Neurotransmitter Release Patterns of VTA Dopamine and Glutamate Neurons

Implications for Reward, Aversion, and Predictive Learning

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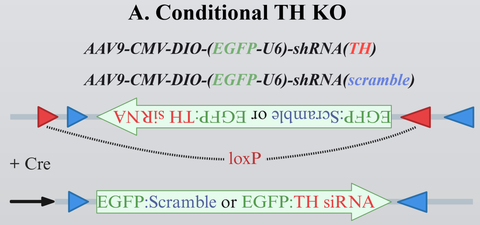
Introduction

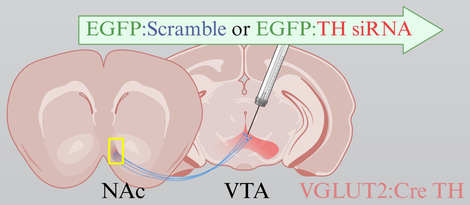
Ventral tegmental area (VTA) dopamine (DA) and glutamate (GLU) neuronal activity have been implicated in a variety of behaviors, including positive reinforcement, motivation, and motor control. Recent advances in neuroscience have shed light on the heterogeneous nature of neurotransmitter release within the VTA. Notably, there exist distinct VTA GLU and DA co-releasing neurons. The function of DA release from these neurons is not well understood. **We seek to characterize how GLU DA co-release from these neurons affects behavior and may differ from VTA GLU-only or DA-only release.**

Objectives & Methods

1. Knockdown DA from VTA GLU-DA releasing neurons
2. Observe behavioral impacts in reward and fear conditioning tasks

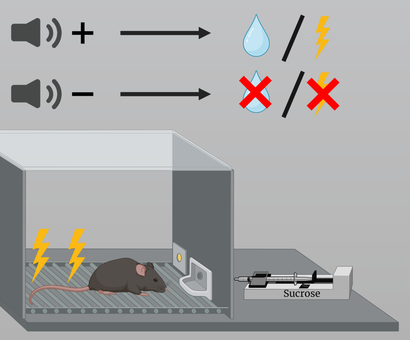
A. Conditional TH KO





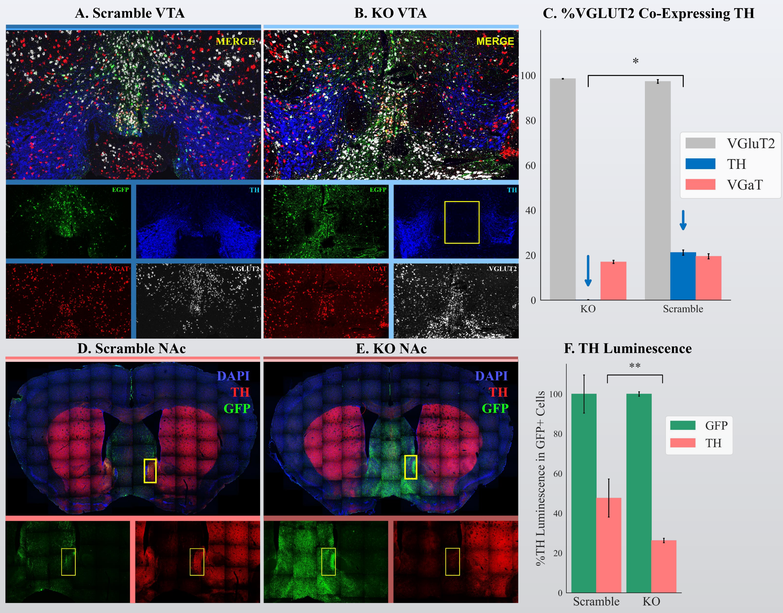
A) Conditional knockdown of DA from VTA GLU-DA releasing neurons via injection of a Cre dependent siRNA (shRNA) AAV vector in VGLUT2:Cre mice.

B. Pavlovian Behavior Tasks



B) Mice were tested using Pavlovian Reward and Fear conditioning paradigms. A CS+ cue is paired with administration of sucrose (reward) or shock (fear) and a CS- cue with nothing.

Knockdown Results



Successful Knockdown of DA from VTA GLU-DA neurons and their terminals in the Nucleus Accumbens (NAc).

n=18

A) VTA imaging of a scramble (control) vector using *immunohistochemistry* (IHC). EGFP: cell marker.

B) VTA IHC imaging of a knockdown vector. Note the yellow box (ROI) indicating visually apparent lack of TH.

C) Percent of VGLUT2 cells co-expressing TH in Knockdown and scramble mice. ($p<0.005$)

D) NAc imaging of a scramble vector using *in situ-hybridization* (ISH). ROI: NAc shell, a major projection target of the VTA. GFP: indicates viral expression.

E) NAc ISH imaging of the knockdown vector.

F) Total luminescence of TH protein in ROI as a percentage of GFP. ($p<0.005$)

Behavior Results

Knockdown (siRNA) mice exhibit worse performance in both reward and shock Pavlovian conditioning tasks when compared to controls.

