

Annex A3
University of Genova – Italian Institute of Technology
Doctoral School on “*Life and Humanoid Technologies*”

Academic Year 2011-2012

Doctoral Course on
“Neuroscience and Brain Technologies”

Research Themes

26 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.

ROLE OF GABA_AR MOBILITY ON GABAERGIC PLASTICITY

Tutor: Andrea Barberis (andrea.barberis@iit.it)

In the past decade it has been established that the weight of GABAergic synapse is shaped by activity, a property commonly referred as synaptic plasticity. This notion is crucial in the understanding of the functioning of neural network. It has been established that GABAergic plasticity is expressed and induced at both pre- and post-synaptic sites. The PhD project will be aimed to study the post-synaptic mechanisms underlying the potentiation of GABAergic synapses by investigating the role of GABA_A receptor (GABA_AR) diffusion. Using the single particle tracking technique, he/she will focus on the interaction of GABA_AR with scaffold proteins such as gephyrin and collybistin in both basal conditions and following plasticity induction. The novelty of this project is that, in order to induce plasticity we will use protocols mimicking physiological patterns, whereas, to date, the GABA_AR mobility in plasticity conditions has been studied under conditions of non-specific chemical-induction. This study will help understanding the relationship between the GABA_AR diffusion and the modification of the synaptic strength.

SYNAPTIC VESICLE DYNAMICS IN HEALTH AND DISEASE

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

Synaptic vesicle (SV) exo-endocytotic cycle is the fundamental process that allows neuron communication in the brain. SV trafficking is orchestrated by a complex protein machinery which precisely regulate each step of the cycle. SV cycling takes place at both inhibitory and excitatory synapses, but little is known on the differences in SV trafficking mechanisms between them, although GABAergic and glutamatergic synapses differ both morphologically and functionally.

The project is aimed at elucidating the different mechanisms of regulation of SV cycle, of the establishment, maintenance and kinetic of depletion of SV pools at inhibitory versus excitatory synapses using live imaging technique.

Particular effort will be posed on the role of the protein products of two epilepsy genes, SYNAPSIN and TBC1D24, on the regulation of SV cycling in GABAergic and Glutamatergic synapses in order to elucidate molecular mechanisms that cause excitation/inhibition imbalance and seizures in patients carrying SYNAPSIN or TBC1D24 mutations.

FUNCTIONALIZED HIGH-RESOLUTION NEUROELECTRONIC INTERFACES.

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

The project will focus on the development and experimental validation of functionalized high-resolution electrode arrays for interfacing neuronal networks and acute brain tissue with unique signal quality. In particular, bio-functionalized nano-materials and micro-/nano-technologies will be investigated for realizing electrodes enabling to achieve very high neuron-electrode couplings on large arrays implemented by CMOS technology. The overall project, in collaboration with commercial and academic EU partners, targets applications in retinal prosthetics. The ideal candidate has an engineering University degree either in physics, bio-medical engineering, micro-/nano-technology, microelectronics or equivalent.

DEVELOPEMNT OF A MULTI-MODAL EXPERIMENTAL IMAGING PLATFORM FOR ELECTROPHYSIOLOGY BASED ON HIGH-RESOLUTION ELECTRODE ARRAYS.

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

Recent developemnts of our laboratory have demonstrated an innovative electrode-based imaging platform for research on neuronal networks and brain tissue. In order to advance the current platform we are looking for a Ph.D candidate with strong computational skills to develop experimental and analysis methods. In particular, we propose to investigate the combination of electrode-based and light-based imaging techniques to better describe functional and anatomical circuits in large networks and cellular assemblies. The work will be carried out within an interdisciplinary team composed by biologists and engineeres and the candidate will be carrying out both experimental and computational developments. Ultimately, this project targets the real-time implementation of advenced processing tools for in-vitro screening applications.

POLYMER ELECTRONICS CATCH THE BEAT: DESIGN AND FABRICATION OF ALL-POLYMER TRANSDUCERS FOR NEUROPROSTHETICS

Tutor: Axel Blau (axel.blau@iit.it).

Neural prostheses target at the capture, modulation or elicitation of neural activity to record the information flow within a neural pathway for its online or later decoding, or to mimic or replace neural functionality that has been compromised or lost. The PhD research activity will be centered on the design, fabrication, post-processing, functionalization, optimization and validation of neural microelectrode arrays entirely made out of polymers. Microstructured templates will be designed and fabricated in IIT's clean room environment (~550 m²), from

which diverse polymer microchannel scaffolds can be molded. They will be rendered electrically conductive by conductive polymers and then characterized optically (microscopy, spectroscopy) and electrochemically (impedance spectroscopy, cyclic voltammetry). A multitude of device designs with add-on functionality (*e.g.*, drug delivery) will be evaluated and validated in diverse neurobiological contexts (*in vitro* and *in vivo* as long-term implants). Their compatibility with optical material and neural network manipulation technologies (optical tweezers, laser microdissector) will be explored. This highly interdisciplinary project offers the opportunity to be trained in various fields including microfabrication technology, material science and neuroscience.

ROLE OF GABA_A-RECEPTORS IN GENERALIZED EPILEPSY

Tutor: Laura Cancedda (laura.cancedda@iit.it).

About 1.0% of general population suffers from a neurological condition called epilepsy. This disorder is characterized by an unbalance of excitation and inhibition tipped towards excess excitation. As GABA_A receptors (GABA_AR) control inhibitory electrical brain activity, it is understandable why different GABA_AR subunits are common targets for mutations/decreased activity associated with epilepsy. However, as GABA plays a pivotal role in normal brain development it is also possible that part of the effects in subjects carrying epileptogenic GABA_A receptor mutations/defective GABA_A transmission be due to brain maldevelopment. In our work, we will focus on different models of generalized epilepsy. Using *in utero* electroporation and electrophysiological methods, we will investigate an influence of altered GABAergic transmission on cortical migration, morpho/physiological neuronal maturation and cortical network wiring.

CELLULAR DETERMINANTS OF DEVELOPING-NETWORK 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. 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.

ALPHA-SYNUCLEIN ROLE IN AXONAL ELONGATION AND REGENERATION AFTER INJURY.

Tutor: Evelina Chierregatti (evelina.chierregatti@iit.it).

Alpha-synuclein (Syn), a cytosolic protein enriched at pre-synaptic terminals, is involved in the pathogenesis of Parkinson's disease. Based on the results showing the interaction of Syn with three cytoskeletal elements, the hypothesis is that Syn would have a role in neuronal differentiation and in axonal regeneration after injury. Although it has been proposed that Syn functions mainly in neuronal maintenance, a recently generated conditional transgenic mouse demonstrated the key role of Syn in adult neurogenesis.

Neuronal cultures derived from Syn transgenic and Syn knock-out mice will be employed to study an effect of wt and mutant A30P Syn on regeneration after laser-assisted axotomy. BDNF-coated beads in combination with the optical tweezers technique will be used to study the role of Syn in growth cone turning. Intermediate filaments and microfilaments remodeling after axotomy will be analyzed following EGFP-tagged vimentin and actin dynamics in neurons before and after transfection.

NEUROGENESIS IN 22Q11.2 DELETION SYNDROME: FOCUS ON microRNAs

Tutor: Davide De Pietri Tonelli (davide.depietri@iit.it).

The 22q11.2 Deletion Syndrome refers to a group of related syndromes associated by a hemizygous microdeletions occurring on chromosome 22. Individuals affected by 22q11DS show a highly variable spectrum of phenotypes that cognitive deficits and increased risk of schizophrenia. Recently, abnormal prenatal neurogenesis has been addressed as possible cause of some neuro-pathological aspects of 22q11DS. MiRNAs are short, noncoding, single stranded RNAs that modulate gene expression at the posttranscriptional level by either repressing translation or stability of targets mRNAs. Interestingly, miRNAs are rapidly emerging as a new layer of regulation of neurogenesis.

