**UNCOVERING THE ROLE OF NOTCH SIGNALING IN EARLY HYPOTHALAMIC FATE CHOICES USING PRIMARY NEUROSPHERES AND NOVEL MICROENVIRONMENT ARRAYS**

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The hypothalamus is a key regulator of homeostatic function within the body. A multitude of cell types including neurons containing distinct neuropeptides and glial cells each arise from a common early progenitor to carry out these complex processes. During embryogenesis, both intrinsic and extrinsic signals instruct early progenitor cells to adopt a certain fate and receive additional cues to further differentiate into their respective mature cell types. Dysregulation of any of the aforementioned cell types can result in physiological consequences persisting into adulthood including obesity and reproductive deficits.

Previous *in vivo* work from our lab has shown that the Notch signaling pathway acts as a critical molecular switch during early development of the hypothalamus. Interestingly, active Notch signaling not only maintains early progenitors as SOX2-positive progenitors of the hypothalamic ventricular zone, but also appears to promote expression of Glial Fibrillary Acidic Protein, a common glial marker, and may play an important role in differentiation of Kisspeptin neurons within the hypothalamus. Additional *in vitro* studies have suggested that extrinsic signals such as leptin or insulin may also regulate Notch signaling, further suggesting a role for its importance. However, the interaction between Notch signaling and extrinsic signals including growth factors and the extracellular matrix is unknown.

In our current study, we developed a hypothalamic progenitor cell culture in which we can activate or inhibit Notch signaling in a controlled extracellular environment. We hypothesized that inhibition of the Notch signaling pathway as well as removal of growth factors may promote primary hypothalamic progenitor cells to adopt specific differentiated fates. To address our hypotheses, primary neurospheres were treated with the γ-secratase inhibitor of Notch (DAPT) in the presence or absence of fibroblast growth factor (FGF) and epidermal growth factor (EGF) to assess their lineage bias. Preliminarily, we have determined that acute treatment with DAPT is sufficient to significantly reduce the downstream Notch target genes *Hes1* and *Hey1* and induce the proneural gene *Mash1*. Additionally, removing the growth factors in the presence of DAPT induces expression of the immature neuronal marker TUJ1, suggesting a more concrete bias towards a neuronal lineage. Taken together, these data provide *in vitro* evidence for the direct role of Notch signaling in cell fate choices in the developing hypothalamus. Next, to activate Notch signaling, we utilized a novel Notch ligand array coupled with multiple extra-cellular matrix (ECM) proteins to determine which Notch ligands may direct the fate of cultured neurospheres. Previous work using this technology has shown that Notch signaling can indeed bias cell fate decisions in bipotential mouse embryonic liver cells. Preliminarily, we find that hypothalamic progenitors prefer to adhere to laminin and fibronectin as their ECMs and that these progenitors robustly express SOX2 when presented with the Notch ligands Jagged1, Delta-like ligand 1 or Delta-like ligand 4. These ongoing studies will help further elucidate the role of Notch signaling in early progenitor fates. Supported by R01 DK076647, T32 ES007326, and F30 DK105760.