

## Introduction

Neuropeptide Y (NPY) is a neuropeptide neurotransmitter in the brain.

- It is found in interneurons & noradrenergic afferent neurons in brain areas enriched with dopamine terminals [1,2].
- It seems to regulate dopamine and glutamate neurotransmission in these area [3,4].

Manipulation of brain NPY induces robust changes in behavior.

- E.g., ICV administration of NPY increases feeding behavior & decreases anxiety [5,6]

Our lab is evaluating NPY as a target for cocaine-dependence treatment using rat models of cocaine-abstinence-induced behaviors (e.g., reinstatement models).

- Recent results (see **Previous Findings** below) support the idea that increasing the levels of brain NPY by lateral ventricles injection will suppress the expression of cocaine mediated behaviors during abstinence [7,8].
- We wonder if chronic elevation of NPY in the lateral ventricle could provide prolonged protection against relapse to cocaine seeking behaviors.

**Problem:** There is no good method to induce a long term (i.e., days or weeks) increase in NPY in the lateral ventricles of the brain.

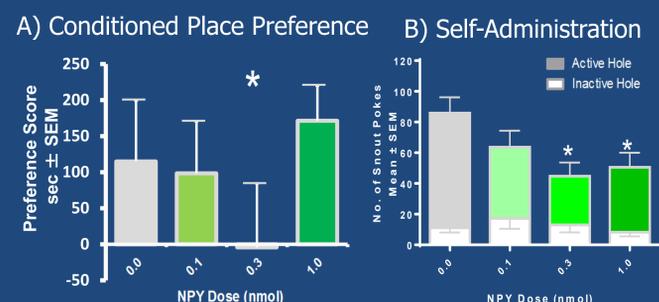
**Solution:** Use a new pharmacogenetics method to recruit and induce cells surrounding the ventricles to produce & release enough NPY to increase its availability to the brain [e.g., 10 & 11].

**Research Question:** Will an injection of an Adeno-Associated Virus (AAV) carrying an NPY gene induce a long lasting

- increase in synthesis of NPY in cells surrounding the ventricles?
- increase in release of NPY from these cells into the ventricles?
- **change in NPY-related behaviors ?**

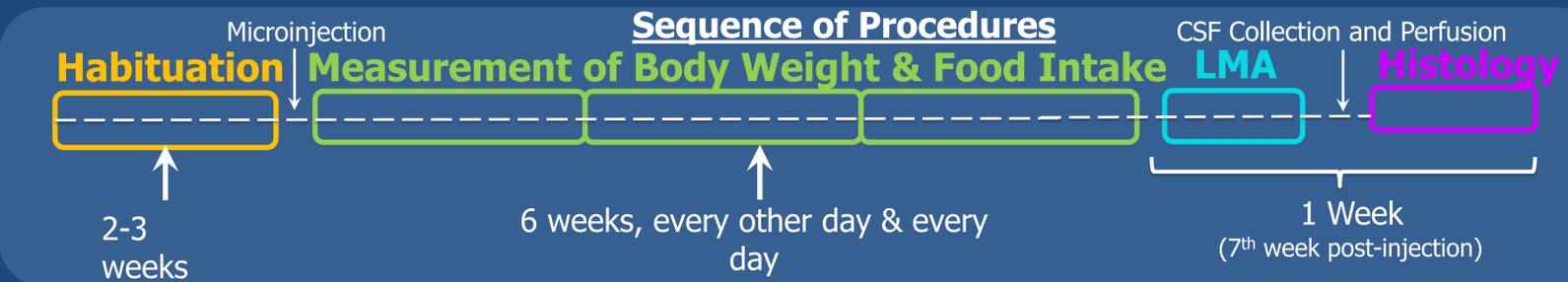
## Previous Findings

↑ brain NPY ↓ cocaine-seeking in cocaine-abstinent rats



NPY injected 1x into the lateral ventricle of cocaine-abstinent rat

- ↓ the rats preference for a context previously associated with cocaine.
- ↓ an operant response for cocaine.



## Methods

### Subjects:

- 28 Long Evans (hooded) rats (~370g)
- Housed in standard stainless steel hanging cages (1/cage).

### Design:

- 2 X 2 (Virus serotype X NPY) – 7 rats/group
  - Virus serotype : AAV10 or AAV1
  - NPY: NPY construct or Green Fluorescent Protein (GFP) construct

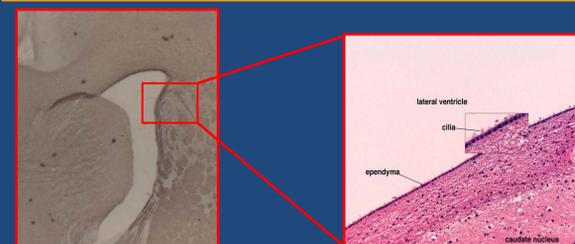
### Procedures: (see Sequence of Procedures above)