The research project will focus on the role played by miRNAs in the control of neurogenesis in both physiological and pathological conditions (e.g. 22q11.2DS). The project will be implemented mostly in animal models (e.g., acute manipulations and genetically-modified rodents).

ROLE OF microRNAs IN MOUSE NEUROGENESIS

Tutor: Davide De Pietri Tonelli (davide.depietri@iit.it).

MiRNAs are short, noncoding, single stranded RNAs that modulate gene expression at the posttranscriptional level by either repressing translation or stability of targets mRNAs. Recently, miRNAs have rapidly emerged as a new layer of regulation of neurogenesis. Currently, more than 600 miRNAs have been identified in the mouse. Many of those have conserved target sites in mammalian mRNAs, of which the vast majority awaits experimental validation. The research project will focus on the role played by miRNAs in the control of neurogenesis and neuronal network formation. The project is primarily aimed at the identification and validation of miRNA target genes. This project will be initially implemented in vitro (e.g., primary stem cell cultures, iPS and 3D cell culture systems) and in a second phase in vivo (e.g., acute manipulations and genetically-modified rodents).

DISEASE MODELING AND CELL THERAPY USING HUMAN FIBROBLAST-DERIVED NEURONS

Tutor: Alexander Dityatev (alexander.dityatev@iit.it).

Modern technologies for generation of human neurons either by direct reprogramming or through production of induced pluripotent stem cells, followed by their differentiation into defined neuronal subtypes, open new possibilities for 1) electrophysiological and pharmacological analysis of synaptic transmission between neurons derived from patients suffering from genetic disorders and 2) transplantation of defined subtypes of human neurons in diseased brains. Here, synaptic physiology of neurons derived from Phelan-McDermid syndrome patients will be studied using patch clamp technique and high-resolution MEA recordings. CDPPB, an agonist of mGluR5 receptors, will be tested to restore synaptic functions in these neurons, as predicted by previous studies in primary murine neurons. Second, new strategies will be developed to promote axonal growth of transplanted induced dopaminergic neurons³, so they could effectively reach their natural targets. Infection of neurons with viral vectors encoding for cell adhesion molecules and enzymes digesting repellent extracellular matrix will be employed.

INTEGRINS AND SYNAPTIC PLASTICITY IN HEALTH AND DISEASE

Tutor: Alexander Dityatev (alexander.dityatev@iit.it)

Recent data demonstrates that beta1 integrins regulate consolidation of long-term potentiation while beta3 integrins are important players in homeostatic synaptic plasticity. This project aims to identify extracellular matrix and cell adhesion molecules which signal via beta1 and

beta3 integrins to induce/support different forms of synaptic plasticity and to characterize the impact of human integrin mutations – found in autism spectrum disorders - for these forms of plasticity. The following methodology will be used: cross-linking of integrin ligands, visualization of integrin distribution and signaling in live cells, mutagenesis, knock-down of integrin ligand expression, and patch clamp recordings of synaptic currents.

OPTICAL DISSECTION OF THE CELLULAR DETERMINANTS OF CORTICAL LOW-FREQUENCY RHYTHMOGENESIS

Tutor: Tommaso Fellin (tommaso.fellin@iit.it)

This project aims at investigating how cortical network dynamics *in vivo* are generated by the activity of single cells. More specifically, this study will focus on slow oscillations, a cortical rhythm that characterizes non-REM sleep, and the somatosensory and prefrontal cortices as model systems. Cell-specific expression of light sensitive proteins will be used to activate or inhibit neuronal firing and to causally test the role of specific subpopulations of neurons in the generation and propagation of slow oscillations. Extracellular local field potential, multiunit, juxtosomal and patch-clamp recordings on anesthetized rodents will be performed in combination with optogenetics, two-photon functional imaging and the use of transgenic animals. Initial studies on anesthetized animals will be extended to freely moving and naturally sleeping mice by combining electroencephalographic recordings with optogenetics

