Genetic Approaches to Dissecting Dopaminergic Anatomy & Function in Mice

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Introduction

Predictable feeding opportunities and its correlation to time, has had long history of ongoing research. In mammals, the suprachiasmatic nucleus (SCN) generates circadian rhythms through photosensitive retinal ganglion cells, enabling synchronization with the daily sunrise (Trzeciak & Steele, 2022). However, the SCN is shown to play a small role in regulating food anticipatory activity, as shown through studies of animals with lesions to the SCN conveying vigorous food-entrained rhythms. Behavioral data of rodents having multiple meal timers and the scarcity of data indicating a specific brain structure that functions as a food-entrained oscillator (FEO), has led to the hypothesis that there are multitude of FEO networks, each with their own behavioral entrainment to scheduled feeding.

Objective

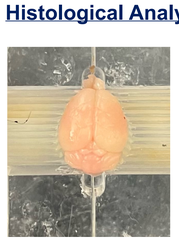
To define the dopamine (DA) neurons required for food anticipatory activity, the lab employs a Cre-lox system to create deletion mutants of tyrosine hydroxylase (TH), an enzyme that is required for catecholamine synthesis. A modified allele of the *TH* gene is introduced into a transgenic mouse that expresses the enzyme Cre-recombinase controlled by a cell-type specific promoter.

Materials and Methods

Mouse #'s:

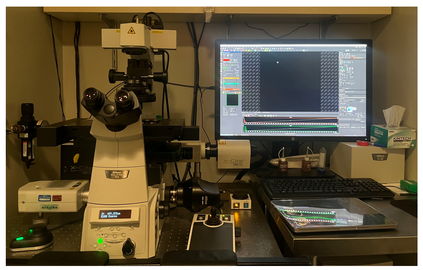
- **Calbindin1-Cre; FloxedTH:** Calb 36 KO, Calb 30 KO, **:** Calb 688 WT, Calb 691 WT, Calb 689 WT
- **Dat-Cre; FloxedTH:** Dat TH 1490 KO, Dat TH 1494 WT, Dat TH 1492 WT

Histological Analysis



Quantification Analysis

Images were captured through the Nikon ECLIPSE Ti microscope equipped with the Nikon DS-Ri2 color camera. ImageJ were used for quantification analyses cell counts,



Results

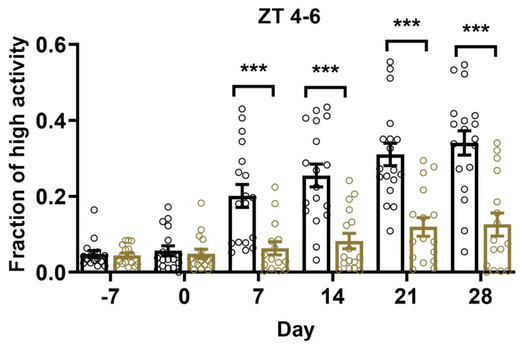


Figure 1: *Behavioral graph of Calbindin1-Cre;FloxTH control and Calbindin1-Cre;FloxTH conditional knockout mice through the duration over their 4 weeks of timed feeding.*

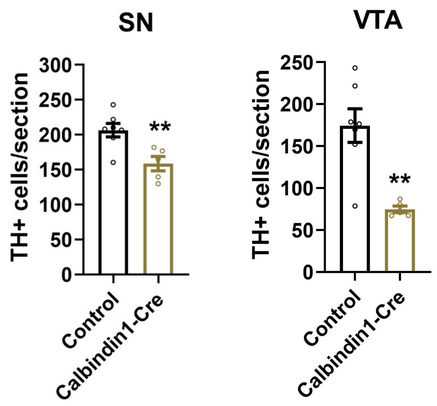


Figure 2: *Image on the left is the quantification of averaged cell counts, located in the substantia nigra region of the midbrain, quantified from Calbindin1-Cre;FloxTH controls and Calbindin1-Cre;FloxTH knock outs (27%, P = 0.0073).*
Figure 3: *Image on the right is the quantification of averaged cell counts, located in the ventral tegmental area region of the midbrain, quantified from Calbindin1-Cre;FloxTH controls and Calbindin1-Cre;FloxTH knock outs.*

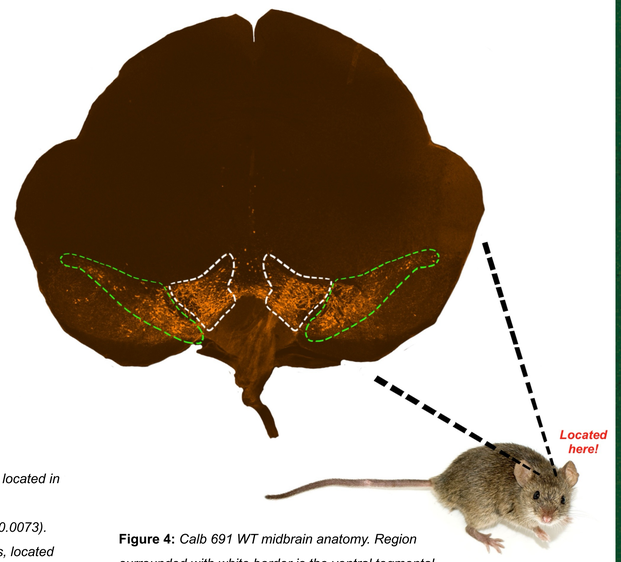


Figure 4: *Calb 691 WT midbrain anatomy. Region surrounded with white border is the ventral tegmental area (VTA). Region surround with green border is the substantia nigra (SN).*

Discussion

Even with their large deletions of DA, a majority of the TH conditional knockout (cKO) mutants were still able predict their scheduled mealtime.

The Calbindin1-Cre TH cKO mice, their trend over the 4 weeks of timed feeding, showed a normalized high activity behavior.

- During ad libitum, the measurements of pre-meal activity showed to have no difference between the cKOs and controls. Yet on day 7 of CR feeding, the controls had an increase in premeal activity, contrasting the cKO mice, who remained inactive before mealtime.
- The Calbindin1-Cre did not have an AAV rescue of TH in the SN and showed little to no amount of FAA. The TH cKO in the Calb1-Cre mice showed a small deletion of TH neurons (27%, P = 0.0073) located in the SN

Re-establishing TH from AAV injections in the SN has help resolved DA neurons in in the SN and is s crucial for FAA. Large deletions(<75%) of TH, in the SN, for Crhr1-, Foxp2-, Ntsr1-, Sox6-, & Slc17a6-Cre, had almost normal levels of FAA..

Summary and Conclusions

By using the Cre-driver for calcium buffering protein, Calbindin1, we were able to create a small deletion of TH-expressing cells. Our observations showed that these mice had normal feeding and body weight. As for the cKO mice, they gravely lacked expression of FAA. This minor deletion in the SN, suggests that the Calb1-Cre is expressed in this brain region's DA neurons. Our experiment also proposed that the Calb1-Cre DA neurons, in the SN, is mostly expressed during the developmental stage, rather than in the adult stage.

Future Work

To further our understanding of the specified dopaminergic neuron subpopulation, in the subject of calorie restriction, partake in regulating circadian rhythms through scheduled feeding:

- Manipulate and trace the connections of Calb1-Cre dopaminergic neurons in the medial portion of the dorsal striatum (DS).
- Obtain a genetic marker that can be expressed in adult DA neurons in the SN.

References

Poulin, J.-F., Zou, J., Drouin-Ouellet, J., Kim, K.-Y. A., Cicchetti, F., & Awatramani, R. B. (2014). Defining Midbrain Dopaminergic Neuron Diversity by Single-Cell Gene Expression Profiling. *Cell Reports*, *9*(3), 930–943. https://doi.org/10.1016/j.celrep.2014.10.008

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Trzeciak, J. R., & Steele, A. D. (2022). Studying food entrainment: Models, methods, and musings. *Frontiers in Nutrition*, *9*, 998331. https://doi.org/10.3389/fnut.2022.998331

Acknowledgments

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Ethics Statement: The experiments described herein were approved by the California State Polytechnic, Pomona Institutional Animal Care and Use Committee under protocols: 13.025, 13.029, 16.029, 17.003, 20.013, and 20.014.

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