Brain activation subsequent to peripheral administration of Exendin-4 in Gcg-knockdown rats

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Background

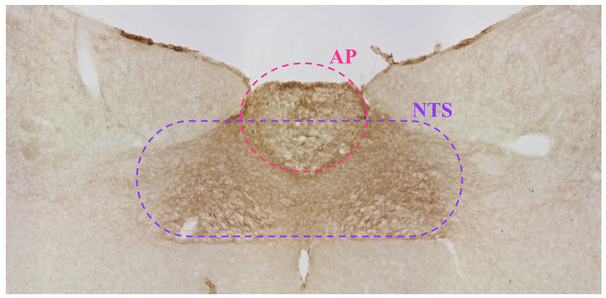
- Glucagon-like peptide 1 (GLP1) is a brain neuropeptide product of the glucagon (*Gcg*) gene.
- GLP1-positive neurons are located in the hindbrain, and their axons project to the spinal cord and to many subcortical brain regions that contain GLP1 receptors (GLP1R).
- GLP1R signaling in the nucleus of the solitary tract (NTS) and area postrema (AP) inhibits food intake and can promote nausea/malaise in rats.
- Our lab developed a transgenic knock-in Gcg-Cre rat model, in which Cre expression is driven by the *Gcg* promoter[1]. In homozygous Gcg-Cre rats, *Gcg* mRNA and GLP1 protein levels are markedly reduced.
- **We hypothesized that GLP1R sensitivity in Gcg-Cre knockdown rats differs from GLP1R sensitivity in wildtype (WT) control rats.**
- To test this, we examined neuronal cFos activation in Gcg-Cre knockdown and wild-type (WT) rats in response to Exendin-4 (EX-4), a GLP1R agonist that crosses the blood-brain barrier after peripheral injection[2].

Methods

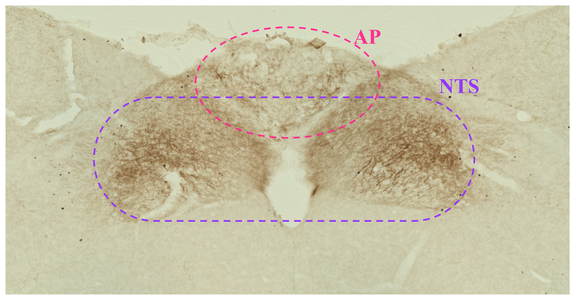
Gcg knockdown rats (16/sex) and wild-type rats (16/sex) received an intraperitoneal injection of either saline (4 per genotype/sex) or Ex-4 (10 µg/kg BW; 4 per genotype/sex). Rats were anesthetized 90 min later and perfused with saline followed by 4% paraformaldehyde. Fixed brains were cryoprotected, frozen, and sectioned at 35 µm thickness using a microtome. Tissue sections were processed for immunoperoxidase localization of nuclear cFos using nickel-enhanced DAB, followed by labeling of GLP1R using plain DAB. Double-labeled sections were mounted on slides, coverslipped, and imaged using a Keyence microscope. Quantitative analysis of cFos and GLP1R labeling will be performed using HALO image analysis software.

Preliminary Results

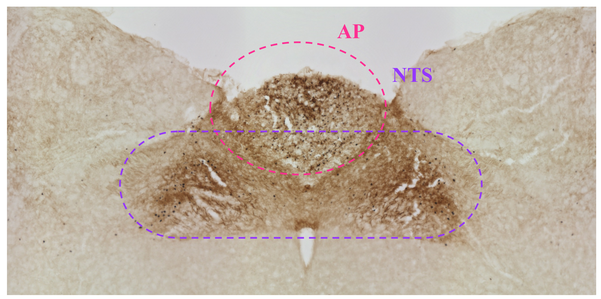
Gcg Knockdown Rats – Saline Injection



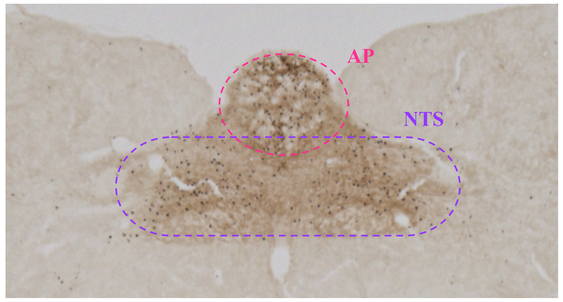
Wild Type Rats - Saline Injection



Gcg Knockdown Rats – Exendin-4 (Ex4) Injection

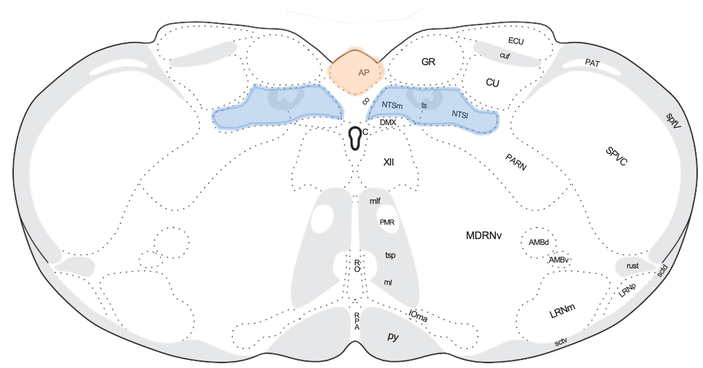


Wild Type Rats – Exendin-4 (Ex4) Injection



Anatomical Labels Guide
- - - represents area postrema (AP) location
- - - represents nucleus of the solitary tract (NTS) location

Swanson Rat Brain Atlas
Orange: area postrema (AP)
Blue: nucleus of the solitary tract (NTS)



Preliminary Conclusions

As previously reported[2], cFos labeling in wild-type rats treated with Ex4 was substantially higher in the NTS and AP compared to cFos in saline-treated controls, indicating activation of GLP1R signaling by Ex4.

Ex4-induced cFos activation within the NTS and AP appeared similar in Gcg knockdown rats, although quantitative analyses have not yet been conducted.

Additional analyses are underway to examine and quantify brain-wide cFos responses in homozygous Gcg-Cre knockdown rats vs. wildtype control rats. Data generated in this cFos study will be integrated with behavioral data examining the ability of Ex4 to suppress food intake in Gcg-Cre knockdown vs. wildtype rats.

Overall results from this project may be relevant for understanding potential individual differences in responses to GLP1R agonist drugs based on individual differences in GLP1 protein production.

References

1. Zheng, et al. (2022) https://doi.org/10.1016/j.molmet.2022.101631

2. Kjærgaard et al. (2019) https://doi.org/10.1016/j.npep.2018.11.004