The Role of Hindbrain GABA-ergic Signaling in the Modulation of Anorexia and Malaise Induced by GLP-1R Agonists

Xing (Serena) Gao[1,2], Allison Pataro[2], C. Daniel Furst[2], Julia Halas[2], Bart C. De Jonghe[2,3], Tito Borner[2,3]

[1] School of Arts and Sciences, Class of 2023, University of Pennsylvania, Philadelphia, PA
[2] Department of Biobehavioral Health Sciences, School of Nursing, University of Pennsylvania, Philadelphia, PA
[3] Department of Psychiatry, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

Introduction

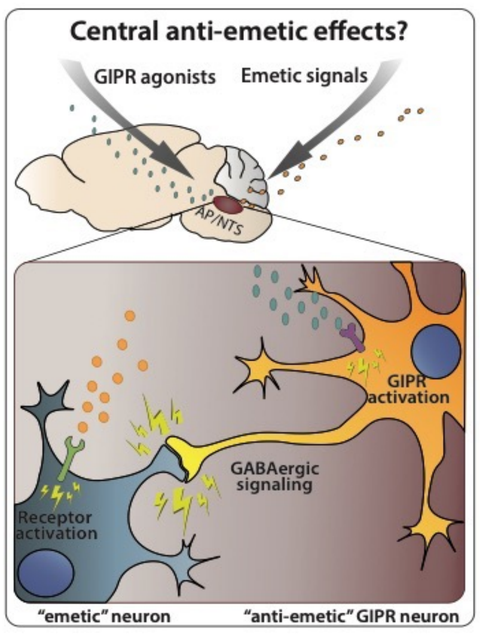

Fig.1: Working model: *GIPR activation may counteract GLP-1/chemotherapy–induced malaise via direct modulation of the AP/NTS circuitry. Given the inhibitory nature of the GIPR-expressing neurons, one can speculate the existence of a local inhibitory network within the caudal hindbrain that could be exploited via GIPR activation to reduce hindbrain GLP-1R–mediated emesis and nausea.*

Nausea and vomiting are two of the most distressing side effects in treatments for diseases such as diabetes and obesity which often leads to **poor quality of life** and **treatment discontinuation**.

Emesis and nausea are largely controlled by the central nervous system (CNS), specifically by the **area postrema (AP)** and the **nucleus of the solitary tract (NTS)**, two adjacent hindbrain nuclei.

Recent work from our lab has shown that glucose-dependent insulinotropic polypeptide receptor (**GIPR**) agonism reduces the occurrence of nausea and emesis induced by Glucagon-like peptide 1 **(GLP-1) based therapeutics**.

A high percentage of the GIPR expressing neurons co-express the **inhibitory neurotransmitter GABA** (*Gad2*) in the AP/NTS, suggesting that GIPR agonism may exert its anti-emetic effects by indirectly inhibiting emetic AP/NTS neurons, through increasing local GABA release.

Aims

1. Determine whether chemogenetic activation of AP/NTS Gad$^+$ neurons attenuates Semaglutide-induced malaise in rats.
2. Determine whether Systemic and Central Administration of Muscimol attenuates Ex4-induced malaise in rats.
3. Quantify in vivo co-localization of c-Fos expression induced by GIPR agonism in AP/NTS Gad$^+$ neurons in rats.

Methods

- Bilateral AP/NTS injections of adeno-associated viruses (AAVs) which cre-dependently encode for excitatory (G_q-coupled) designer receptors exclusively activated by designer drugs (DREADDS) were performed in the Gad-cre rats.
- Fourth intracerebroventricular cannula implantation surgery was conducted on Sprague Dawley rats for central infusion of muscimol.
- Food consumption, kaolin intake, and body weight were measured in the Gad-cre rats and the Sprague Dawley rats.
- GIP-532-induced c-Fos expression on AP/NTS GABAergic neurons was anaylzed through immunohistochemistry assay.