- Viruses were inserted by stereotaxic surgery; cannula aimed at the right lateral ventricle (LV).
- 20 µl of virus was injected at 0.5 µl/min
- Behavioral Measures
  - Body weight – every other day
  - 24 h Food intake – every day
  - Open Field Locomotor activity (LMA) - once
- Neurochemical Measures of NPY
  - Cerebral spinal fluid (CSF) NPY
    - CSF collected 1x under deep anesthesia, just before euthanasia.
    - Planned analysis by enzyme immunoassay (EIA)
  - Brain tissue NPY around the ventricles
    - Post-mortem in sectioned paraformaldehyde perfused tissue
    - Planned analysis by immunohistochemistry (IHC)

- Histological examination of brain tissue
  - to validate placement of injection site.
  - to visualize the target tissue for cell integrity & composition.
    - Brains harvested after perfusion (paraformaldehyde) and exsanguination.
    - Sectioned in a cryostat (40 µm) and stored in a sodium azide solution at 4 °C.
    - Some sections treated with a Hematoxylin & Eosin (H & E stain).

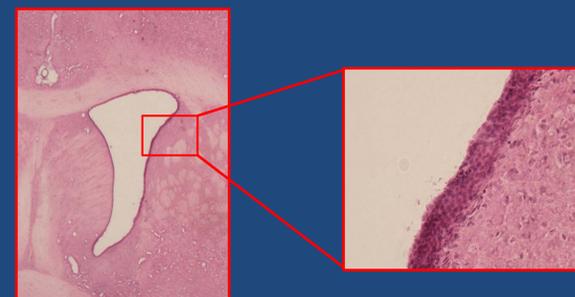
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## Neuroanatomical Target



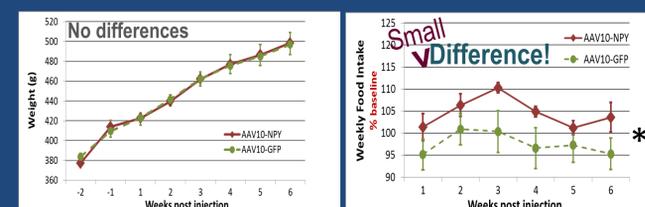
Ependymal cells, Neurons, and Glia [9]

## Results: Histology

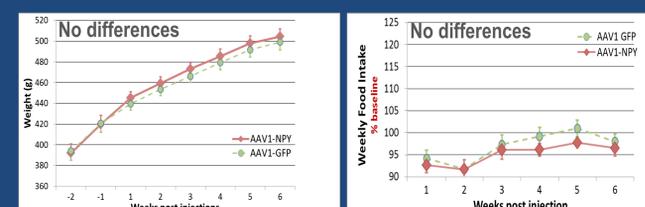


- General appearance similar to “prototype”, but less well organized.
- Missed placements\* were identified in 4 rats: dorsal (N=3) or lateral (N=1).
- \* removed from the analysis of behavioral data

## Results: Body weight & Food Intake



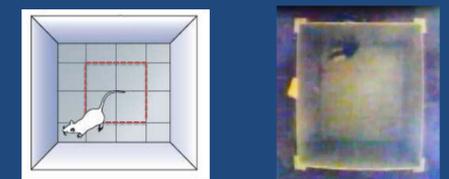
\* Significant Main Effect of Construct



- AAV10-NPY, but not AAV1-NPY, increased food intake.
  - Consistent with the known effects of ↑ NPY in LV [e.g., 5]
- Effect size too small to evaluate time course.

## Results: Locomotor Activity

60 min exposure to a novel open field



	Whole field		Center Field		
	Total Distance	Maximum Velocity (m/s)	Total Distance	Maximum Velocity	Latency (s) to Enter
AAV10-NPY	34.5 ± 5.6	0.13 ± 0.005	4.6 ± 1.3	0.15 ± 0.02	21.0 ± 8.2
AAV10-GFP	43.0 ± 11.2	0.14 ± 0.009	7.3 ± 3.1	0.19 ± 0.04	304.6 ± 285.5
AAV1-NPY	28.9 ± 5.7	0.13 ± 0.005	3.2 ± 1.0	0.13 ± 0.04	58.5 ± 26.1
AAV1-GFP	34.5 ± 5.6	0.13 ± 0.005	3.8 ± 1.0	0.15 ± 0.04	47.6 ± 30.4

No differences in general LMA or approach to the center of the box

## Discussion

1. The small persistent and significant increase in food intake after injection of AAV10-NPY treatment is supportive of the hypothesis, but more information is needed conclude that the change in food intake is mediated by an increased in CSF NPY.

The critical next steps in the current study are to

- determine if NPY synthesis in cells around the lateral ventricle is both increased and novel using IHC.
  - determine if NPY concentration in CSF is increased, supporting an increase in release of NPY into the lateral ventricle, using EIA.
2. We also expected, but did not observe, a change in center field behavior, a purported measure of anxiety.
- This contradictory result suggests caution in the interpretation of these results.

## References

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