TITLE: **Investigating sensory processing in migraine models: from cellular and circuit mechanisms to brain-wide dynamics**

ABSTRACT:
Migraine is a common brain disorder characterized by a global dysfunction in multisensory information processing and an alteration of the gain in sensory and/or higher-order associative areas (1). Genetic mouse models of familial hemiplegic migraine (FHM) represent a unique opportunity to obtain mechanistic insights into the brain dysfunctions causing migraine. Our previous studies in brain slices, showing enhanced excitatory neurotransmission in FHM models (2,3), point to pathogenic mechanisms where an impaired excitation/inhibition (E/I) balance in specific cortical circuits, including those that reciprocally connect different cortical areas involved in sensory processing, could lead to both altered sensory gain/perception and to susceptibility to the cortical spreading depression (CSD) at the origin of migraine symptoms.
To investigate FHM alterations in these cortico-cortical circuits and the underlying mechanisms, the project adopts a multiscale approach covering the range from cellular and microcircuit analysis in brain slices to the characterization of the brain-wide signals in awake animals. Experimental methods including high resolution and mesoscale functional imaging, patch-clamp and multisite electrophysiology and optogenetic manipulations will be combined with advanced analytical and computational approaches to achieve two aims.
Aim 1. It has been shown that top-down inputs to sensory cortices from higher order and motor areas mainly target the apical dendrites of pyramidal cells; NMDA glutamate receptors (NMDAR) located in these dendrites play a critical role in amplification and plasticity of synaptic inputs, in sensory perception, and in CSD initiation. **Experiments in acute brain slices and awake animals will be combined to investigate whether, upon either electrical or optogenetic stimulation of cortico-cortical projections or sensory stimulation, activation of NMDARs and generation of NMDA and calcium spikes in apical dendrites of pyramidal cells are facilitated in barrel cortex (S1) of FHM mice. We will also investigate the consequences of the FHM alterations on the spatiotemporal dynamics of spontaneous and sensory-evoked network activity in S1.**
Aim 2. To link the FHM alterations at the cellular and circuit level to the brain-wide dynamics associated with sensory processing (and possibly CSD), **we will investigate whether the FHM models show alterations in the long-range spatiotemporal patterns of cortical activity in resting state conditions and during sensory stimulation in awake animals**. Resting state functional connectivity and **analysis of sensory-induced brain-wide activity will be complemented with optogenetic mapping methods to identify non-sensory related or secondary areas recruited by the activation of specific regions and to evaluate the alterations in effective connectivity for FHM models**.

REFERENCES:

1. Brennan KC and Pietrobon D. A systems neuroscience approach to migraine. (2018) Neuron 97:1004-21
2. Tottene et al. Enhanced excitatory transmission at cortical synapses as the basis for facilitated spreading depression in Ca(v)2.1 knockin migraine mice. (2009) Neuron 61:762-73
3. Capuani et al. Defective glutamate and K+ clearance by cortical astrocytes in familial hemiplegic migraine type 2. (2016) EMBO Mol Med 8:967-86

PARTICIPANTS (PI and co-PIs):

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EXPERIMENTAL DATA:

To be acquired	x
Already acquired (ready to be used)	

Methods
Aim 1.
-**Patch Clamp Recordings** in acute cortical slices using electrical or optogenetic stimulation of specific subpopulation of neurons are ongoing and have approved protocols.
-**Local field potential and multiunit activity (LFP/MUA) recordings** using implantable multisite neural probes (a commercial multielectrode array with 32 recording sites distributed along the cortical depth and a novel probe with 256 microelectrodes arranged in a two-dimensional array) and parallel recordings of spike activity from specific neurons with juxtacellular patch-clamp electrode in awake animals in resting state condition or during optogenetic activation of specific sub-populations of neurons.
The required protocols for recordings in awake animals are not yet approved (expected response time 2-3 months) and the experimental setup is expected to be available in 3 months.
-**2P functional imaging** of the spatial patterns of calcium dynamics in slice and in awake animal in combination with optogenetic or sensory stimulation. The experimental setup is available (except for optogenetic stimulation); the required protocols are not yet available (expected response time 2-3 months).
Aim 2
-**Mesoscale brain-wide recordings**, for collection of brain-wide cortical activity (area of 15x15mm^2 sampled at 250 fps with a resolution of 512x512, bandwidth of the reconstructed signals with intrinsic optical imaging+iGluSnFr: 0.01-20Hz) in resting state condition and during sensory stimulation (whisker/visual flash) in awake animals.
-**Optogenetic Brain Mapping**: for collection of brain-wide cortical activity (area of 15x15mm^2 sampled at 250 fps with a resolution of 512x512, bandwidth of the reconstructed signals with intrinsic optical imaging+iGluSnFr: 0.01-20Hz) in response to the targeted optogenetic activation of 2D-subregions of interest on the cortical surface of awake animals (ChR2 and Chrimson-R)
-**LFP/MUA Brain Recordings** at 256 (16 x16) sites along the cortical depth at 650Hz whole frame sampling rate over an area of 250 μm^2 in awake animals in resting state condition or during the presentation of sensory stimuli, possibly in combination with a targeted optogenetic activation of specific sub-populations of neurons.
The required protocols for recordings in awake animals are not yet approved (expected response time 2-3 months) and the experimental setup for the Aim 2 recordings is expected to be available in 3 months.

ETHICS COMMITTEE:

Obtained	
Conditioned submission*	Expected time response (in months):
Not required	

* request will be submitted only if a PhD student will be associated to the project