ABSTRACT

NOVEL IMPACTS OF HOST-ENVIRONMENT INTERACTIONS IN ENTERIC GLIA THROUGH SEQUENCING AND *IN-SITU* EXPRESSION

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The enteric nervous system (ENS) is comprised of enteric neurons and glia that facilitate essential gastrointestinal (GI) function including motility, visceral sensation, absorption, and gut permeability. Enteric neurons and glia are responsive to environmental cues and stressors ranging from the local gut microenvironment to the host's psychosocial state and understanding how the ENS integrates these cues to modulate local and systemic function is critical. Novel roles for enteric neurons in host-environmental interactions have been discovered using specialized sequencing technologies but these tools have not yet readily investigated enteric glia. The goal of this dissertation was to develop and utilize genetic technologies to characterize enteric glial responses to environmental mediators.

First we adapted existing genetic tools to study molecular changes in the ENS and specifically enteric glia. We developed effective means of characterizing enteric glial expression within complex *in vivo* models using the RiboTag model with RNA-sequencing and subsequently visualized changes in gene expression within enteric ganglia *in situ*. We then utilized these techniques to investigate sex-specific responses to early life stress in enteric glia. Enteric glia from male and female mice have contrasting expression profiles including differences in GPCR signaling that could contribute to sex-specific ENS signaling mechanisms and ultimately GI disease outcomes. This supports recent findings of sexual dimorphism in glial functional connectivity and may highlight a critical difference in the way enteric glia communicate with other cell types between males and females. Additionally enteric glia from male mice 'feminize' following early life stress through altered expression of GI and neurological disease genes including mechanisms of glial-immune communication like type I interferon signaling. Together these data

highlight striking differences in the physiologic molecular patterns and nature of stress response in enteric glia between males and females that likely contribute to sexually dimorphic GI disease patterns and symptom presentation.

Next we investigated ENS type I interferon responses through the stimulator of interferon genes (STING) pathway. STING responds to both microbial and host mediators to contribute to GI inflammation. However the role of STING signaling in the gut is complex and can either exacerbate or ameliorate inflammation likely dependent on complex microenvironmental factors. We provide the first known investigation of STING expression and signaling within the ENS. STING is expressed in both enteric neurons and glia but IFNB is only expressed in enteric neurons. ENS STING is activated by its canonical ligands to produce type I interferons. However this is likely primarily mediated through canonical activation of enteric neuronal STING and the contribution of enteric glial STING to type I IFN response is minor. Additionally enteric glial STING does not alter gastrointestinal outcomes during acute colitis within the DSS colitis model. Taken together these findings suggest enteric glia do not utilize STING for canonical type I IFN signaling or contribute to disease pathology in acute DSS colitis. Enteric glial STING may instead utilize primordial and specialized signaling pathways that more selectively alter local function.

Together our data provide novel genetic tools and data to further uncover molecular functions in enteric glia and their role in GI and systemic health. Using these we discovered entirely novel molecular interaction effects between sex and early life stress that shift the framework of these risk factors in GI disease. Furthermore we highlight a novel potential mediator of ENS-microbe communication with STING. Our findings further characterize the molecular patterns used by glia in response to complex environmental factors and highlight unique heterogeneity in glial intercellular communication.