# Proof of Concept Application of ATLAS to GLP-1 Neurons in Gcg-Cre Rats

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## Introduction

Mapping neural circuits is essential for understanding how the brain regulates physiological state, stress responsiveness, motivation, and behavior. However, identifying true monosynaptic postsynaptic targets of widely projecting neurons has historically been limited by available tracing approaches

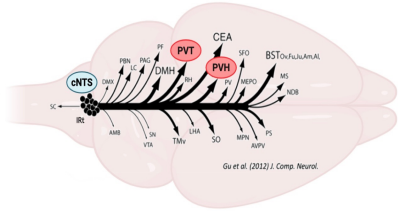
- Glucagon like peptide 1 (GLP-1) neurons in the caudal nucleus of the solitary tract (cNTS) regulate appetite, metabolic homeostasis, autonomic output, and interoceptive stress and project to key forebrain regions including the paraventricular hypothalamus (PVH) and paraventricular thalamus (PVT).
- Conventional axonal and retrograde tracing methods cannot reliably distinguish monosynaptic postsynaptic targets from fibers of passage or indirect connectivity.

ATLAS (Anterograde Transsynaptic Labeling with Activity dependent Secretion) is a protein based tracing system that enables exclusive anterograde, monosynaptic transfer of a FLP recombinase payload from genetically defined neurons. Although validated in other neural systems, we sought to validate its application to GLP-1 circuitry in Gcg-Cre rats.

Gcg-Cre rats provide cell type specificity for targeting GLP-1 neurons in the cNTS. Because GLP-1 neurons are glutamatergic, they are theoretically capable of activity-dependent ATLAS-mediated FLP release to their postsynaptic targets.

**Question:** Can ATLAS be used to identify monosynaptic postsynaptic targets of cNTS GLP 1 neurons in Gcg Cre rats?

**Projection targets of GLP-1 neurons**




Previous studies demonstrate that cNTS GLP-1 neurons project broadly to multiple forebrain and limbic regions, including the paraventricular nucleus of the hypothalamus (PVH) and paraventricular nucleus of the thalamus (PVT). These regions were selected as candidate targets to test whether ATLAS can identify their direct monosynaptic postsynaptic targets.

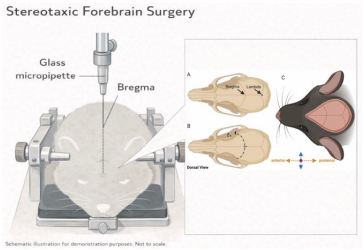
## Approach

• **Animal model:** Gcg-Cre::tdTomato reporter rats were used to verify specificity and accurate targeting of ATLAS protein to Cre-expressing GLP1-producing neurons, visualized by red tdTom fluorescence in the cNTS. To visualize postsynaptic target neurons (which should express a red FLP-dependent reporter), non-reporter Gcg-Cre rats will be used.

• **Caudal brainstem injections:** The Cre-dependent ATLAS virus (AAV8-DIO-ATLASsnFLP) was injected bilaterally into the cNTS to transfect GLP1 neurons and enable monosynaptic anterograde transfer of FLP to their postsynaptic targets.

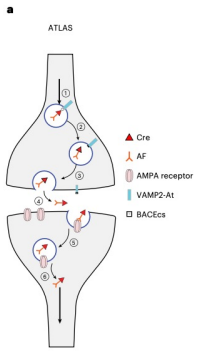
• **Forebrain targeting:** Forebrain regions receiving GLP1 projections were targeted with a FLP-dependent reporter virus (AAV8-CAG-FLPX-rc) mixed with a tracer virus (pAAV-hSyn-DIO-EGFP) to confirm injection accuracy.

• **Target regions:** Injections targeted the paraventricular hypothalamus (PVH) or paraventricular thalamus (PVT).



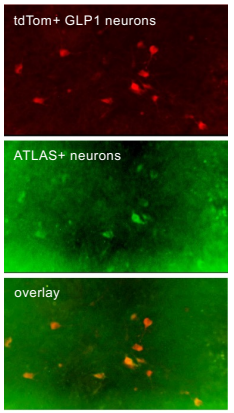

## References

**Figure 3** (*at right*) ATLAS-Cre is first made inside the presynaptic neuron and packaged into synaptic vesicles using a targeting signal. When the neuron releases neurotransmitters, the ATLAS construct is also released into the synaptic cleft after being cleaved. The released fragment, which carries Cre, binds to receptors on the postsynaptic neuron. It is then taken up into the cell through endocytosis and transported inside. From there, Cre moves into the nucleus, where it activates expression of a reporter gene in the postsynaptic cell.




## Results

**Figure 1** *(at right):* Evidence for selective expression of AAV8-DIO-ATLASsnFLP in tdTom+ GLP1 neurons of the cNTS 3 weeks after bilateral cNTS injections in Gcg-Cre/tdTomato rats. ATLAS reporter labeling (green) was restricted to tdTom+ neurons, confirming Cre dependent targeting.



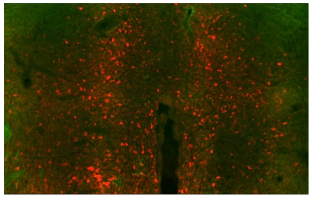



**Figure 2** *(above)* shows labeling in the paraventricular nucleus of the hypothalamus (PVH) in a Gcg-Cre rat that received cNTS injection of ATLAS coupled with bilateral PVH injection of a FLP-dependent red reporter virus. Red neurons "should" be only those that receive synaptic input from cNTS GLP1 neurons.

***However,*** similar labeling was observed in control animals that lacked ATLAS-mediated FLP expression. These results indicate that the FLP dependent reporter virus in this initial study produced non-specific red reporter expression, preventing reliable identification of monosynaptic targets.

Ongoing experiments are testing a newly developed FLP dependent reporter virus with improved specificity to reduce background labeling and enable accurate mapping of GLP1 postsynaptic targets.

## Discussion

These findings demonstrate that the Cre dependent ATLAS construct can be selectively targeted to GLP-1 neurons in the cNTS of Gcg-Cre tdTomato rats, supporting its use for cell type specific circuit mapping. However, the FLP dependent reporter produced strong forebrain labeling even in the absence of ATLAS mediated FLP, indicating nonspecific activation. Similar labeling in both experimental and control groups suggests leak expression of the reporter, preventing reliable identification of true monosynaptic postsynaptic targets.

Future studies will use an updated FLP dependent reporter with improved specificity and reduced background, with continued targeting of forebrain regions including the PVH and PVT. Together, these results highlight successful targeting of cNTS GLP-1 neurons while identifying a key technical limitation that must be resolved for accurate circuit mapping.

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