Scramble n=7, siRNA n=6

A) Percent of times mouse entered reward port during CS+ per day.

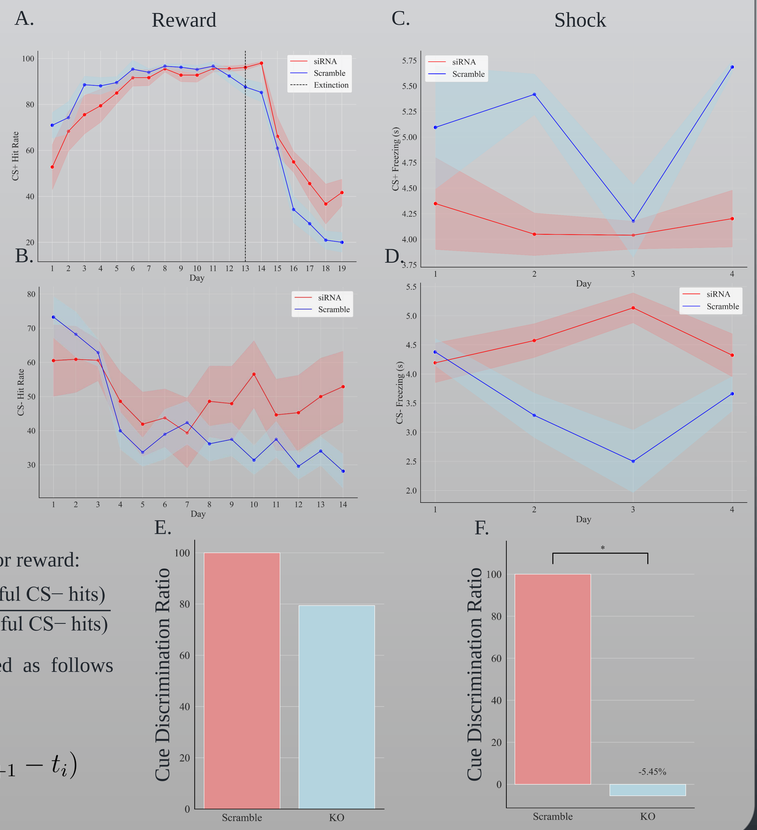
B) Percent of times mouse entered reward port during CS- per day.

C) Amount of time in seconds spent frozen during the CS+

D) Amount of time in seconds spent frozen during the CS-

E) The area under the curve of the daily discrimination ratio for knockdown mice compared to controls.

F) Similar to E except the discrimination ratio is calculated using time spent frozen during a given cue. ($p<0.02$)

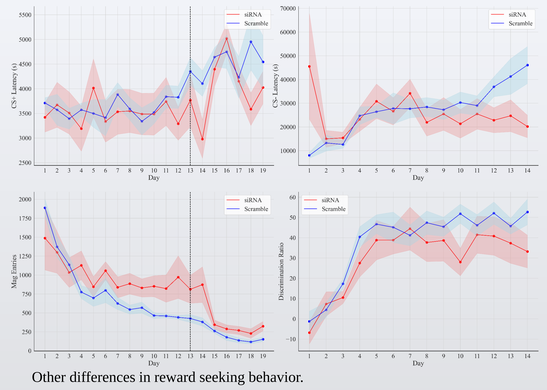


The discrimination ratio is calculated as follows for reward:

$$D(t) = \frac{(\%\text{ of successful CS+ hits} - \%\text{ of successful CS− hits})}{(\%\text{ of successful CS+ hits} + \%\text{ of successful CS− hits})}$$

The Area Under the Curve (AUC) is calculated as follows where t_i is the day at a given point (i).

$$\text{AUC} = \sum_{i=1}^{n-1} \frac{D(t_i) + D(t_{i+1})}{2} \times (t_{i+1} - t_i)$$



Other differences in reward seeking behavior.

Conclusions & Discussion

Thus far our data shows that conditional knockdown of DA from GLU-DA VTA neurons results in learning deficits across both reward and fear conditioning tasks. With a statistically significant impact to fear cue discrimination.

Upshot: VTA DA release from VTA GLU-DA neurons appears to play a crucial role in fear learning and reward association distinct from the established role of VTA DA only release.

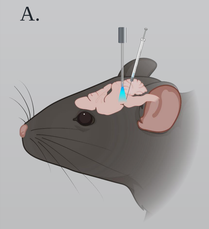
Current & Next Steps

A) Current: Fiber Photometry analysis of VTA subpopulations during behavioral tasks to characterize activation patterns.

B) Current: Optogenetic activation and inactivation of VTA subpopulations during behavioral tasks.

Next: Conditional DA Recovery from GLU-DA VTA neurons.

A.



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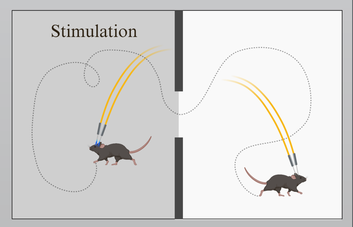

References & Acknowledgements

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Graphics were created with BioRender.com

References and More information can be found here: