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

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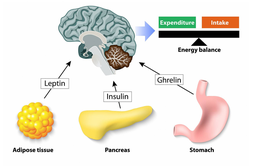
BACKGROUND

Enteroendocrine cells (EECs) are rare cells located through out 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-protine-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 tend 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.



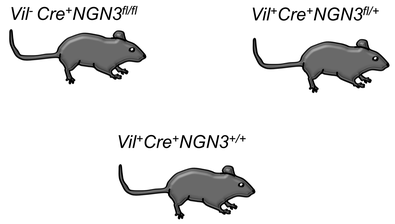
Figuer 1. Visualization of the communication that the brain has with the stomach through used of ghrelin, also know as an enteroendocrine cell.

INTRODUCTION

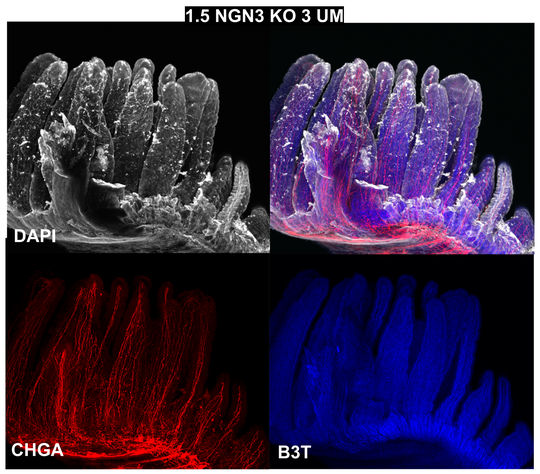
The aim is to understand if the presence of EECs is required for the normal development of enteric neurons and muscle within the small intestine. Within the small intestine EECs are found through the mucosa and submucosa layer of the small intestine. More specifically in the mucosa layer the lamina propria 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 recombinase, as well as Vilinan1 are used with the mice models to test for presence and absences of NGN3.

METHODS

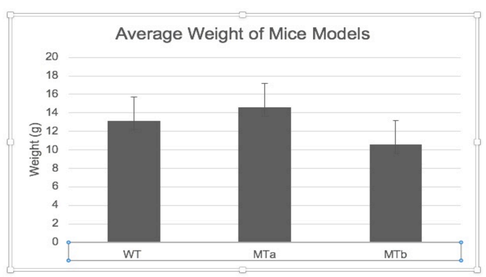
Three different mice modes were made and measured for weight averages. Utilization of sectioning through staining for whole-mount and immunofluorescent for neurons (Chromogranin A (CHGA) , Beta-III Tubulin (B3T)).



Figuer 2. Villin1 (Vil), Cre Recombinase (Cre) NGN3 (Neurogenin 3).
Vil- Cre+NGN3fl/fl - NGN3 Kockout (MTb)
Vil+Cre+NGN3fl/+ - NGN3 heterozygous (MTa) *Vil+Cre+NGN3+/+* - NGN3 Wild type (WT)



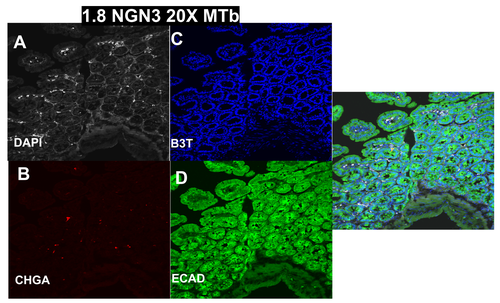
Figuer X. Neurons are still prevalent in NGN3 KO mice through whole mount staining for CHGA. CHGA i



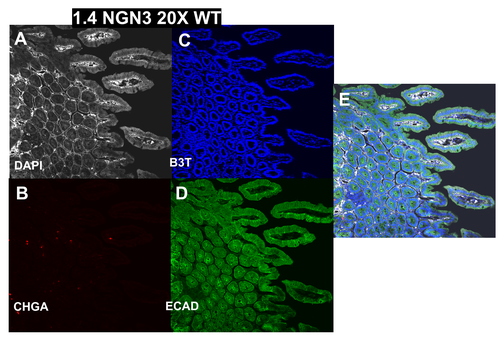
Figuer 3. Absence of NGN3 causes significant decrease in weight. Weight averages of mice models at p0.

RESULTS

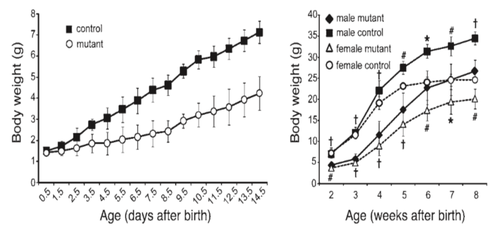
The neural markers CHGA and B3T were detected in both MTb and WT mice with no distinct differentiation in quantity present. Whole mount stain provides clearer identification of neurons still being present.
Weight variation between mouse models act as a form of verification for removal of NGN3 in MTb.



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



Figuer 5. All necessary markers are present in WT mice for DAPI , B3T, CHGA , and ECAD.

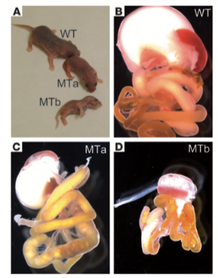


Figuer 6. Mutant mice gain less body weight than control littermates. During the first two weeks of life, the weight of WT and Mta, MTb was taken every day (left graph) and there after once a week for a period of 6 more weeks (right graph). Mutant mice do gain lees weight than control mice and keep, at adult stages about 36% lowere body weight then WT. WT,*n*=66; MTb+Mta, *n*=35 *(different group of mice than Figuers X-X)*

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 litters 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.

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



FUTURE DIRECTIONS

1) **Immunofluorescent staining for additional enteric neurons as well as enteric muscles.**
2) **Quantitative differention 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

Mittal, R., Woo, F. W., Castro, C. S., Cohen, M. A., Karanxha, J., Mittal, J., ... & Jhaveri, V. M. (2019). Organ-on-chip models: Implications in drug discovery and clinical applications. *Journal of cellular physiology*, *234*(6), 8352-8380.

Kim, T. H., & Shivdasani, R. A. (2016). Stomach development, stem cells and disease. *Development*, *143*(4), 554-565.

Mellitzer, G., Beucher, A., Lobstein, V., Michel, P., Robine, S., Kedinger, M., & Gradwohl, G. (2010). Loss of enteroendocrine cells in mice alters lipid absorption and glucose homeostasis and impairs postnatal survival. *The Journal of clinical investigation*, *120*(5), 1708-1721.

Kaelberer, M. M., Buchanan, K. L., Klein, M. E., Barth, B. B., Montoya, M. M., Shen, X., & Bohórquez, D. V. (2018). A gut-brain neural circuit for nutrient sensory transduction. *Science*, *361*(6408), eaat5236.