Lateral Septum GLP-1R Neuron Effects on Feeding Behavior

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Introduction

- Glucagon-like peptide-1 (GLP-1) is a neuropeptide made in neurons in the hindbrain that play a role in meal-related satiation, the feeling of fullness that leads to the decision to stop eating, in many species.
- Stomach fullness and ingested nutrients activate GLP-1 neurons
- GLP-1 neurons project to many other brain areas that are involved in food intake control
- When GLP-1 is released by these neurons, it acts at GLP-1 receptors (GLP-1R) expressed on other cells.
- The GLP-1R is a G-protein-coupled receptor that increases neuron excitability when it is activated by GLP-1 binding.
- Our lab uses rodent models to address how brain GLP-1Rs work to suppress food intake.
- The lateral septum (LS) is a forebrain nucleus where GLP-1Rs are densely expressed.
- Previous studies in our lab showed that activation of GLP-1Rs in the LS suppresses chow, sugar, and high fat food intake, and blocking LS GLP-1Rs increases intake.
- We hypothesize that GLP-1R-induced food intake suppression occurs because the activated GLP-1Rs -increase the firing rates of LS neurons, which leads to neurotransmitter release.
- To test this hypothesis, we used a new approach to prevent LS GLP-1R-expressing neurons from releasing neurotransmitters ("silencing" these neurons) in mice and examined how this affects their feeding behavior.
- Hypothesis: Silencing GLP-1R neurons in the LS will increase food intake and promote weight gain because this will prevent these neurons from mediating the satiating effects of GLP-1.

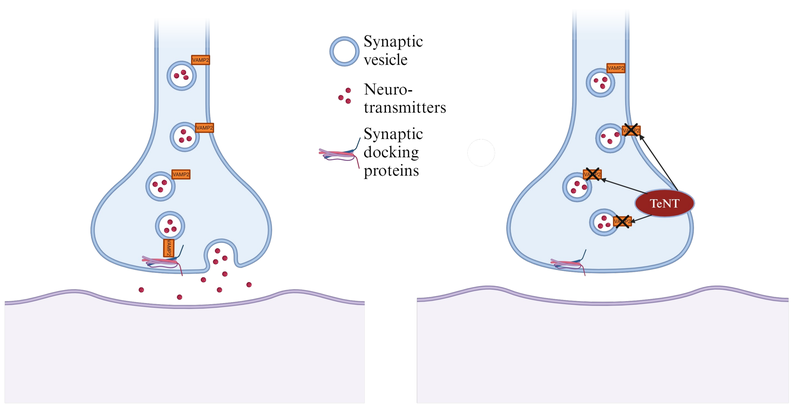
Methods

Cre-lox system: Cre = Causes Recombination of DNA.

- Cre and Locus of cross-over (x) P1 (LoxP) sites come from a P1 bacteriophage recombinase.
- In order for Cre to work, it needs the target gene to be surrounded by two loxP sites. Cre is an enzyme that recognizes the loxP site and promotes recombination.
- We use transgenic mice to take advantage of this system. Our GLP-1R-Cre mice express Cre recombinase in all cells that produce the GLP-1R.

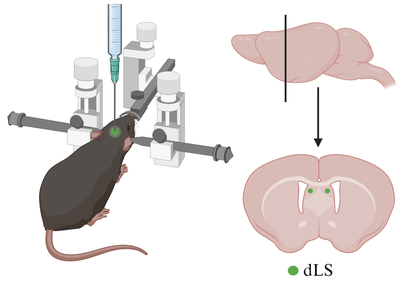
Adeno-associated viruses (AAV): We used Adeno-associated viruses (AAV) that induce new gene expression in LS neurons that express GLP-1R.

- These AAVs induces a gene for a green fluorescent protein (GFP) that is under the control of Cre. This means that only cells that have Cre recombinase will be able to express GFP from these AAVs.
- Both our control and experimental AAVs induce GFP expression, but our experimental AAV induces another gene as well. The experimental AAV includes a gene that encodes Tetanus Toxin (TeNT) that is controlled by Cre.
- In neurons, TeNT prevents synaptic release of neurotransmitters by destroying a synaptic vesicle docking protein called VAMP2. This protein helps the synaptic vesicle that stores neurotransmitters fuse to the membrane for release into the synaptic cleft. Destruction of VAMP2 "silences" the synapse because the neuron is then unable to release neurotransmitters.



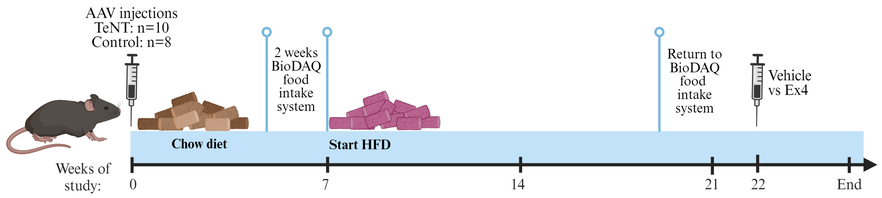
Behavioral Methods

AAV-DJ-CMV-DIO-eGFP-2A-TeNT or AAV1-CAG-FLEx-eGFP-WPRE (Control)



Subjects: 18 Naïve adult males and female GLP-1R-Cre Mice
Stereotaxic Surgery: Under isoflurane anesthesia, mice received bilateral injections targeting the dorsal LS, either AAV-DJ-CMV-DIO-eGFP-2A-TeNT (synaptic silencing of GLP-1R neurons) or AAV1-CAG-FLEx-eGFP-WPRE (control)

