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"Characterization of Ependymal Cells and CSF-Contacting Neurons in the Cerebral Aqueduct"

BACKGROUND: Ependymal cells line Cerebral Spinal Fluid (CSF) filled brain ventricles and the spinal cord central canal forming a barrier between CSF and neural tissue while also facilitating CSF circulation. CSF-contacting neurons (CSF-cNs) are interspersed within and around the ependymal layer and possess an axon extending into the CNS parenchyma, allowing them to detect changes in CSF composition and flow. A novel population of prodynorphin (pDyn)-expressing ependymal cells was recently found to be closely associated with kappa opioid receptor (KOR) expressing CSF-cNs. Dynorphin (Dyn) is the endogenous ligand for KOR. Dyn/KOR signaling in the spinal cord regulates injury-induced ependymal proliferation into astroglia. Dyn/KOR signaling in the brain regulates stress and alcohol withdrawal (WD). We have identified abundant pDyn+ cells surrounding the midbrain cerebral aqueduct suggesting that the cerebral aqueduct represents an unexplored ventricular niche.

OBJECTIVE: To characterize pDyn-expressing ependymal cells and CSF-cNs around the midbrain aqueduct and to determine if the architecture between the two cell populations parallels that in the spinal cord. Additionally, this study will investigate whether alcohol withdrawal alters ependymal cell proliferation and pDyn expression.

METHODS: Male and female *Pdyn-Cre;Ai14* mice, which express tdTomato reporter in pDyn+ cells, or male C57BL/6J mice were used for all experiments. CSF-cNs were identified using immunohistochemistry (IHC) for Pkd2l1, intracerebroventricular (ICV) AAV2-CAG-GFP injection, and cholera toxin B (CTB) injections that retrogradely trace CSF-cNs. RNAscope was performed on fresh frozen brain sections using probes for ependymal markers *Foxj1*, *pDyn*, and *Sntn*. Colocalization between CTB-labeled CSF-cNs and *pDyn* and/or *Oprk1* was determined. To assess cell proliferation after alcohol injections, mice received EdU injections after they received an ethanol/saline injection for 4 days followed by a 24 hr withdrawal. Mice were sacrificed 4hrs later during WD, and brains were processed for EdU detection.

RESULTS: *Foxj1* labeled all ependymal cells surrounding the aqueduct, and most co-expressed Sntn, indicating mature ependymal cells. pDyn+ cells were enriched along the ventral ependymal lining. Pkd2l1+ and CTB-labeled CSF-cNs were closely associated with pDyn+ cells. CTB-labeled neurons also expressed *Oprk1*, consistent with the spinal cord central canal. No EdU+ cells were detected after ethanol withdrawal, although pDyn expression increased in ependymal cells.

CONCLUSION: We identified a novel population of mature pDyn-expressing ependymal cells surrounding the cerebral aqueduct that are near *Oprk1*-expressing CSF-cNs, suggesting Dyn released into the CSF may regulate ventricular stress signaling. This establishes the cerebral aqueduct as a previously unrecognized dynorphin sensitive ventricular niche and lays the groundwork for future studies on Dyn/KOR signaling in ependymal cells in disease states.