Annex A3

University of Genova – Italian Institute of Technology

Doctoral School on "*Life and Humanoid Technologies*"

Academic Year 2012-2013

Doctoral Course on

"Neuroscience and Brain Technologies"

Research Themes

17 positions available with scholarship

INTRODUCTION TO RESEARCH THEMES

Candidates are asked to prepare a research project of their choice related to one or more topics of the themes listed below. The soundness and originality of the project will be part of the evaluation process.

Presynaptic disorders in a model of human hereditary epilepsy

Tutor: Pietro Baldelli (pietro.baldelli@iit.it)

A new form of familial epilepsy characterized by a non-sense mutation in the SYN1 and 2 gene was recently reported. Given that, mice lacking Synapsin I and II show an epileptic phenotype, with a late onset of the spontaneous seizures it is likely that these mutations are the cause of the human phenotype. The main research aim of the project will be clarify the role played by the SynII deletion in epileptogenesis characterizing SynII KO mice at various developmental ages. The epileptic phenotype of the Syn KO mice suggests the existence of neuronal excitability imbalance attributable to a differential contribution of Syn II at inhibitory versus excitatory synapses. Inhibitory GABAergic transmission will be studied in hippocampal slices prepared from WT and Syn II KO mice by patch-clamp recordings. Moreover, we further intend to characterize the possible effects of a synaptic dysfunction on the hippocampal network using a high-density microelectrode array.

Optogenetically driven homeostatic plasticity in epileptogenesis

Tutors: Pietro Baldelli, Fabio Benfenati (pietro.baldelli@iit.it fabio.benfenati@iit.it)

Homeostatic processes have been proposed to explain the discrepancy between the dynamics of synaptic plasticity and the stability of brain function. Recently developed optogenetic technologies offer the promise of non-invasive controlling of specific neuronal functions. Although network-wide excitation triggers compensatory homeostatic changes, it is unknown whether neurons initiate homeostatic synaptic changes in response to cell-autonomous increases in excitation. We plan to employ optogenetic tools to cell-autonomously excite dissociated excitatory and inhibitory neurons to investigate the compensatory homeostatic processes. Using optogenetic approach and patch clamp-recordings, we intend to explore mechanisms of homestatic plasticity activated during epileptogenesis and seizures, to develop alternative treatment strategies for epilepsy based on the up-regulation of homeostatic processes. This research will help in better understanding of the mechanisms for epileptogenesis and might lead to development of new gene therapy-based strategies for epilepsy prevention.

Development and application of opto-neural interfaces and prosthetic devices based on organic electronics (2 positions available)

Tutor: Fabio Benfenati (fabio.benfenati@iit.it)

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The goal of the project is to create an opto-neural interface made of organic semiconducting polymers allowing light-evoked excitation/inhibition of targeted neural cells populations, both *in vitro* and *ex vivo* in explanted retinas. The proposed activity is of the upmost importance toward the realization of a broader project, i.e. the fabrication of an implantable Organic-based Prosthetic Device (OPD) for the treatment of genetic degenerative diseases of the retina, aiming at restoring vision in blind people. We expect to set-up an efficient bio-organic photovoltaic interface to mimic the light sensitivity, transduction efficiency, spectral characteristics, spatial and temporal resolution of the intact neuronal network of the retina. We also expect that this prosthesis activates retinal ganglion cell activity upon physiological illumination in explants of degenerated retinas and that, once coupled to a suitable substrate for implantation, can rescue vision in experimental models of retinal degeneration. The project will study the impact of photovoltaic organic polymers on primary neuron physiology in vitro and in vivo, adopting standard live imaging and electrophysiological techniques, i.e. patch-clamp and multi-electrode array recordings. The same opto-neural interfaces will be tested on retinal explants and eventually implanted in vivo in experimental models of photoreceptor degeneration. The ideal applicant should have a background in cell and slice electrophysiology extended to the visual system and to the neurophysiology of the retina.

Investigating the inhibitory-excitatory balance in neuronal networks by multi-modal electrophysiological imaging

Tutor: Luca Berdondini (luca.berdondini@iit.it)

The balance among excitation and inhibition within cortical microcircuits plays an important role in information-processing. Furthermore, it is altered by several brain diseases. However, the interplay among these network constituents is not completely understood and the investigation at the cellular scale within large networks is precluded by their complexity and accessibility.

Based on our recent development of an innovative electrode-based imaging platform, we intend to investigate at cellular/sub-cellular scales the balance among excitatory and inhibitory circuits within

large neuronal networks of controlled size. This can be achieved by combining our high-resolution MEA platform with fluorescence microscopy to identify and characterize the electrical activity of the tightly interrelated cellular circuits.
We are looking for a Ph.D candidate with computational skills and with a strong interest in developing experimental and analysis methods to dissect these network components and to characterize them over long-term timescales and under basal and altered conditions.

Cellular determinants of brain circuit development and wiring
Tutor: Laura Cancedda (laura.cancedda@iit.it)
The goal of the laboratory is to understand the molecular determinants of brain circuit development. In particular, we are interested in how extracellular factors and especially GABAergic signaling modulate events such cell proliferation, polarization, migration, morphological maturation and network wiring in health and disease. To achieve this goal, we use a combination of *in vivo* and *in vitro* approaches (*in vitro* electrophysiology, biochemistry, confocal microscopy, molecular biology and behavioral testing), and focus on different brain areas.

Presynaptic targeting of optogenetic probes
Tutor: Fabrizia Cesca (fabrizia.cesca@iit.it)
Optogenetics offers the possibility of high-speed mapping of brain circuitries with high spatial and temporal resolution. The range of currently available optogenetic constructs still lacks functional opsins targeted to the presynaptic compartment. We engineered a range of presynaptic-targeted excitatory and inhibitory opsins (i.e. ChETA, CatCh, Arch) by employing the synaptosomal-associated protein 25 (SNAP-25) as a targeting motif. The project aims at characterizing these probes in a range of applications. We will test the ability of our constructs to modulate the excitability of neural networks *in vitro*. In addition, we will evaluate how they can modulate neuronal circuits *in vivo*, by injecting them in specific brain areas in living rodents. We are also planning to expand the panel of presynaptic-targeted probes, to include the recently described LiGluR (*l*igand- and *l*ight-activated *i*onotropic *g*lutamate *r*eceptor), a synthetic photoswitchable ion channel whose activity can be reversibly modulated by illumination.

Characterization of the neurodynamics of in vitro engineered networks to be interfaced with computational artificial systems
Tutor: Michela Chiappalone (michela.chiappalone@iit.it)
The study herewith proposed is part of the BrainBow project (www.brainbowproject.eu), funded by the European Commission. The BrainBow project will provide a proof-of-concept for next generation neuroprostheses aimed at restoring lost functions at the level of the Central Nervous System. The PhD project will aim at i) building single or multiple in vitro engineered networks and ii) perform experiments on those networks, either in physiological or pathological condition (i.e. in presence of a lesion disconnecting one cell population). The project requires expertise in culturing and maintaining neurons from different brain districts, use of the micro-electrode array set-up, development of software tools for data analysis of multichannel signals and good attitude towards manual work. The ideal candidate should hold a degree in biomedical engineering or a related discipline, be a highly motivated and creative individual who wants to work in a dynamic, multi-disciplinary research environment. Former lab experience will be highly considered.

Alpha-synuclein interaction with the plasma membrane of neurons.
Tutor: Evelina Chieregatti (evelina.chieregatti@iit.it)
Alpha-synuclein (Syn), a cytosolic protein enriched at pre-synaptic terminals, is released from neurons and participates in the spreading of Parkinson's disease pathology. Based on our results that show distinct effects of Syn on actin cytoskeleton, whether Syn was electroporated in neurons, or delivered as a purified protein in the medium, we hypothesize that Syn may interact with a component of the external surface of the neuronal membrane. Syn binding to the membrane and its possible localization in specialized micro-domains would activate an intracellular pathway leading to changes in cytoskelatal dynamics and to synaptic dysfunction. Neuronal cultures derived from Syn knocked-out mice incubated with recombinant purified human Syn will be employed to analyze the levels and the phosphorylation state of proteins of the signaling cascade mediating the observed cytoskeletal alterations. We have identified a possible receptor for Syn, and its involvement in Syn effect will be analyzed by the use of blocking antibodies and silencing RNAs. The domains of the two proteins necessary for their binding will be defined by two hybrid system and point mutations will be introduced in the two proteins sequences to inhibit their interaction and to reverse Syn-induced phenotype.

Two-photon holographic microscopy for the optical dissection of cortical networks
Tutor: Tommaso Fellin (tommaso.fellin@iit.it)
The project focuses on the development of a two-photon microscope combined with structured light illumination for the functional investigation of neocortical microcircuits. The work is based on recent technical developments achieved by our group (Dal Maschio et al. Optics Express 2010, Dal Maschio et al. Optics Letters 2011). The PhD project will require hardware and the software development to efficiently integrate liquid crystal spatial light modulators with experimental setups for electrophysiology and imaging recordings. The project requires good knowledge of optics, optical engineering (in particular for fluorescence imaging application), established programming skills, graphic processor unit experience and good attitude towards manual work. The ideal candidate should hold a degree in applied physics, engineering or a related discipline and be highly motivated and creative individual who wants to work in a dynamic, multi-disciplinary research environment.

Modeling and simulations of optogenetic probes to modulate gene expression and neuronal excitability
Tutor: Luca Maragliano (luca.maragliano@iit.it)
The project will involve structural modeling and molecular dynamics simulations of optogenetic probes, and it is part of a joint task with the experimental group to ultimately clone the probes and use them in primary neuronal cultures and in vivo. Specific applications will be the control of gene expression and of extracellular pH levels. In the first case, chimeric probes will be engineered by fusing light-activatable domains to peptides interacting with silencing factors, while in the second case pH-dependent fluorescence proteins will be fused to rhodopsins. The computational study involves 3D modeling of the structures followed by molecular dynamics simulations, in particular potential of mean force calculations, to obtain information on the ensemble of conformations explored by the chimeras. Results from the simulations will guide experimental engineering of the proteins, while feedback from the experiments will be utilized to rationally improve their design

Cellular and synaptic mechanisms of lesion-driven plasticity in cortical circuits in vivo
Tutor: Paolo Medini (paolo.medini@iit.it)
Scientific project: We are searching a motivated and talented Phd student to investigate the plasticity of neuronal circuits after both peripheral and central lesions in the visual system in vivo within the multidisciplinary environment of the Italian Institute of Technology. At this aim, the candidate will use state-of-the-art techniques such as in vivo patch clamp recordings, two-photon microscopy and optogenetics. In particular, we will compare the plasticity in the different cell types composing visual cortical circuits with the aim to design future behavioural read-outs. The candidate should have completed her/his master studies in Medicine, Physics or Biology or Bioengineering.

Role of cell-type specific cortical connectivity in circuit functioning and plasticity
Tutor: Paolo Medini (paolo.medini@iit.it)
Scientific project: We are searching for a talented and smart student willing to learn different, state of the art approaches (in vivo patch and calcium imaging) to study the functioning and plasticity of the connections emanating from identified cell types in cortical microcicuits in vivo. In particular, the study will focus on the role of long-range, interareal connections in driving the animal behaviour. The Phd program and the courses will be within the multidisciplinary environment of the Italian Institute of Technology. The candidate should have completed her/his master studies in Medicine, Physics or Biology or Bioengineering.