DOPAMINE TRANSPORTER KNOCK-IN MICE CARRYING LOSS-OF-FUNCTION MUTATION FOUND IN PATIENTS WITH DOPAMINE TRANSPORTER DEFICIENCY SYNDROME (DTDS)

Tutor: Raul R. Gainetdinov (raul.gainetdinov@iit.it)

Recent identification of hereditary deficiency in the dopamine transporter (DAT) in patients with Dopamine Transporter Deficiency Syndrome (DTDS) (Kurian et al., 2011) provided a unique opportunity for the development of humanized knock-in mouse lines bearing these functional mutations in DAT gene (DAT-KI mice). DTDS is a novel autosomal recessive disorder related to impaired DAT function that characterized by hyperkinesias, parkinsonism or mixed hyperkinetic and hypokinetic movement disorder. At the initial stage, we plan to focus on mutation (Leu224Pro) that causes the most severe phenotype, however in future we plan to develop additional knock-in models with other mutations as well. Development and characterization of these KI mice will allow us to perform detailed investigation of the pathological molecular mechanisms involved in this disorder and generate experimental test systems for finding new treatments for disorders related to DAT dysfunction in general.

AUTOLOGOUS TRANSPLANTATION OF iDA CELLS DEVELOPED FROM FIBROBLASTS TO ANIMAL MODELS OF PARKINSON'S DISEASE

Tutor: Raul R. Gainetdinov (raul.gainetdinov@iit.it)

Recent demonstration of the possibility of direct generation of dopaminergic neurons from fibroblasts opens new opportunities for treatment of Parkinson's Disease (PD). To validate the potential efficacy of autologous transplantation in PD, we plan to perform transplantation of DA neurons developed from the fibroblasts of the same animals to mice with lesion of dopamine system. Different strategies will be attempted to achieve significant transplantation efficiency. Detailed anatomical, neurochemical and behavioral characterization will be performed in these mice. This project will be performed in collaboration with Dr. Vania Brocoli (San Raffaele Institute, Milan, Italy).

MOLECULAR MECHANISMS OF TAU-DRIVEN NEURODEGENERATION

Tutor: Laura Gasparini (laura.gasparini@iit.it)

Intraneuronal accumulation of tau protein is a hallmark of Alzheimer disease and related neurodegenerative disorders termed ‘tauopathies’. Emerging evidence in transgenic models of tauopathy suggests that abnormal post-translational modifications of tau and alterations of neuronal function occur before tau inclusion formation. Indeed, our results in a retinal model of tauopathy support this view indicating that accumulation of hyperphosphorylated tau and axonopathy precede the development of intracellular deposits of tau filaments. However, it remains unclear to what extent such molecular and anatomical alterations affect neuronal physiology and network connectivity. The student will elucidate functional changes typical of initial stages of tauopathy and identify the underlying molecular mechanisms. To this end, in an integrated approach, he/she will combine state-of-the-art *in vitro* and *in vivo* electrophysiology, molecular/cell biology and high-resolution imaging techniques to detect single cell and network physiological abnormalities arising at different stages of tau pathology in a relevant model of tauopathy.

CIRCUIT PLASTICITY UPON CENTRAL AND PERIPHERAL LESIONS IN VISUAL CORTEX

Tutor: Paolo Medini (paolo.medini@iit.it)

We will explore neuronal plasticity in the visual cortex upon cortical focal lesions or peripheral denervation (retinal scotoma), searching for differential plasticity of excitatory and inhibitory neurons by means of two-photon targeted patch clamp recordings *in vivo*. We will test the hypothesis that compensatory receptive field enlargement is due to reduced activation of cortical inhibitory cells and the role of reactive gliosis in this process.

