Anatomical Analysis of the LDTg CTR to VTA Projection

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COL 2026

PURM Penn Undergraduate Research Mentoring Program

INTRODUCTION

The study of amylin as an anorexigenic hormone has had an oversaturated focus on hypothalamic and hindbrain structures.

Recent work from our lab demonstrated that amylin acts in the laterodorsal tegmental nucleus (LDTg), an under-studied location, to reduce both food intake and body weight via activation of calcitonin receptors (CTRs). However, the downstream targets of these LDTg CTR cells remain unknown.

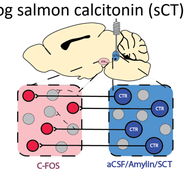
Here, we demonstrate that a subpopulation of LDTg CTR expressing cells ($LDTg^{CTR}$) project to the ventral tegmental area (VTA) and lead to increased activation of VTA neurons.

Therefore, we will anatomically characterize this pathway by phenotyping $LDTg^{CTR}$ as well as downstream VTA neurons.

METHODS

1. Surgical Approach

- A bilateral cannula was surgically implanted into rats at the level of the LDTg to allow for delivery of vehicle, amylin, and the long-acting amylin analog salmon calcitonin (sCT)



2. Pharmacological Manipulation

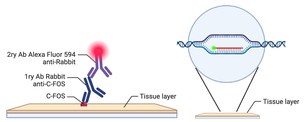
- Use of a micropump allows for precise administration of small volumes of drugs directly into the LDTg

3. Tissue Collection and Processing

- Rats were euthanized and perfused 90 minutes after drug administration
- Brains were collected and sliced at 20µm and 50µm for the VTA and LDTg, respectively
- VTA was collected for further tissue processing
- LDTg was collected to verify accurate cannula placement

4. Immunological Staining

- Immunohistochemistry (IHC) was performed on mounted VTA slices to stain for c-Fos, a marker for activated neurons
- Fluorescence in situ hybridization (FISH) performed on LDTg slices to stain for neuronal markers



RESULTS

1. LDTg CTR cells have mono-synaptic projections to the VTA

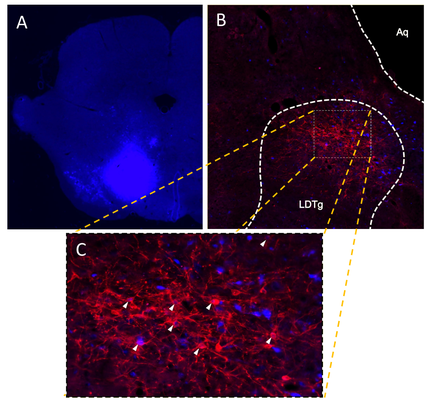


Figure 1. LDTG CTR cells project to the VTA. (A) Rats were unilaterally injected 50nL of Fluoro-Gold (blue) into the VTA to retrogradely label LDTg cells that project to the VTA. (B-C) Then, IHC was used to stain for CTR (red) in the LDTg.

2. Activation of LDTg CTR signaling increases activation of downstream VTA neurons

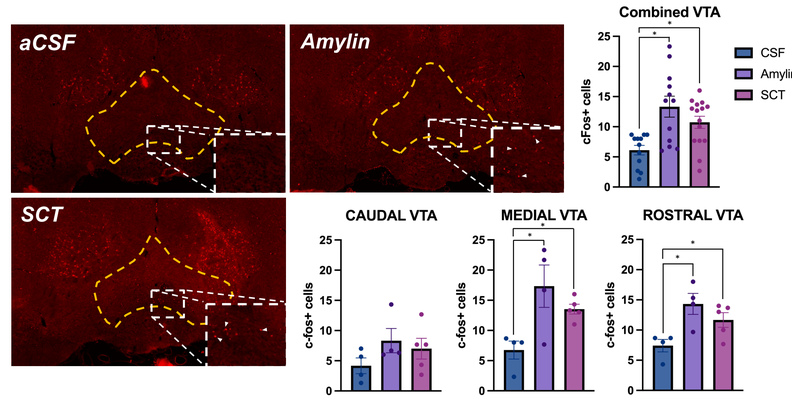


Figure 2. Activation of LDTg CTR signaling causes an increase in VTA activity. Rats were injected CSF (n = 4), amylin (n = 4), or sCT (n = 5) in the LDTg to activate LDTg CTR cells. Coronal slices of caudal, rostral, and medial slices of the VTA were collected and stained for c-Fos expression. The number of activated VTA cells between CSF, amylin, and SCT were quantified within the VTA and across the three regions.