Results

Activation of AP/NTS GABA-ergic neurons enhances semaglutide-induced hypophagia while reducing malaise

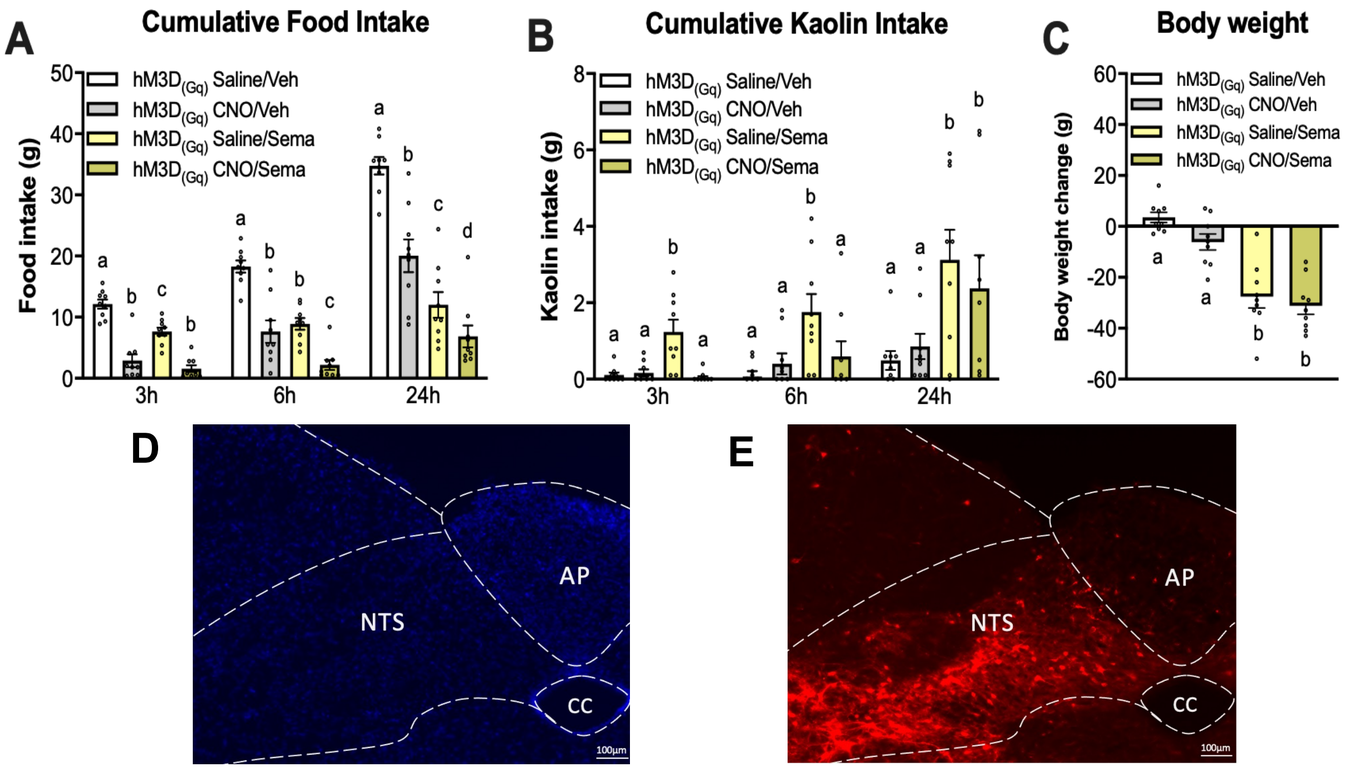

Fig.1: Chemogenetic activation of AP/NTS GABA-ergic neurons enhances semaglutide-induced hypophagia while reducing malaise.
(A) *CNO-induced (1mg/kg IP) activation of AP/NTS GABA-ergic neurons alone induces anorexia. When CNO is combined with semaglutide, the treatment results in a more profound anorexia in rats than semaglutide alone.* ***(B)*** *Comparing to semaglutide administration, the combined treatment also reduced pica behavior (the consumption of non-nutritive kaolin clay, a well validated a proxy for nausea in animals that, like rodents, lack the emetic reflex).* ***(C)*** *Changes in body weight 24h post injection. The suppression of food intake and reduced pica suggests that the excitatory neurotransmitter GABA signaling within the AP/NTS can inhibit emesis/nausea without reducing the ability of semaglutide to reduce feeding.* ***(D)*** *Representative image of the AP/NTS using the nuclear staining DAPI (blue) in Gad-cre rats.* ***(E)*** *Representative image depicting the spread and intensity of the expression of the excitatory DREADD (Gq) (red). Data in (A-B) analyzed with 2-Way ANOVA followed by* Tukey post hoc test. *Data in (C) analyzed with* One-Way ANOVAs followed by Tukey post hoc test. All data expressed as mean ± SEM. Means with different letters are significantly different from each other ($P < 0.05$).

Systemic and Central GABA-A Receptor Agonism by Muscimol has Minor Effects on Ex4 induced Anorexia, Pica and Body Weight Loss in Rats

