Doctoral thesis/dissertation Digest Form

Title of Doctoral Thesis: Application of *in vivo* CMOS imaging device with microdialysis and optogenetics for studying serotonergic neurons in pain modulation

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(Summary)

This dissertation focuses on establishing the methodology for probing the activity of serotonergic neurons in a deep brain region. The primary goal of this work is to achieve multi-region imaging coupled with microdialysis and optogenetics for studying serotonergic pathways in the pain circuitry of a freely behaving mouse, which has been difficult using other commercial devices due to their size and weight.

In this dissertation, **Chapter 1** presents the background of the study and reviews the recent studies in calcium imaging and optogenetics, especially in regard to pain research. **Chapter 2** introduces the imaging-microdialysis platform. This method allows us to observe the activity of G-CaMP6 imaging and neurotransmitters during formalin-induced nociceptive pain. This is the first report to simultaneously measure calcium signaling and serotonin release during acute pain in three different locations in the brain (DRN, CeA, and ACC) under contralateral and ipsilateral conditions. Furthermore, the correlation between brain activity and behavior has been assessed, and the utility of imaging devices has also been examined by a neural blockade in the spinal cord.

Chapter 3 focuses on the study of the DRN-ACC pain circuitry. To specifically observe serotonergic neurons, the experiment used SERTCre mice. Two imaging devices were implanted to concurrently observe the activity of GCaMP6-expressing cells in the DRN and GRAB 5HT in the ACC. GRAB 5HT was used to observe serotonin dynamics. The activity of serotonergic neurons in the DRN and the ACC between pain and PBS groups has been evaluated.

In contrast to the previous chapter that used the GCaMP sensor for imaging, **Chapter 4** introduced the RGECO imaging sensor. The device has been modified for a red-shifted imaging sensor. Channelrhodopsin-2 (ChR2) was used for photo-stimulation. This chapter demonstrated the RGECO imaging in the DRN and the ACC and revealed the serotonin release and activity using opto-microdialysis and opto-dialysis systems. **Chapter 5** summarizes the important experimental results, including the future prospects of the study. The development of a multi-region imaging system in this study may hold immense potential for enhancing our understanding of serotonergic networks, which could contribute to a better approach to elucidate the fundamentals of pain.