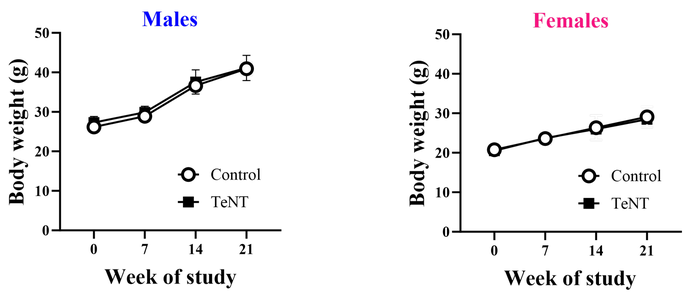
- Mice were maintained on a 12:12 light:dark schedule and were habituated to all experimental procedures in advance.
- Body weight was measured several times per week.
- After AAV injection surgery, mice were maintained on standard low-fat chow diet for 7 weeks.
- During the last 2 weeks on chow diet, mice were placed into the BioDAQ continuous food intake monitoring system. This system houses mice in a normal home cage, with a food hopper resting on a sensitive balance so that each bite of food taken is recorded at the moment the food intake occurs.
- Next, mice moved back to regular home cages and were switched to high-fat diet (45% fat; HFD) for the remainder of the study. They were placed in the BioDAQ system again during week 19 of the study and remained there until the end.
- During week 22, after mice had been on HFD for 15-weeks, we tested the effectiveness of the GLP-1R agonist Exendin-4 (Ex4) at suppressing food intake, hypothesizing that it would be less effective after synaptic silencing of GLP-1R neurons. Mice received intraperitoneal (IP) injection of vehicle or EX-4 before dark onset, in counterbalanced order separated by at least 48 hours.



Statistical Analysis: Effects on cumulative food intake and meal pattern variables during the dark phase were assessed via ANOVA. Meals were defined as at least 0.02 g consumed with an inter-meal interval of at least 300 s.

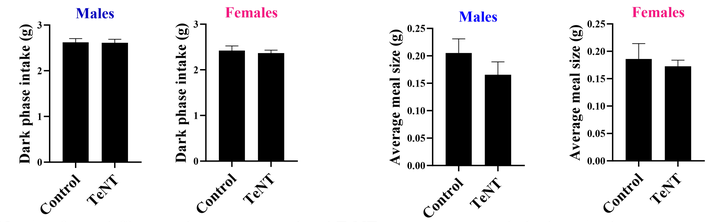
Results

Body Weights



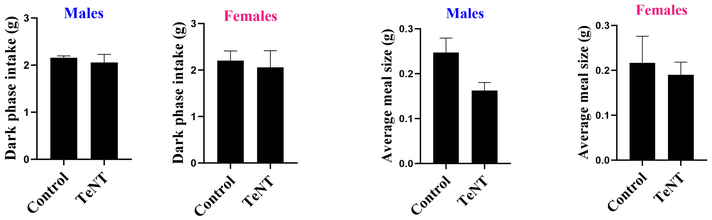
- Males and females gained weight throughout the study.
- Males gained weight faster than females when put on HFD.
  * This sex difference has previously been reported in the literature.
- No difference between controls and TeNT mice.

Dark Phase Chow Pattern



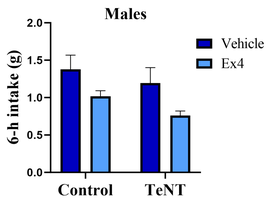
- No significant differences between control and TeNT mice on average dark phase intake or on average size of meals. No effect on number of meals (data not shown)

Dark Phase HFD Pattern



- No significant differences between control and TeNT mice on average dark phase intake or on average size of meals. No effect on number of meals (data not shown)

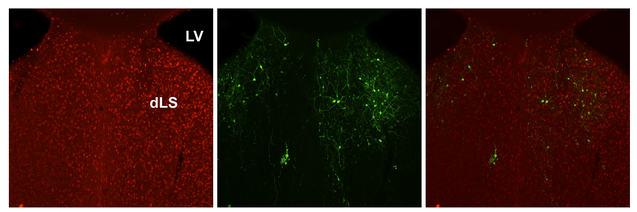
Ex-4 effects on 6-H HFD Intake



- EX-4 does suppress food intake in both control and TeNT

Histological Validation

Representative images of a coronal section through the brain of a TeNT mouse showing the dLS. LV = lateral ventricle. All subjects included in the analysis were histologically verified to have TeNT expression.



NeuN staining in red to identify neurons

GFP in green labels neurons expressing TeNT

Merged image to show both NeuN and GFP

Conclusion

- This preliminary study did not find any effect of preventing LS GLP-1R neuron neurotransmitter release on food intake, body weight, or response to a GLP-1R agonist drug.
- We observed that all groups of mice gained significant body weight on HFD, and we replicated a previously reported sex difference. Female mice took longer to gain weight than males.
- Histological analysis of the brains of the TeNT mice suggested that we may not have achieved ideal levels of expression of this protein, and this may explain the lack of behavioral effect.
- Alternatively, neurotransmitter release by these neurons may not be crucial for normal control of food intake and body weight.
- Further investigation will be required to determine which of these is correct.