Developmental programming of neural circuits integrating drinking and feeding in female mice

Gina Yu[1], Serena Sweet[1], Jessie Zimmermann[1], Richard Simerly[1]
1. Vanderbilt University, Department of Molecular Physiology & Biophysics

INTRODUCTION

- Metabolic disorders are very common and are associated with deviations to neural circuit development.
- Hypothalamic neural circuits develop early in postnatal life, but our understanding of their developmental timeline remains rudimentary.
- Previous rat studies show drinking and feeding are coordinated homeostatic processes (i.e. dehydration-induced anorexia).
- The PVH receives inputs from neurons in the MePO and AgRP neurons from the arcuate nucleus of the hypothalamus.

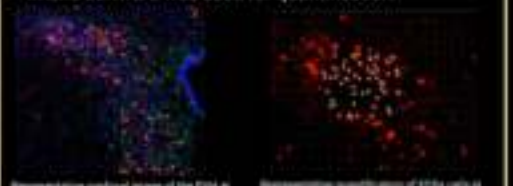
- I hypothesize that osmotic signals reach the MePO and PVH prior to AgRP projections in neonatal mice, with no significant sexual dimorphism between females and males.
- I hypothesize perturbations to drinking circuitry during development will impact the formation of feeding circuitry.

METHODS

- Determine the developmental time course of neural circuits controlling drinking.

- Determine if the organization of the AgRP feeding circuitry is dependent upon drinking stimuli during neonatal development.

- Imaris software was used for quantification.



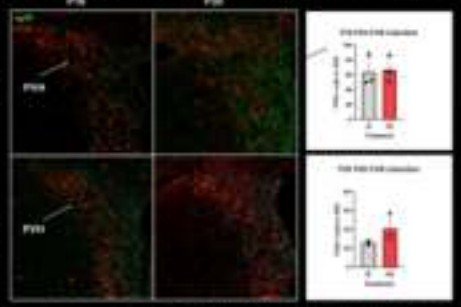
Representative confocal image of the PVH at 20x of a P30 female mouse + HS.

Representative quantification of FOS+ cells in an ROI of the PVH

RESULTS

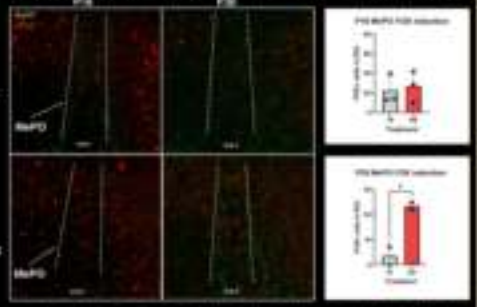
Determine the developmental time course of neural circuits controlling drinking.

Fos is induced in the PVH across all neonatal timepoints in response to HS



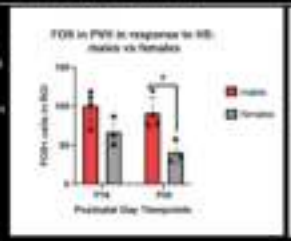
Representative confocal images at 20x of the PVH of P14 and P30 mice treated with IS as a control and HS as a dehydration stimulus with quantification of AgRP fiber densities. Includes quantification of Fos+ cells in IS and HS treated mice.

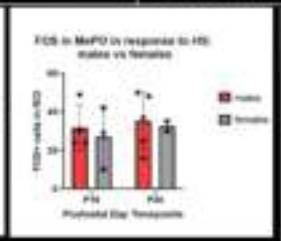
Fos is induced robustly in the MePO across all neonatal timepoints in response to HS



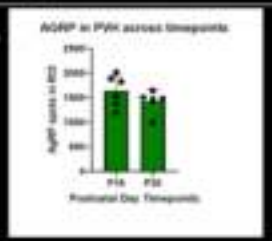
Representative confocal images at 20x of the MePO of P14 and P30 mice treated with IS as a control and HS as a dehydration stimulus with quantification of AgRP fiber densities. Includes quantification of Fos+ cells in IS and HS treated mice.

Sexual dimorphism in the PVH for IS treated mice. No sexual dimorphism in the MePO.

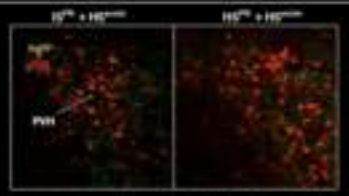




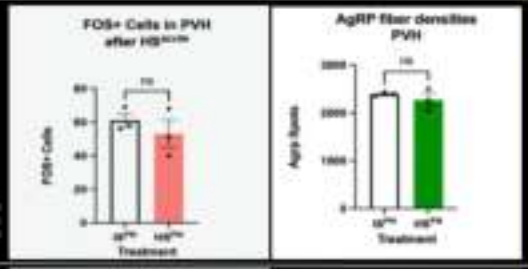
AgRP fiber density data from P14 and P30 female mice in the PVH

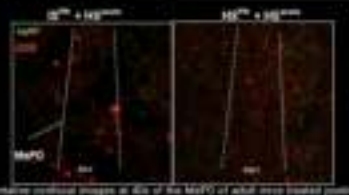


Determine if the organization of AgRP feeding circuitry is dependent upon drinking stimuli during neonatal development.

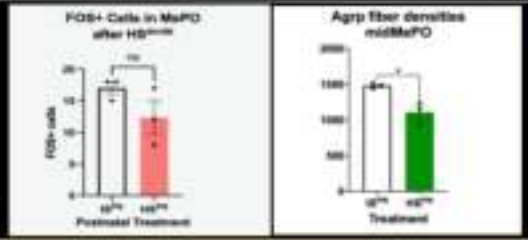


Representative confocal images at 40x of the PVH of adult mice treated postnatally from P5-P15 with IS as a control (IS^{P5}) and HS as a dehydration stimulus (HS^{P5}), with quantification of Fos+ cells and AgRP fiber densities following acute HS injection 90 mins before tissue collection and in IS before tissue collection.



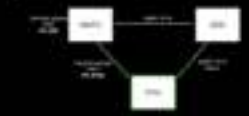


Representative confocal images at 40x of the MePO of adult mice treated postnatally from P5-P15 with IS as a control (IS^{P5}) and HS as a dehydration stimulus (HS^{P5}), with quantification of Fos+ cells and AgRP fiber densities following acute HS injection 90 mins before tissue collection and in IS before tissue collection.



CONCLUSIONS

- Fos is robustly induced in the PVH and MePO in response to dehydration stimuli in females at P16 and P30.
- In the second week of life, multiple signals converge onto the PVH, which could explain the high Fos induction in IS-treated animals.
- There are sexually dimorphic differences in the PVH, but not the MePO, in response to dehydration stimuli.
- AgRP fiber densities have reached the PVH in mature patterns by P16.



- In adult female mice, there are no changes in AgRP fiber densities in the PVH in HS^{P5} animals, and no changes in the dehydration response.
- In adult female mice, there is a significant difference in the AgRP fiber densities in the MePO of HS^{P5} mice, and there is a trend of an attenuation of the dehydration response.

- Implications:
  - Energy balance regulation is further studied through defining timelines of thirst.
  - Data can support treatment and prevention of metabolic disease in areas prone to droughts or with little access to water.
  - Clarify understandings of hunger and thirst regulation in correlation with each other.

FUTURE DIRECTIONS

- Include more timepoints for comparisons (P1, P8).
- Increase numbers of animals in studies.
- Consider factors for sexual dimorphism (hormone levels, different developmental timelines).
- Explore differences in developmental programming in neural circuits between males and females.
- Define areas of overlap with behavior.

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- Jessie Zimmermann

Some images made with Biorender

NIH

deCOOL stuff SyBBURE

Development of neural circuits integrating drinking and feeding in female mice

Gina Yu[1], Serena Sweet[1], Richard Simerly[1]

Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN[1]

Metabolic disorders are very prevalent and are associated with deviations to neural circuit development. Hypothalamic neural circuits develop early in postnatal life, but our understanding of the timeline of their development remains rudimentary. Previous studies in rats show that drinking and feeding are coordinated homeostatic processes, as indicated by dehydration-induced anorexia: a condition where dehydration results in reduced food intake and consequential decreased body weight. In mice, it is known that Agouti-related peptide (AgRP) neurons originating in the arcuate nucleus of the hypothalamus respond to nutritional cues and reach downstream targets, such as the paraventricular nucleus of the hypothalamus (PVH), in mature patterns during the second week of life. The PVH also receives inputs from the median preoptic nucleus (MePO), which is an integrative center for modulating fluid intake. Thus, the PVH is a likely node of convergence for drinking and feeding. While drinking circuitry appears to be intact early on in life, the timeline of responsiveness remains undefined in neonatal mice. I hypothesize that osmotic signals reach the MePO and PVH prior to AgRP projections in neonatal mice, and that there will be no significant sexual dimorphism between females and males. To test this hypothesis, we perfused neonatal mice exposed to hypertonic saline (HS) as a dehydration stimulus and isotonic saline (IS) as a control at postnatal days 8, 16, and 30. The brains were processed through immunohistochemistry and imaged using a confocal microscope. FOS as a marker of neuronal activation and DAPI as a counterstain will be used to examine the activation of neurons in the MePO and PVH in response to dehydration stimuli. Using Imaris software, FOS+ cells in the females and males of the HS and IS treatment groups across ages were compared. I found that HS treatment significantly induces FOS in the MePO at P30 in female mice, and that there is robust induction in the PVH as well. FOS induction in the PVH in response to HS is significantly higher in males compared to females with no significant differences in the MePO, indicating a sexual dimorphism in how dehydration signals are conveyed to downstream targets. Our research will provide further insight into corresponding metabolic phenotypes, dehydration-induced anorexia, and other eating and drinking conditions.