BEHAVIOURAL AND PHYSIOLOGICAL EFFECTS OF OPTOGENETIC MODULATION OF IDENTIFIED CORTICAL CONNECTIONS IN VIVO

Tutor: Paolo Medini (paolo.medini@iit.it)

We will use viruses capable of labelling neuronal connections to photo-activate or photo-inhibit identified connections in the visual cortex. In particular, we will evaluate: a) the consequences of opto-modulation of cortical connections emanating from the main output cortical layer 5 in simple forms of visually-driven behaviour; b) the impact of horizontal connections among primary and association, multisensory cortices on the animal capability to integrate inputs from different sensory modalities

EARLY DETECTION OF BEHAVIORAL ABNORMALITIES AND NEURONAL CORRELATES IN GENETICALLY MODIFIED MICE

Tutor: Dr. Papaleo Francesco (francesco.papaleo@iit.it).

Early detection and therefore early intervention in schizophrenia and related cognitive deficits might bring new hope for preventing and curing the debilitating features of this neurodevelopmental disorder. Thus, effective tools for detecting high-risk individuals and prodromal stages are much needed. The aim of this project will be to determine the developmental trajectories of specific cognitive/behavioral abnormalities associated with schizophrenia-clinically-relevant genetic mutations. This will be attained through early neurobehavioral characterization of genetically modified mice during critical developmental periods. Ontogenetic studies will be performed first during periods equivalent to infant-childhood ages in human. This will include the analysis of somatic growth indexes, development of somatosensory reflexes, ultrasonic vocalizations and juvenile social responses. It will follow the assessment of cognitive functions during periods equivalent to adolescent-young adulthood, when schizophrenia often start to manifest. The same mice will be also studied for early neurochemical and synaptic dysfunctions directly correlated to the behavioral endpoints.

COGNITIVE IMPAIRMENT IN POLYGLUTAMINE DISEASES

Tutor: Maria Pennuto (maria.pennuto@iit.it)

Polyglutamine (polyQ) diseases are neurodegenerative disorders caused by expansion of polyQ tracts in the coding regions of specific genes. These disorders include Huntington's disease, SBMA and various types of spinocerebellar ataxia. Several genes contain a number of repetitions of the nucleotide triplet CAG, which is translated into a polyQ tract in the native protein. Evidence from psychological studies suggests 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 polyQ tracts can cause cognitive impairments, we plan to use a translational and multimodal approach. Transgenic mice carriers of variable lengths of polyglutamine tracts will undergo cognitive testing. Similarly, we will investigate structural and functional neural correlates of analog mutations in human healthy subjects using an imaging genetics approach

GLUTAMATERGIC AND GABAERGIC INTERICTAL SPIKES AND THEIR RELATIONSHIP TO ICTAL TRANSITION

Tutor: Stefano Taverna (stefano.taverna@iit.it).

Despite the variety of available therapies, the treatment of seizures is not adequately controlled in one third of epileptic patients. New approaches and strategies to cure these treatment-resistant forms of epilepsy are therefore needed. A better understanding of the ground rules that control the transition toward seizures (i.e, the *mechanisms of ictogenesis*) might help develop novel effective remedies for these resistant cases. This project aims to identify biological mechanisms responsible for the interictal state and ictal onset in focal drug-resistant temporal lobe epilepsies. An involvement of GABAergic inhibitory circuits in seizure generation is a novel hypothesis which is supported by evidence obtained by our research team. Based on this evidence, we hypothesize that inhibitory circuits are critically involved in the generation of interictal events promoting transition to ictal discharges and in the generation of seizures in focal epilepsies. Our work will focus on the entorhinal cortex (EC), a parahippocampal region that plays a role in temporal lobe epileptogenesis and ictogenesis.