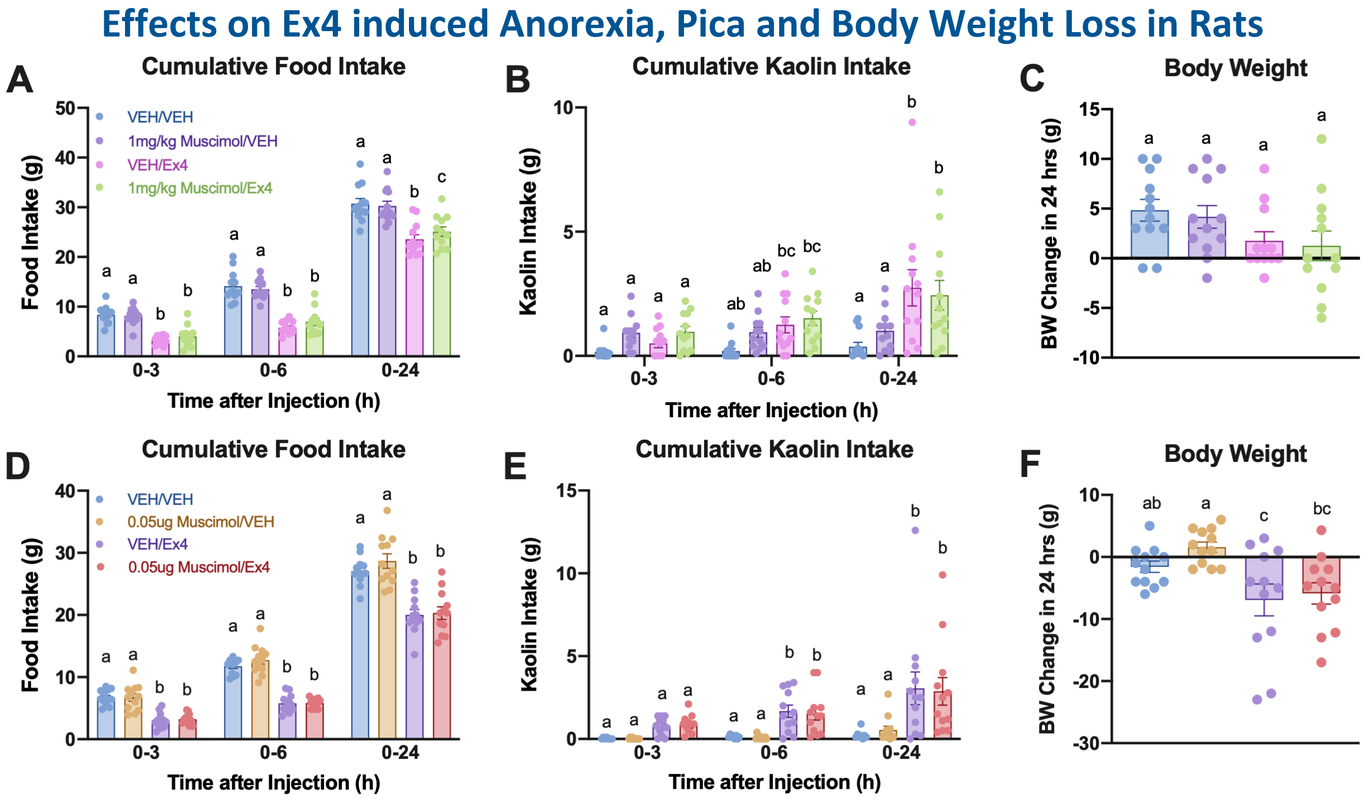

Fig.2: GABA-A receptor agonism by muscimol has little effects on Ex4-induced hypophagia and malaise.
(A) *Compared to control rats, there is no significant change in food intake following systemic muscimol injection (1 mg/kg). Muscimol pre-treatment alleviated hypophagia induced by systemic Ex4 (10 µg /kg) in rats (n = 12 per group).* ***(B)*** *Ex4 significantly induced kaolin consumption, but muscimol pre-treatment was ineffective in reducing pica behavior.* ***(C)*** *No significant changes in body weights has been observed 24h post injection across all conditions.* ***(D-F)*** *Muscimol icv pre-treatment (0.05 µg/µl) infused into the 4th ventricle failed to prevent Ex4 induced anorexia (D), pica (E), and body weight loss (F) (n = 12 per group). Data in (A,B,D,E) analyzed with RM 2-Way ANOVA followed by* Tukey post hoc test. *Data in (C,F) analyzed with* One-Way ANOVAs followed by Tukey post hoc test. All data expressed as mean ± SEM. Means with different letters are significantly different from each other ($P < 0.05$).

