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“Characterizing a Novel Population of Dynorphin Ependymal Cells in the Ventral Aqueduct”

Background: Ependymal cells (ECs) are an important, yet understudied, cell type found lining the cerebrospinal fluid (CSF)-containing cavities in the brain and spinal cord. Recently, a novel population of ECs in the spinal cord were found to participate in a dynorphin/kappa opioid receptor (KOR) signaling mechanism alongside CSF-contacting neurons (CSF-cNs), which influenced glial proliferation following injury. The Maiya lab has shown certain populations of ECs in the mouse brain that express the dynorphin precursor, prodynorphin (pDyn), particularly near the ventral cerebral aqueduct (CA). Additionally, the kappa/dynorphin system elsewhere in the brain has been implicated in stress-induced increases in alcohol consumption and alcohol withdrawal.

Objectives: This study aims to describe the location of dynorphin-expressing ECs and CSF-cNs in the mouse brain. Furthermore, we conducted a preliminary study investigating the impacts of alcohol on this signaling pathway and on EC proliferation around the CA.

Methods: All experiments were conducted with pDyn-Cre;Ai14 mice that label all the cells expressing the pDyn gene. RNAscope was performed on sections to investigate (1) the type of ECs that express *pDyn* using markers *Sntn* and *Foxj1* and (2) colocalization of *pDyn* and KOR (*Oprk1*) with CSF-cNs which were labeled by Cholera toxin -B subunit (CTB) that had been injected intra-cerebroventricularly. CSF-cNs and their projections to the DRN were mapped along the AP axis using CTB. The effect of ethanol withdrawal on EC and glial cell proliferation in the CA was investigated using EdU/Click iT chemistry on mice that were injected with ethanol (experimental) or saline (control) intraperitoneally (IP) for 4 days. Immunohistochemistry (IHC) was used to evaluate differences in *pDyn* expression of ECs (using acetylated α -tubulin as a marker) following ethanol withdrawal. Fluorescence density and colocalization were analyzed using FIJI and QuPath, respectively.

Results: Significant overlap was observed between EC marker (*Foxj1*) and *pDyn*. Interestingly, most of these ECs also expressed *Sntn*, a marker for mature ECs. CSF-cNs were labeled by CTB, some of which expressed *Oprk1* but minimal *pDyn* expression. The Click-EdU experiment revealed no proliferation. A significant difference was found between controls and experimental mice in expression of *pDyn* within a specific population of ECs in the ventral CA following alcohol withdrawal.

Conclusions: We identified a novel population of ECs expressing *pDyn* in the ventral CA that were located in close proximity to CSF-cNs expressing *Oprk1*. These ECs modulate their *pDyn* expression in ethanol withdrawal, suggesting the possibility of these cells participating in ventricular stress signaling through CSF. Our work positions this new population of ECs in the CA as a possible participant in dynorphin signaling, warranting future studies in various disease states.