prdm proteins contain a N-terminal PR domain related to the SET methyltransferase domain, and zinc finger motifs. Prdm factors may either act as direct histone methyltransferases or recruit histone modifying enzymes to target promoters (2).

In this work, we investigate the role of Prdm12 in Xenopus laevis development. We show that at neurula stage, Prdm12 is expressed in the neural tube and in cephalic placodes. Using gain and loss of function approaches, we show that Prdm12 is important for both placodal development and neural crest migration.

Cécile Milet et al., PNAS 110(14), 5528-33 (2013)
Tobias Hohenauer and Adrian W. Moore, Development 139, 2267-2282 (2012)

Keywords: Prdm12, Pax3, neural crest, placode, xenopus laevis

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Gustatory Sensory Perception in Zebrafish Larva

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Zebrafish larva represents an excellent model organism for studying behavior at the neuronal level because of its smaller nervous system, optical transparency and diverse behavioral repertoire. To study gustatory neural responses and the corresponding motor behavior, we developed an innovative microfluidic chip that enabled the presentation of different gustatory stimuli simultaneously in a well-controlled environment, at different durations with unprecedented spatio-temporal accuracy. This chip was integrated into two-photon microscope to monitor the dynamics of large number of neurons with single-cell resolution in transgenic zebrafish expressing GCaMP.3 in hindbrain and forebrain gustatory areas. Additionally, a behavioral set-up was integrated to record the kinematics of tail movements, enabling direct correlations between gustatory-driven neural changes and the motor outputs. Using these methodology, we find that in the vagal lobe, compounds with different hedonic values activated non-overlapping neural circuits. An increase in the duration of the stimulus linearly correlated with the number of activated neurons in the vagal lobe, and with the probability of inducing a motor behavior. No correlation was observed between stimulus duration and the level of neuronal activity, suggesting that the probability of generating a gustatory-induced behavior depends on the number of neurons activated rather than the relative activity of a specific group of neurons. Altogether, the combination of innovative, cutting-edge techniques allowed us to have a comprehensive and causal correlation between a natural sensory stimulus and the dynamics of significantly larger neural population with single-cell resolution in intact and behaving vertebrate.

Keywords: Zebrafish, Two, photon imaging, GCaMP, Microfluidics, Gustatory stimuli

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Compressed sensing for high resolution MR microscopy of neuronal tissue

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Despite the fact that the modern magnetic resonance imaging (MRI) hardware available today often results in sufficiently high signal-to-noise ratio (SNR) for a single signal average to be acquired, the total experimental time, dictated only by the requirement for sufficient k-space coverage, can be extremely long, prohibiting the very high resolution imaging of live biological systems. In such cases compressed sensing (CS) approaches hold great potential. The use of CS methods to the reconstruction of magnetic resonance images has been reported mainly in clinical settings where it shows great promise in applications requiring fast acquisition such as cardiac imaging [1].
In this work we present the implementation of CS on a high field preclinical scanner and its application to high resolution magnetic resonance microscopy (MRM). Undersampled masks were generated based on the diffusion limited aggregation (DLA) random growth model [2] starting from a fully encoded rapid acquisition with relaxation enhancement (RARE) pulse sequence (distributed by Bruker Biospin). The new acquisition scheme (CS-RARE) reduced the experiment time by more than a factor of two (47%) while preserving the spatial resolution and image contrast. We acquired fully encoded and under-sampled 3D images of buccal and abdominal ganglia of Aplysia californica (25 microns isotropic resolution). For image reconstruction we used a 3D version of the Sparse MRI toolbox [1]. An automatic cell segmentation algorithm allowed us to compare the two sets of images and evaluate the performance of the CS-RARE acquisition. We find that compressed sensing is applicable to imaging live neuronal tissues, allowing significantly shorter acquisition times while providing the image quality necessary for identifying the majority of neurons.