Study of cognitive dysfunctions relevant to schizophrenia in mice
Tutor: Francesco Papaleo (francesco.papaleo@iit.it)
The overall goal of this project is to understand the genetic bases that cause the development of cognitive abnormalities relevant to schizophrenia neuropathology. While several schizophrenia-susceptibility genes have been identified, effect sizes are very small and replication is inconsistent, likely because of the complexity of human polymorphisms, genetic and clinical heterogeneity and the potential impact of gene-gene and gene-environment interactions. In this context, mutant mice bearing targeted mutations of schizophrenia-susceptibility genes are unique tools to elucidate the neurobiological basis of this devastating disorder. Using genetically modified mice for genes relevant to schizophrenia, we will employ a combined approach beginning at the behavioral level and culminating at the cellular and molecular levels. Cognitive abnormalities are core enduring symptoms in schizophrenia, dramatically contribute to poor functional outcomes in patients and currently represent a great “unmet therapeutic need”. This project is then focused on behavioral cognitive analyses and relative neurobiological correlates.

Cognitive Impairment in Polyglutamine Diseases
Tutor: Maria Pennuto (maria.pennuto@iit.it **)**
Spinal and bulbar muscular atrophy (SBMA) is a motor neuron disease caused by expansion of the CAG trinucleotide repeat, encoding a polyglutamine (polyQ) tract, in the androgen receptor (AR). SBMA belongs to the family of polyQ diseases, which also includes Huntington’s disease, DRPLA, and six types of spinocerebellar ataxia. Evidence from psychological studies indicate that patients with these disorders show cognitive symptoms even before the onset of a full blown neurological disorder. To identify the brain mechanism through which the expanded polyQ tracts cause cognitive impairment, we plan to use

a translational and multimodal approach. We will investigate structural and functional neural correlates of analog mutations in human healthy subjects using an imaging genetics approach. We expect to identify novel genetic variants in polyQ diseases and motor neuron disorders.

Transcription dysregulation in motor neuron diseases
Tutor: Maria Pennuto (maria.pennuto@iit.it **)**
Brain misfolding diseases are a large family of neurodegenerative disorders, which includes Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS) and spinal and bulbar muscular atrophy (SBMA). These disorders share several features, including late age at onset, accumulation of misfolded proteins, and neuronal toxicity. ALS and SBMA are motor neuron diseases, characterized by the progressive loss of motor neurons (upper and lower in ALS, lower in SBMA), and skeletal muscle weakness, fasciculation, and atrophy. The mechanisms underlying neuronal dysfunction and death remain to be elucidated. In this project, we propose to test the hypothesis that transcription dysregulation is responsible for neuronal damage. We will explore the details of gene transcription dysregulation in the pathogenesis of ALS and SBMA, as models of neurodegeneration. We expect to identify classes of gene whose expression is altered in cell and animal models of these diseases. Using microarray, real-time PCR, and seq-PCR analyses, we will identify changes in gene expression that occur early in disease pathogenesis. The effect of dysregulation of specific genes will be explored in details with molecular, cellular, and structural analyses.

Neuronal circuit integration in the physiology and pathophysiology of action control
Tutor: Raffaella Tonini (raffaella.tonini@iit.it **)**
Our research interest is to study how neuronal circuits integrate to control behaviour. We are primarily focused on the neuromodulatory mechanisms regulating the functional connectivity between the cortex and striatal regions, which play a major role in sensory-motor integration and in reward-based learning.
The specific aims of our research address how neuromodulatory pathways (dopamine and endocannabinoids) act in a coordinated manner to shape synaptic- and network plasticity at defined corticostriatal circuits and their impact on action plans and adaptation (e.g. learning of goal-directed actions, their flexible use and how they become habitual). Addressing this issue is instrumental to understand how these behaviours are formed and maintained, but also how they are modified in diseases which affect aspects of cognition and motivation, such as drug addiction or compulsive disorders.
Experiments will be performed by an integrative approach combining neurophysiological techniques (electrophysiological recordings, optogenetic and calcium imaging) with *ex-vivo* and *in-vivo* pharmacology and behavioural analysis.