Enteroendocrine Cells Effect on Neurons and Muscle Development In the Small Intestine

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BACKGROUND
Enteroendocrine cells (EECs) are rare cells located throughout the gastrointestinal tract. EECs secrete several different hormones that help control feeding, nutrient absorption, gut motility, and glucose homeostasis. Ghrelin, also known as the hunger hormone, is an example of a hormone that is secreted by EECs. Ghrelin interacts with the brain via a G-protein-coupled receptor that is highly expressed in the brain and known as growth hormone secretagogue receptor (GHSR). Upon bind of Ghrelin to this receptor one tends to experience modulation of the growth hormone secretion, blood glucose homeostasis, stress responses and gastrointestinal tract motility. Lack of EECs or irregular hormone secretion alter the communication between the gut and other organs leading to malnutrition, obesity and diabetes. How EECs communicate with the brain, either through the circulation and/or direct synaptic connections between the enteric neurons and the vagus nerve, is an active area of research.

METHODS
Three different mice modes were made and measured for weight averages. Utilization of sectioning through staining for whole-mount and immunofluorescent for neurons (Chromogranin A (CHGA), Beta-III Tubulin (B3T)).

RESULTS
The neural markers CHGA and B3T were detected in both MTb and WT mice with no distinct differentiation in quantity present. Whole mount stain provides clearer identification of neurons still being present. Weight variation between mouse models act as a form of verification for removal of NGN3 in MTb.

CONCLUSIONS
Results suggest that the absence of EECs does not compromise the neural and muscular development and organization of the mice. However according to size comparison of the litter NGN3 MTb held an over all smaller size average then that of NGN3 MTa and NGN3 WT. This leads to the idea that while there is not compromises to neural and muscular development and organization there is a compromise in the amount of nutrition and or signaling that is taking place between gastrointestinal tract and brain in the mice if they are not able to develop in the same amount of time to the same size as the other mice. Further testing will aid to determine what type of signaling or lack there of , that may be compromising the nutrition absorbance.

FUTURE DIRECTIONS
1) Immunofluorescent staining for additional enteric neurons as well as enteric muscles.
2) Quantitative differentiation of neuron production in NGN3 KO vs. NGN3 WT.
3) Genotyping for NGN3 KO mice.
4) Modeling of NGN3 KO in small intestine organoid.
5) Elucidate the direct and indirect mechanism of how EECs regulate neuron development within the small intestine.

REFERENCES

INTRODUCTION
The aim is to understand if the presence of EECs is required for the normal development of enteric neurons and muscle within the small intestine. Within the small intestine EECs are found through the mucosa and submucosa layer of the small intestine. More specifically in the mucosa layer the lamina propria and the muscularis mucosa hold the blood vessels and the muscles. Proceeding validation, the mechanisms behind this development must also be understood.

To address this hypothesis, the production and development of EECs, requires the presence of Neurogenic 3 (NGN3). Thus genetically deleting NGN3, a transcription factor required to turn on most genes unique and required for EECs. Allows for the specific identification of genes required by EECs for normal function. Deletion of intestinal NGN3 cells has been successfully completed in the past with mice and organoid models, confirmed by the lack EECs. Cre recombination, as well as Villin1 are used with the mice models to test for presence and absence of NGN3.

Figure 1: Visualization of the communication that the brain has with the stomach through used of ghrelin, also know as an enteroendocrine cell.

Figure 2: Wildtype (VII), Cre Recombinase (Cre) NGN3 (Neurogenin 3). VIl-Cre/NGN3+/−, NGN3 Knockout (MTb) VIl-Cre/NGN3+/−, NGN3 heterozygous (MTa) VIl-Cre/NGN3−/+ , NGN3 Wild type (WT).

Figure 3: Absence of NGN3 causes significant decrease in weight. Weight averages of mice models at p0.

Figure 4: MTb immunofluorescent staining does not have significant staining from that of WT. Neuron cells appear to be heavily prevalent.

Figure 5: All necessary markers are present in WT mice for DAPI, B3T, CHGA, and ECA1.

Figure 6: Mutant mice gain less body weight than control littermates. During the first two weeks of life, the weight of WT and MTa, MTb was taken every day (left graph) and there after once a week for a period of 6 more weeks (right graph). Mutant mice do gain less weight than control mice and keep, at adult stages about 35% lower body weight than WT, WT, n=46; MTa-MTb, n=25 (different group of mice than Figure Xb).

Figure 7: A photography taken p3.5 of a wild-type, heterozygous (MTa) and mutant (MTb). B-D Photographs of the dissected intestinal laccus from the wild-type and mutant mice shown in A taken with the same magnification.