A novel optical platform for in vivo imaging of opioid-induced structural plasticity in the pain neural circuits

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1. ABSTRACT

This RF Seed Fund application will foster a seamless collaboration among three PIs to address the broad challenge of the chronic use of opioid analgesic in pain management leading to addiction. The central goal of this proposal is to tailor a unique, novel, multimodal optical platform (fl-ODM) for in vivo imaging to measure the responses of critical cell components in the pain neural circuits in the spinal dorsal horn (SDH) of mouse models of opioid-induced hyperalgesia (OIH). Using this approach, we will monitor the reaction of neurons, astrocytes, and microvascular vessels to opioid exposures and subsequently to elucidate the molecular pathways underlying the responses during OIH pathogenesis.

Funded by an NIH BRAIN Initiative grant, we have succeeded in prototyping a fl-ODM (US Patent pending) that integrates 1.3um swept-source 3D optical coherence Doppler microscopy and dual-channel time-sharing fluorescence microscopy. This approach enables concurrent imaging of large-scale cell-specific fluorescence features (e.g., identification of glial vs neuronal Ca^{2+} signaling) and 3D vasculature and blood flow networks *in vivo*, at cellular/capillary resolutions and over a FOV (e.g., 3×4mm^2 and >1.4mm of depth). More importantly, our recent advances in deep-learning-based denoising and motion-artifact removal further permit fl-ODM to image capillary flow networks of awake/behaving animals rather than animals under anesthesia. With the combination of a chronic spinal window technique and isotropic viral delivery of green/red fluorescence proteins (GFP/RFP) expressing neuronal/astrocytic Ca^{2+}, fl-ODM will allow us to image *in vivo* the spatio-temporal evolution of neuronal, astrocytic and microvascular changes in the SDH pain neural circuit induced by opioids during OIH development.

Emphasis will be on: **Aim 1** – modifying/optimizing fl-ODM to enable spectral imaging to the structural changes in the SDH of mice ($Ca^{2+}{}_N$/$Ca^{2+}{}_A$: neuronal/astrocytic Ca^{2+} fluorescence signaling from spinal cord; HbO_2/HbR: tissue oxygenated hemoglobin/deoxygenated hemoglobin, and CBF: vascular blood flow); **Aim 2** – characterizing and validating the efficacy of fl-ODM for image tracking morphine-induced astrogliosis in the SDH of the OIH mouse models. The inclusion of metabolic imaging (HbO_2/HbR) in fl-ODM will enable us to investigate the mechanisms underlying the complex hemodynamic interactions among neurons/astrocytes, microcirculatory blood flows, and metabolic changes during OIH development. Moreover, the technological advances in fl-ODM tailored for spinal cord uses will enable 3D imaging in awake animals, circumventing the confounding artifacts of anesthesia (e.g., isoflurane induced vasodilation, basal metabolic states, cellular functions such as neuronal/glial activities). Previous work in Dr. Tang's lab has elucidated a Wnt5a-mediated neuron-to-astrocyte signaling pathway that controls morphine-induced astrogliosis. We will use fl-ODM to visualize the Wnt5a-regulated astrogliosis and the blood flow changes in the SDH of OIH models.

This project harnesses the interdisciplinary collaboration of 3 laboratories with unique and complementary expertise, led by Drs. Pan (a biomedical engineer), Du (a neuroimaging scientist for drug addiction), and Tang (a pain and analgesia research expert). The successful development and validation of the new fl-ODM will establish a novel approach to study cell-specific activities and the resultant hemodynamic functions in the pain neural circuits and will have a broad impact on both mechanistic and translational research on opioid-induced structural alterations of essential cell components in the SDH, including neurons, astrocytes, and microvascular capillaries. Our platform will facilitate the development of therapeutic approaches to intervene opioid-induced pathogenesis in neural circuits.

This RF Seed Fund will allow us to generate preliminary data to apply for a major NIH grant (R01) for a comprehensive study of opioid-induced hyperalgesia and addiction.