Differences in Brain Activation Between Wild-Type and *Gcg* Knockdown Rats Following Exendin-4 Administration

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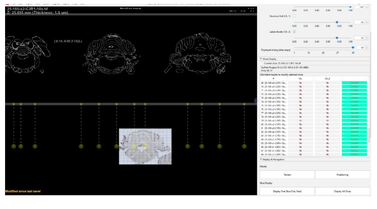
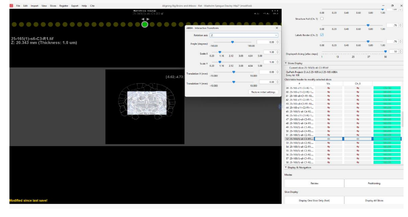
Background

- Glucagon-like peptide-1 (GLP-1), encoded by the *Gcg* gene, is a neuropeptide and hormone that regulates energy balance, stress responsivity, appetite, and reward-related behaviors through activation of GLP-1 receptors (GLP-1R).
- GLP-1–producing neurons are located in the hindbrain and project to the spinal cord and multiple subcortical regions that express GLP-1R.
- Our laboratory 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 GLP-1 peptide levels are markedly reduced.
- This *Gcg* knockdown (KD) model enables investigation of the effects of reduced endogenous GLP-1 on behavior and physiology.
- The present study examines whether developmental reduction of GLP-1 alters neuronal activation following pharmacological stimulation with the GLP-1R agonist Exendin-4 (Ex-4).
- We hypothesize that *Gcg*-KD rats will exhibit altered c-Fos activation following Ex-4 administration compared to wild-type (WT) controls.

Method

Adult Gcg-KD and wild-type (WT) rats received an intraperitoneal injection of Exendin-4 (Ex-4; 1 µg/kg) or saline prior to perfusion.
Rats were perfused transcardially with saline followed by 4% paraformaldehyde (PFA). Brains were post-fixed and cryoprotected in 20% sucrose at 4°C, then sectioned coronally (35 µm) using a freezing sliding microtome. Sections were stored in cryoprotectant until processing. Brain tissue was processed for immunohistochemical detection of GLP-1R and c-Fos as a marker of neuronal activation.
Quantification focused on regions implicated in feeding and reward, including the nucleus of the solitary tract (NTS), parabrachial nucleus (PBN), and ventral tegmental area (VTA). Brain sections were imaged using a Keyence digital microscope to capture high-resolution, stitched images of immunolabeled tissue. Images were aligned to a standardized rat brain atlas using ABBA to identify regions of interest and guide consistent analysis.

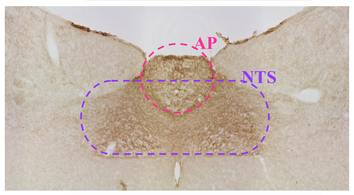




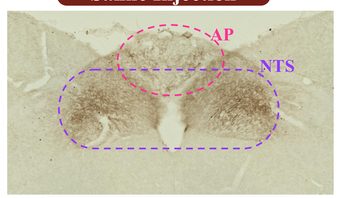
Preliminary Results

C-fos & GLP-1R

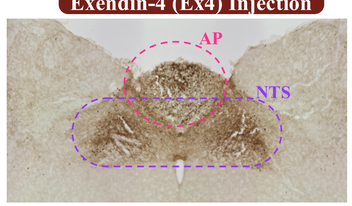
Gcg Knockdown Rats – Saline Injection



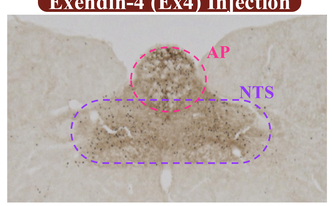
Wild Type Rats - Saline Injection



Gcg Knockdown Rats – Exendin-4 (Ex4) Injection



Wild Type Rats – Exendin-4 (Ex4) Injection

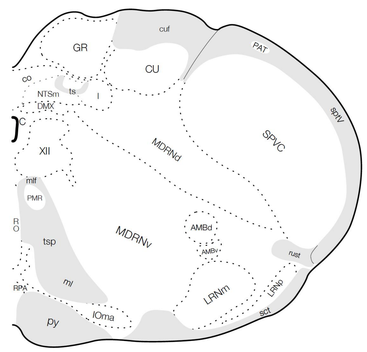


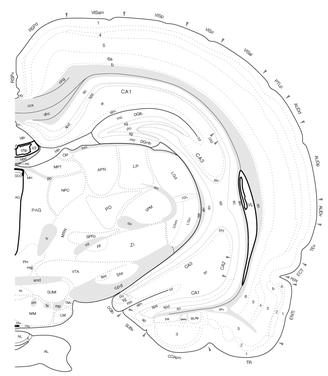
C-Fos

Brainstem

Midbrain

Gcg Knockdown Rats Saline injected case





Gcg Knockdown Rats Ex-4 injected case

Preliminary Conclusion

Although full quantification of neuronal activation is ongoing, preliminary immunohistochemical observations are consistent with prior reports[2]. In wild-type (WT) rats, Exendin-4 (Ex-4) treatment produced greater c-Fos labeling in the nucleus of the solitary tract (NTS) and area postrema (AP) compared to saline-treated controls, indicating activation of GLP-1R signaling.
Ongoing analyses will quantify brain-wide c-Fos responses in homozygous Gcg knockdown (KD) rats relative to WT controls. This approach may identify additional brain regions beyond those traditionally associated with feeding that are modulated by GLP-1R activation.
These data will be integrated with behavioral measures assessing the effects of Ex-4 on food intake in Gcg-KD and WT rats. Together, these analyses will help determine how developmental reductions in endogenous GLP-1 influence GLP-1R-related neuronal activation across circuits involved in feeding, stress, and reward.

References

1. Zheng, H., López-Ferreras, L., Krieger, J.-P., Fasul, S., Cea Salazar, V., Valderrama Pena, N., Skibicka, K. P., & Rinaman, L. (2022). A Cre-driver rat model for anatomical and functional analysis of glucagon (Gcg)-expressing cells in the brain and periphery. *Molecular Metabolism*, *66*, 101631. https://doi.org/10.1016/j.molmet.2022.101631

2. Kjaergaard, M., Salinas, C. B. G., Rehfeld, J. F., Secher, A., Raun, K., & Wulff, B. S. (2019). PYY (3-36) and exendin-4 reduce food intake and activate neuronal circuits in a synergistic manner in mice. *Neuropeptides*, *73*, 89-95. https://doi.org/10.1016/j.npep.2018.11.004