Gian Michele Ratto: Short Term Mobility 2016

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Final Report

During my staying in Boston I have laid down the basics of a future collaboration with the group led by Prof. Michela Fagiolini and Takao Hensch. This collaboration is based on a study that is being completed in my lab in Pisa, where we have developed and demonstrated a novel technique for the measurement of the intracellular pH and concentration of Chloride. Chloride ($[Cl^-]_i$) and pH (pH_i) are fundamental regulators of neuronal excitability, and they exert wide-ranging effects on synaptic signaling and plasticity, and on development and disorders of the brain. The ideal technique to elucidate the underlying ionic mechanisms is quantitative and combined two-photon imaging of $[Cl^-]_i$ and pH_i, but this has never been performed at the cellular level *in vivo*. Here, in Pisa we have demonstrated a genetically-encoded fluorescent sensor that includes a spectroscopic reference (an element insensitive to Cl^- and pH). We have designed a method that fully corrects for this source of error. By using this tool we have shown that ratiometric imaging is strongly affected by the optical properties of the brain. In Pisa we have employed in vivo two photon microscopy to measure changes in during post-natal development, data that have been eagerly awaited for about 20 years. My activities in Boston have been directed to three goals: 1) Extending this method to wide field imaging to evaluate the spatial spread of inhibition in sensory cortices; 2) Using this method to resolve GABAergic activity during sensory activity in the visual cortex; 3) Opening up the way to the study of Chloride homeostasis during the postnatal development of the cortex in murine models of autism.

1) We have defined a method that allow the computation of the fractional change of intracellular chloride by observing changes of the sensor fluorescence upon 1-photon stimulation at a single wavelength. With this method we have observed the timing and spatial extension of inhibition during visual activity and the effect of diazepam.
2) We collected preliminary data indicating the putative role of Cl currents in the definition of orientation selectivity.
3) We have designed a study on the role of chloride regulation in the etiology of Rett syndrome, a genetic disease carrying significant similarities to most diseases of the autistic spectra. There are several indirect biochemical evidence that chloride homeostasis might be altered in patients, but there is no direct evidence of this important fact either in patients or in mouse models of the disease. In this study we will collect the first direct evidence of an involvement of chloride regulation in a brain disease. One preliminary experiment suggested that, as predicted by our working hypothesis, chloride concentration might be abnormally high in late juvenile Rett mice, leading to altered inhibitory synaptic transmission. This preliminary studies are leading to the preparation of a joint grant application, that hopefully will support these studies in the next few years.