nanobodies). High-resolution confocal imaging of intact GFP-RyR2 heart sections and brain slices revealed highly ordered arrays of GFP-RyR2 in various regions of the heart, but disperse distribution of GFP-RyR2 in hippocampus and other regions of the brain. Using Alexa-Fluor 647 (AF647)-conjugated GFP-specific probes, we will perform super-resolution imaging to further define the subcellular distribution of GFP-RyR2 in the heart and brain (Supported by NSERC, CFI, CIHR, and LCIA).

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Immunoimaging with Light Sheet Microscopy: Microglial Dynamics in the Developing Zebrafish Brain

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The immune system is integral to the natural development and homeostasis of the brain, and in its response to injury and disease. These processes are highly sensitive to cell position and dynamics, and live imaging is important for quantification of these aspects of the immune response. The zebrafish is well established as a model organism for developmental biology due to its relative transparency and ease of study by microscopy. Recent zebrafish transgenic lines are extending its use to the study of the innate immune system. However, the immune system is characterized in part by the fast, organism-scale motility of its cells, presenting challenges for proper observation of its roles, both in the development of the organism and in immune responses. We apply 1- and 2-photon light sheet microscopy, or selective plane illumination microscopy (SPIM), to achieve rapid, large volume imaging of the developing zebrafish brain and the dynamics of microglia - the main innate immune cell of the brain. The high-speed, low-photodamage imaging by SPIM enables the direct tracking of microglia throughout the entire zebrafish brain in early development. Further, the high spatio-temporal resolution allows observation of the microglial processes during development, as well as of the active state in response to pathological stimuli. In capturing these dynamics, across the full organ, we begin to gain a more complete understanding of neuroimmunology not available with other approaches. This work lays the foundation for the measurement of neuroimmune dynamics and their relationship to developing brain circuitry and vasculature as well as activity and function.

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Influence of Nanoparticle Exposure on Nervous System Development in Zebrafish Studied by Means of Light Sheet Fluorescence Microscopy

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Zebrafish has a remarkable similarity in the molecular signaling processes, cellular structure, anatomy and physiology to other higher order vertebrates, making it an excellent vertebrate model organism (1). Recently, zebrafish has been used for neurotoxicity screening of numerous nanomaterials with a focus on the developmental effects due to the possibility of in vivo visualization of specific neurons and axon tracts by injecting dyes in live animals as well in fixed ones (2). Here we propose Light Sheet Fluorescence Microscopy (LSFM) (3) (4) to perform neurotoxicity studies, in order to study the nervous system architecture and to image 3D structures in the brain of live larvae during the development. It is worth noting that the 3D imaging capabilities and the fast imaging speed provided by LSFM, combined with the low photo damage levels indicate this imaging technique as an 'election' method for imaging neurons distribution during embryo development. Specifically, to evaluate the neurotoxicity of nanoparticles on zebrafish, we characterized the apoptotic processes and axon tract disruption in the brain during development, that correlated cerebral malformations and anomalies in the behavior. Here, we report about the correlation of nanoparticle exposure with the neuronal number and related brightness using light sheet fluorescence microscopy. LSFM is also suitable for 3D super resolved investigations (5).

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Role of AL, FE, CU in the Alterations of Mechanical Properties of Cortical Neurons Probed by Atomic Force Microscopy

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Neurons are highly specialized cells primarily responsible for transmitting information through chemical and electrical signaling in both the central and the peripheral nervous system. Over the last few years there has been significant progress in understanding the role played by the substrate stiffness in neuronal growth, of the cell-substrate adhesion of forces, of the relationship between the neuron soma elastic properties and its health. The structural and mechanical properties of living neurons and their interaction with the surrounding environment such as the growth substrate, extracellular matrix, glial cells or other neurons can be evaluated by atomic force microscopy (AFM). This technique is able to image and interact with cells in physiologically relevant conditions at high spatial resolution, control forces applied to cells, measure cellular elastic properties and monitor variation in elastic modulus across living cells. Recent studies have demonstrated that Aluminum (Al), Iron (Fe), Copper (Cu) ions are involved in some neurodegenerative disorders. In particular Al concentration is increased in degenerating neurons in Alzheimer's disease (AD), Copper is involved in neurodegenerative disorders like familial amyotrophic lateral sclerosis, Alzheimer's or prion diseases, and accumulation of iron within senile plaques and neurofibrillary tangles is found in brains affected with AD. In this study we realize micro and nano scale maps of elastic modulus, adhesion forces and hysteresis of cortical neurons in the absence and in the presence of Al, Fe, Cu, ions at different concentrations by using AFM. We demonstrate that metal absorption causes variation of mechanical properties of neurons, implying a possible modification of cytoskeletal components, together with changes in cell-substrate adhesion forces and elastic properties.

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New Insights into Biophysical Mechanisms of the nsPEF-Induced Neuronal Response

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Simulation studies of neuromuscular incapacitation using high-intensity electric pulses (EPs) were previously reported. These studies hypothesized that a reversible action potential (AP) block can be achieved based on energy deposition causing neuronal electroporation. However, theoretical concepts were presented without elaboration of specific details on possible biological mechanisms. Recently, we discovered that nanosecond pulsed electric fields (nsPEF) could initiate phosphatidylinositol-4,5-bisphosphate (PIP2) depletion in non-excitable cells. PIP2 is the precursor for important second messengers and is a key modulator of the neuronal ion channels involved in AP generation. By using primary hippocampal neurons (PHN) and the PLCδ-PH-EGFP optical probe of PIP2 hydrolysis, we demonstrated that electric field (EF) exposure induced PIP2 depletion in the PHN, and defined EF exposure parameters necessary to safely elicit reversible effects without neuronal damage. Results show that five days after neuronal dissociation (D5), the pre-exposure level of the cytoplasmic PLCδ-PH-EGFP fluorescence is significantly higher in D5 neurons than in D1 neurons, likely due to higher levels of tonic inositol1,4,5-trisphosphate (IP3). Such biological sensitization caused D5 neurons to respond intensely following a single 7.5 kV/cm 600 ns EP, while the D1 neurons did not respond. Despite age of development, the stronger 15 kV/cm 600 ns or longer 7.5 kV/cm 12 μs EPs initiated profound PIP2 depletion in all neurons studied, outlining direct impact on the neuronal plasma membrane during electroporation. Accordingly, D1 neurons exposed to such EPs had significant post-exposure propidium iodide (PI) uptake. In the more sensitive D5 neurons, PIP2 recovery was achieved within 10 min after all 600 ns EPs exposures, but 12 μs EPs caused irreversible PIP2 depletion. Thus, nsPEF-induced PIP2 depletion in neurons could be the primary biological mechanism responsible for both stimulation and latent inhibition of neuronal tissues.