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Single-Cell Spatial Sequencing and Connectomic Mapping of the Interpeduncular Nucleus Reveal Sex-Dependent Mechanisms of Opioid Reward

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Opioid use disorder (OUD) affects 2.1 million people in the US, with women more prone to mood disorders, suggesting sex-specific brain circuit changes. The interpeduncular nucleus (IPN) is an understudied midbrain region relaying information between the basal forebrain and hindbrain. The IPN neurons contain high levels of μ opioid receptors and regulate the motivational properties of substances of abuse. The IPN also regulates female reproductive behaviors and is sensitive to ovarian hormones.

We investigated the IPN's function in sex-specific physiological and behavioral responses to opioids. We employed Visium and CurioSeeker platforms to compare the spatially-resolved transcriptional profiles of midbrain cells between female and male mice. The IPN exhibits high densities of sex-dependent differentially expressed genes (DEGs). Gene ontology analysis of these DEGs in the IPN predicated sex differences in axon guidance, synaptic transmission, and circuit connectivity. Therefore, we used MAP-Seq to analyze the efferent connectome of IPN neurons in males and females with single-cell resolution. We found that most IPN neurons in both sexes project to the medial raphe nucleus (MnR). Electrophysiological recordings revealed robust sex differences in the connectivity strength in the IPN-MnR circuit. Finally, our single-cell RNA sequencing of the IPN cells detected robust transcriptional responses to oxycodone, with distinct differences observed between males and females across various cell types.

In summary, our high-throughput, single-cell sequencing, and connectivity analyses reveal striking sex-dependent differences in IPN neurons and their responses to opioid exposure. These insights may be relevant to non-mesolimbic and sex-dependent mechanisms of OUD.