RESULTS

3. Approximately half of LDTg CTR cells are glutamatergic or GABAergic

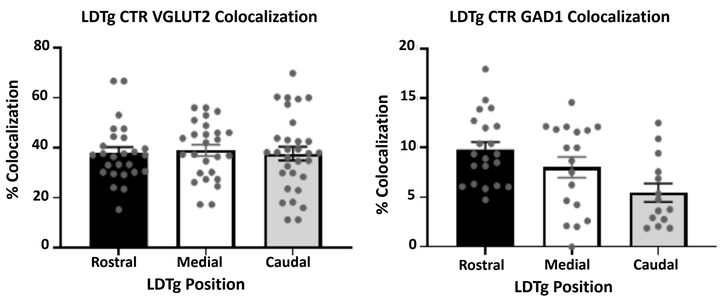
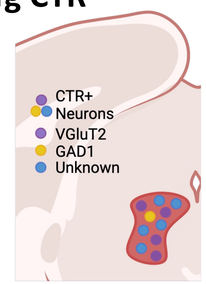


Figure 3. Approximately half of LDTG CTR cells are glutamatergic or GABAergic. Fluorescence in situ hybridization (FISH) was performed on rostral, medial, and caudal slices of the LDTg for VGLUT2 and GAD1. The numbers of VGLU2 and GAD1 expressing LDTg CTR cells were then compared between the three regions.

ONGOING EXPERIMENTS AND FUTURE DIRECTIONS

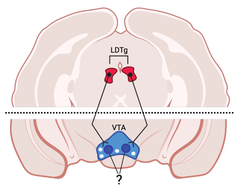
1. Identifying remaining LDTg CTR neurotransmitters

- 50% of CTR neurons in the LDTg remain unidentified
- Identifying the neurotransmitter produced by the remaining 50% of cells will provide insights into the functional role of this pathway
- Therefore, we will perform the following staining combinations (among others), and determine the proportion of LDTg CTR cells that express each marker
  - CTR/GAD1/GAD2
  - CTR/VGLUT1/VGLUT2



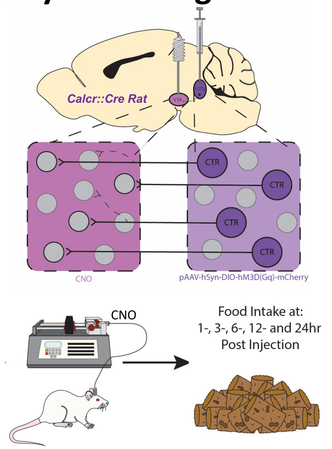
2. Phenotyping VTA neurons activated by LDTg CTR signaling

- Building on our results of increased c-Fos expression in rostral and medial planes of the VTA, we will stain for the neuronal identity of those activated VTA neurons
- Studies have shown that varying populations of neurons are present amongst the rostro-caudal axis, and dopaminergic neurons have been identified to populate more rostral and medial areas



3. Evaluating the functional role of the LDTg → VTA pathway in feeding

- With the establishment of the pathway's existence, we will evaluate its behavioral role in feeding
- Transgenic *Calcr*::cre rats will receive bilateral injections of a cre dependent excitatory DREADDs virus into the LDTg. Then, intra-VTA CNO injections will be performed to activate $LDTg^{CTR}$ axon terminals that project to the VTA
- Following injections, food intake and body weights will be assessed at 1, 3-, 6-, 12-, and 24-hour timepoints.



CONCLUSIONS

LDTg CTR cells project to the VTA, and activation of CTR signaling increases VTA neuronal activity.

Rostral and medial VTA activity, as opposed to caudal, is more strongly modulated by LDTg CTR signaling.

Around 40% of LDTg CTR cells are glutamatergic and 5-10% are GABAergic at any given point in the LDTg rostro-caudal axis.

Overall, our results identify a novel pathway with the ability to modulate VTA neuronal activity, suggesting a potential role in the control of the rewarding value of food and energy balance.

ACKNOWLEDGEMENTS

Thanks to Halcyon Hu and Drew Belser for technical support