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Witten, T. A. and Sander, L. M., Phys. Rev. Lett., 47, 1981.

Keywords: magnetic resonance imaging (MRI), compressed sensing (CS), magnetic resonance microscopy (MRM), cell segmentation, diffusion limited aggregation (DLA)

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Characterization of cholinergic Ca2+-response of the Kenyon cells of the Mushroom-Bodies reveals a PKA-independent effect of cAMP, by in-vivo functional calcium brain imaging, in Drosophila.

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In Drosophila, the Mushroom-Bodies (MBs) have been implicated in multiple functions, as olfactory learning and memory, locomotor activity, spatial orientation, sleep, and decision making. Notably, the MBs, which express the nicotinic Acetylcholine Receptor (nAchR), receive their main inputs from the cholinergic olfactory pathways, through the Projections Neurons (PNs). In a former study, we have reported that the stimulation of the MBs with the nicotine (an agonist of the acetylcholine, which mimics an olfactory input), generates in addition to the primary response in the dendrites, a delayed secondary response occurring exclusively in the axons (1). Moreover, we have shown that the cAMP signaling pathway is crucial in the modulation of this response (1).
In the continuity of this study, we are characterizing, at the cellular and molecular levels, the nicotine effect on the Kenyon cells (the intrinsic neurons) of the MBs. We use the in-vivo brain imaging approach, based on the Ca2+-sensitive bioluminescent sensor (GFP-aequorin), to record the nicotinic induced Ca2+-response. More specifically we investigate the role of cAMP pathway (e.g.: dnc, rut, PKA), as well as the different channels (VGCC, CNG, K+, etc.) and their relationship to the cAMP pathway, in order to dissect their contribution to the different components of the Ca2+-response and in its modulation. Interestingly, our results bring evidence that cAMP could act independently of the PKA, through CNG.
1) Martin JR, Rogers KL, Chagneau C, Brûlet P (2007). In-vivo Bioluminescence Imaging of Ca2+-Signaling in the Brain of Drosophila. PLoS ONE 2(3): e275.

Keywords: Drosophila, cAMP, bioluminescent sensor, mushroom bodies, invivo calcium imaging

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The neural basis of Motion After Effect in zebrafish larvae

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Visual illusions are useful tools for study neuronal mechanisms associated with sensory perception. An example is the motion after effect (MAE) illusion, where visual exposure to continuous coherent motion (conditioning stimulus, CS) for a period of time induces, following stimulation offset, the illusory perception of motion in the opposite direction.
Towards a more comprehensive theory to explain the mechanisms underlying MAE we used behavioral essays, optogenetics and two-photon imaging of transgenic zebrafish larvae expressing GCaMP3.

Our results showed that following the presentation of a CS, zebrafish larvae generated optokinetic movements in the opposite direction of those induced by CS. This optokinetic MAE-like behavior was independent of the CS velocity, but strongly dependent on CS duration.

Using optogenetics to transiently block eye movements during the presentation of the CS, we showed that neither muscular fatigue nor eye-muscle proprioception feedback played a role in the generation of the optokinetic MAE-like behavior. Furthermore, the MAE-like behavior was also observed at the level of tail movements, suggesting that the MAE-like behavior is most probably generated in a sensory rather than a motor brain area.

Using two-photon calcium imaging of the two main visual centers (retina and optic tectum) we showed that following CS offset, direction selective (DS) neurons in CS direction were strongly habituated in the tectum but not in the retina, suggesting that the MAE-like behavior is generated at the tectal circuit. Furthermore, we observed rhythmic neuronal activations specific to DS tectal neurons in MAE direction. Frequency analysis of these rhythmic activities showed higher power modulations specific for frequencies matching those of the eye movements during the MAE-like behavior.

Based on the obtained results, we created a model in which neurons representing a tectal directional motor command compute the balance between the spontaneous activities of two DS tectal sub-populations.

Keywords: zebrafish, visual perception, two, photon calcium imaging

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High temporal resolution confocal imaging of new near infrared voltage sensitive dyes

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Communication between cells in the central nervous system is encoded in the form of membrane potentials and electrical transients. Optical recordings of these transmembrane potentials have been recorded using Voltage Sensitive Dyes in the recent years. However the optical recording faces multiple challenges in recording spontaneous neuronal activity and small transients without electrical stimulations with high spatiotemporal resolutions. Major challenges include sensitivity, phototoxicity, cytotoxicity and absorption and autofluorescence from the tissues in cases of in vivo recordings. To overcome the challenges, a new set of VSDs in the near infrared spectrum have been developed and the functional validation of the new VSDs are performed using high temporal resolution confocal microscopy. The spectral scan performed using the confocal microscopy equipped with a White light Laser on the new VSDs calibrates and confirms the absorption and emission wavelengths in the near infra red spectrum with an approximate stroke shift of 50-80nm. Preliminary staining and confocal imaging of the VSDs performed in primary neuronal culture shows high fluorescence intensities for low laser power hence reducing the phototoxicity and photobleaching effects. The resonant scanner equipped with the confocal microscope allows high sampling frequency (up to 8KHz in line scanning) with frame rates of 7-10 frames per second (1 frame/100ms). The membrane depolarization is induced in the culture by change in ionic concentration of the environment hence avoiding the use of invasive stimulation electrodes. With this confocal microscopy technique, optophysiological recordings of transmembrane potentials using the new voltage sensitive dyes can be performed with high spatial (uM) and temporal (ms) resolutions with simple and minimally invasive procedure.

Keywords: Voltage sensitive dyes, infrared, confocal imaging

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Functional imaging at neuron scale by MRI

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While the majority of functional magnetic resonance neuroimaging investigations are currently restricted to averaging the signal from thousands of neurons, in the present study we report activation maps with single neuron resolution [1]. By using manganese enhanced magnetic resonance imaging (MEMRI) in combination with custom built coils, optimized acquisition strategies and an ultra-high magnetic field (17.2T) we are able to obtain high resolution 3D functional maps (25μm isotropic resolution) of intact neuronal networks generating the feeding behavior in Aplysia.
First, we established that Mn2+ accumulates intracellularly when injected into the living annimals, or when the isolated ganglia are perfused in vitro. Injected animals were used to study the neuronal responses to sensory stimuli, whereas the in vitro ganglia permitted to image the modulatory effects of specific transmitters. Second, we demonstrated that the intracellular Mn2+ concentration was different among the identified neurons. Third, we found that the method implemented here can be used to distinguish different levels of activity in a single identified neuron in response to different stimuli. Electrophysiological measurements confirmed that the functional properties of the ganglia and of the identified neurons were not altered and therefore that the Mn2+ concentration was nontoxic [2].

To conclude, we introduce MEMRI as a functional imaging method that enables high-spatial resolution recordings of individual neurons within a functional network. Future perspectives include the application of this technique to probe how different neuronal networks interact to generate goal-directed behaviors and how these behaviors are regulated by sensory inputs and learning. Ultimately this approach will help to identify the neuronal plasticity which underlies the transition from goal-directed to compulsive behaviors.
References: [1] G. Radecki, et al, Proc. Natl. Acad. Sci. U.S.A., vol. 111, no. 23, pp. 8667–8672, 2014. [2] I. O. Jelescu, et al, NeuroImage, vol. 76, pp. 264–271, 2013.

Keywords: MEMRI, MRI, ultra, high field, aplysia, neurons, manganese, fMRI, functional imaging, high resolution

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SPECT and DESI brain imaging for the study of a new mouse model for leucopathy and linked neurodegenerative diseases

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Unpublished patch-clamp experiments allowed the identification of an intracellular lactate-activated sodium channel. This putative lactate sensor was located in the axonal membrane and may be involved in the regulation of lactate use. As glucose is also an energy substrate for the axon, we hypothesized that this sensor is rather involved in oxidative stress control than in energetic balance. As predicted, histological studies revealed that knockout mice (sensor KO) have gradual white matter rarefaction with age. We also found lower glutathione concentrations in the brains of KO mice if compared to those of WT mice. In this work, we compared the GSH distribution in the brains of WT and KO mice using in vivo SPECT imaging. We used 99m-Tc-meso-HMPAO as radiotracer for GSH localization and cerebral perfusion, and 99mTc-d,l-HMPAO for perfusion control experiments. Surprisingly, we found a lower uptake of both radiotracers in cerebellum and caudoputamen indicating hypoperfusion in both regions. We therefore propose that the white matter rarefaction in KO mice may be related to a chronic cerebral hypoperfusion. This hypothesis was supported by typical histological features. Analysis of systolic pressure indicates that KO mice were hypotensive but this finding is not sufficient to explain the appearance of brain hypoperfusion. Lactate is an important energy source but it is also a signaling molecule for vasoreactivity in the neurovascular unit. We propose that the altered cerebral blood flow regulation is related to an increased extracellular lactate concentration in our mutant mice. As it has been shown that the gene encoding for this sensor could be methylated at a high frequency, expression loss could be linked to human vascular leucopathy associated to aging and neurodegenerative diseases. To further study the involved molecular mechanisms, we performed MS imaging experiments (DESI-MS) with slides of brains of WT and KO mice.

Keywords: Brain, Neurodegenerative diseases, Leucopathy, SPECT, DESI

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General principles governing the development of tectal neurons receptive fields in the zebrafish larva.

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During development functional properties of visual neurons are highly plastic, undergoing progressive refinement before reaching a mature state. However, despite the large and continuous neurogenesis processes, developing organisms need to maintain stable neuronal representation of object features (position, size or motion direction) to perform relevant visually guided behaviors. Here we asked what are the governing principles that rule the development of visual properties of tectal neurons at the population level.
For that purpose, we used two-photon calcium imaging of HuC:GCaMP5G zebrafish larvae to study the stability and dynamics of receptive fields (RFs) and direction selectivity (DS) changes of large neuronal ensembles in the optic tectum (up to ~500 neurons). The experiments were performed in developing 6-7 days post fertilization (dpf) larvae every 4 hours for a period of up to 12 hours.

Preliminary results obtained at single time points indicate that tectal neurons that are sensitive to discreet portions of the visual field displayed robust RFs (low variance across trials). Conversely, neurons that were close to the neurogenic sites of the OT showed variable RFs across trials and their RFs were not tuned towards specific regions of the visual field (non-single gaussian multimodal RFs).
Our working hypothesis is that cells displaying robust RFs already underwent synaptic pruning and posses few strong synaptic connections. On the contrary, neurons with variable responses (immature neurons) posses numerous weak synaptic connections. Ongoing studies will shed light on neuronal principles underlying the maturation of functional neuronal properties (RFs and DS) along the development of the visual system.

Keywords: zebrafish, 2, photon imaging, visual system, development

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Harnessing multiplex genetics to actuate and archive neural activity in the circadian clock circuit of Drosophila

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Photo-actuators and optical sensors of neural activity galvanized circuit neuroscience research. By trapping into the unparalleled sophistication of the genetic toolkit that the fruitfly offers, the existing limits of optophysiology are being pushed forward. We harnessed heat to acutely alter neural activity and employed GFP for longer-term monitoring of circuit activity at single-cell resolution in the circadian clock network of flies, which controls the sleep-wake cycles. We will present behavioral and physiological data specifically assessing the logic of hierarchical network operation in a rhythmogenic model microcircuit.
Please consider only for ORAL PRESENTATION

Keywords: CaLexA, thermogenetics, TANGO mapping, Drosophila

*Speaker

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