GIPR Agonism Activates AP/NTS GAD$^+$ Neurons in Rats

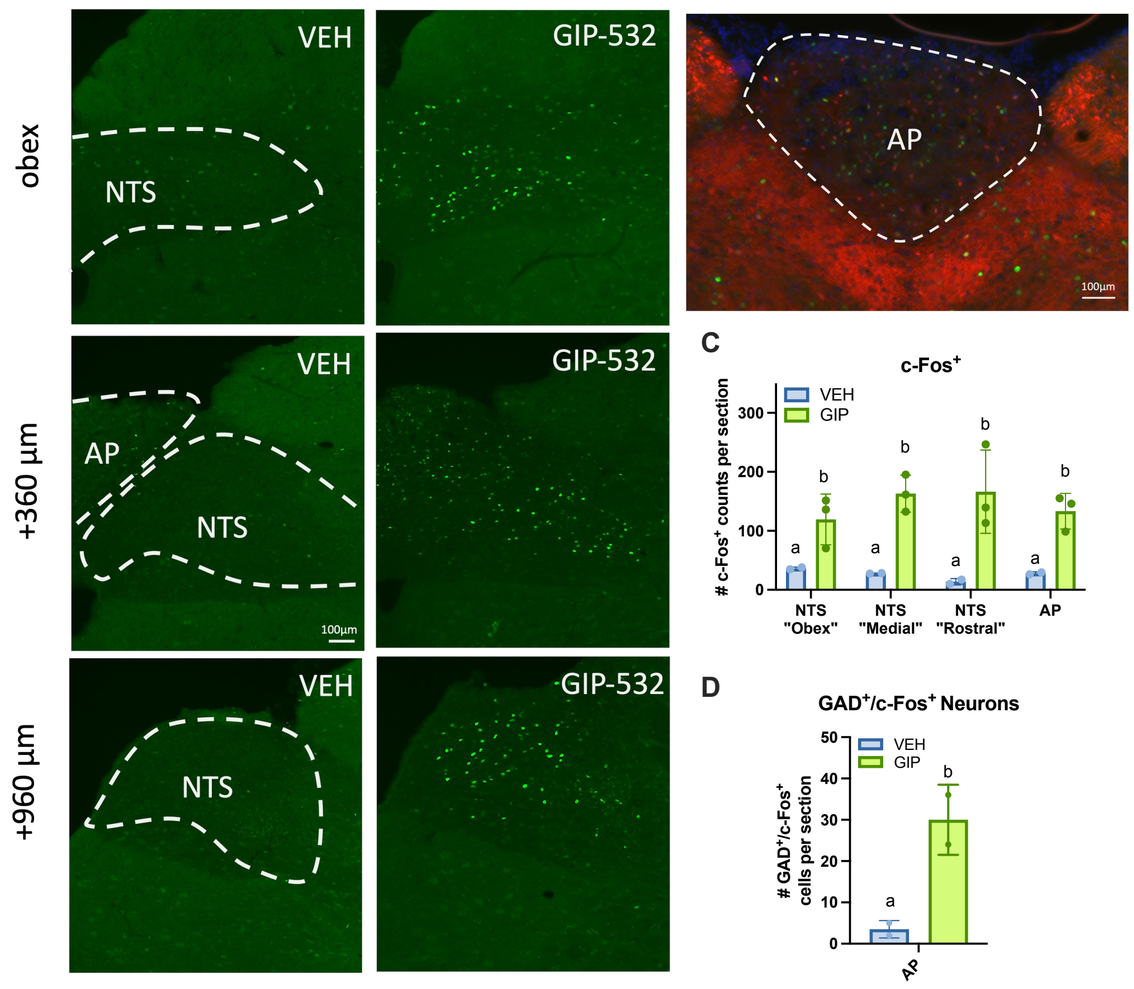

Fig.3: GIP-532 Activates GAD$^+$ Neurons in the AP/NTS of the Gad-cre tdTomato Rats.
(A) *Representative immunofluorescent images showing c-Fos-positive across different coronal plane levels (0, 360, and 960 µm rostral to the obex) and in the medial part of the AP, 3 h after potent GIP analog GIP-532 ip (30nmol/kg) injection.* ***(B)*** *Representative immunofluorescent image of the AP showing the nucleus using nuclear staining DAPI (blue), neuronal activation (c-Fos expression; green), Gad$^+$ neurons (tdTomato expression, red).* ***(C)*** *Quantification of c-Fos positive neurons in the rostral, medial, and caudal NTS and medial AP.* ***(D)*** *Number of Gad$^+$ neurons in the AP/NTS that co-express c-Fos 3 h after treatment (n = 2). All Data analyzed with paired t-test. Data expressed as mean ± SEM. Means with different letters are significantly different from each other ($P < 0.05$). Scale bar, 100 µm.*

Conclusions and Future Directions

Our results point to a key role of GABA-ergic neurons as understudied modulators of feeding and illness-like behaviors and provide a neuroanatomical and mechanistic explanation for the anti-emetic effects of GIPR agonisms.

- Chemogenetic activation of AP/NTS GABA-ergic neurons attenuated semaglutide-induced malaise and enhanced the hypoghagic effects.
- The potent and selective GIP analogs GIP-532 induced neuronal activation across the whole AP/NTS. Importantly, 25% of the activated neurons are GABA-ergic neurons.
- The selected doses of muscimol were largely ineffective in counteracting Ex-4-induced effects. On going studies aim at adjusting the dosage of muscimol and evaluate the effects of other types of GABA-R analogs on GLP-1-based therapeutics side effects.

Acknowledgement

This work was supported by the Ernest M. Brown, Jr. College Alumni Society Undergraduate Research Grant.