Ictal patterns can be reproduced in epileptic animal models, permitting studies on how the epileptic zone is organized. Using a variety of electrophysiological techniques on the *in vitro* intact brain preparation of the guinea pig, we will study GABAergic inhibition and interictal events in experimental models of seizures and of epilepsy.

Knowledge on mechanisms derived from this work will be used to identify the most effective sites and patterns of local brain stimulation that will be utilized as a therapeutic tool in controlling seizure occurrence.

miRNAs AND HOMEOSTATIC PLASTICITY

Tutor: Tatiana Tkatch (tatiana.tkatch@iit.it)

Homeostatic synaptic plasticity is important for maintaining the neural circuit activity within a stable range critical for optimal network operation. Adaptations to excessive excitation or inhibition can manifest as changes in the quantity of neurotransmitter release, alterations to the number of neurotransmitter receptors, and changes in synapse morphology caused by reorganization of the actin cytoskeleton. These cellular events require the rapid synthesis of mRNAs and their translation in proteins as well as the local translation of pre-existing mRNAs. Such an on-site protein synthesis is especially important for locally mediated adaptive mechanisms. There is emerging evidence that miRNAs could play a key role in regulation of local synaptic protein synthesis. We have identified a set of dendritically enriched miRNAs, the abundance

of which is changed in response to prolonged inactivity. The goal of the project is to elucidate the role of these miRNAs in homeostatic plasticity.

SYNAPTIC AND NETWORK CORRELATES OF BEHAVIORAL PLASTICITY

Tutor: Raffaella Tonini (raffaella.tonini@iit.it)

The ongoing research projects are designed to specifically address how neuromodulatory pathways (e.g. dopamine and endocannabinoids) act in a coordinated manner to shape bidirectional synaptic plasticity at the level of neuronal circuits relevant for action planning and adaptation. We are primarily focused on the synaptic mechanisms regulating the functional connectivity between the cortex and subcortical regions (dorsal and ventral striatum) in order to understand how goal-directed and habitual behaviours are formed and maintained, and how they are modified in diseases which affect aspects of motor control (such as Parkinson's disease) or cognition and motivation (such as drug addiction).

Experiments will be performed by an integrative approach combining neurophysiological techniques (patch-clamp recordings, optogenetic and calcium imaging) with *in-vitro* and *in-vivo* pharmacology and behavioral analysis.

EPIGENETICS OF SLEEP AND COGNITION

Tutor: Dr. Valter Tucci (valter.tucci@iit.it)

A crucial integrative property of the brain is to combine temporal information across a wide range of timescales (seconds, hours) with a well-organized physiology that regulates inner biological processes (e.g. sleep homeostasis). Evolution has favored biological clocks by means of homologous genetic sequences that sustain both self-sustained periodic oscillations (e.g. circadian) and non-periodic (e.g. timed behaviours) mechanisms. Several evidence suggests that epigenetic (e.g. genomic imprinting) mechanisms play a role in sleep and cognition. The goal of this project is to select novel epigenomic elements which play a role into the modulation of sleep and cognition, to develop appropriate mouse models and to investigate them, by means of electrophysiological, molecular, behavioural and computational approaches. Overall, the project involves *in silico*, *in vitro* and *in vivo* studies and the ideal candidate should be willing to embark in a multidisciplinary research experience.

GENETICS OF CIRCADIAN ABNORMALITIES

Tutor: Dr. Valter Tucci (valter.tucci@iit.it)

A restricted number of so called core clock genes modulates the molecular feedback loop that underlies circadian rhythms in the organism. The importance of such core genes in generating and protecting the circadian oscillations of sleep-wake cycling has been largely demonstrated by constructing genetically modified mice which carry targeted mutations within the circadian molecular circuitry. Within this project we will study, by 24 hours continuous monitoring, mice with single point mutations that delay or advance circadian period. The information gained from this project will advance the understanding of the mechanisms that cause diseases such as Delayed or Advanced Sleep-Phase syndrome.

For any further information please write an email to:
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