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CONTRACTING ORGANIZATION: Baylor College of Medicine, Houston, TX

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None.

14. ABSTRACT

Abnormal eating habits that drive excessive food seeking- or avoidance can manifest as health-threatening and socially impactful eating disorders. The scope of research includes 3 main aims: 1) to determine how basal forebrain ACh+ and Glu+ neurons are connected; 2) to determine how Glu+ neurons in the basal forebrain influence feeding behavior; and 3) to identify downstream targets of basal forebrain Glu+ neurons that govern feeding behavior. Specific results from this reporting period have identified that specific activation of DBB Glu+ neurons severely reduced feeding, and importantly, specific activation of DBB Glu+ fibers in the lateral hypothalamus (LH) also induced similar reduction in feeding, suggesting LH as one major downstream target mediating the feeding effect. However, specific inhibition of DBB Glu+ fibers in LH failed to increase feeding, suggesting a sufficient but not necessary role for LH neurons in mediating the feeding effect. The remaining planned experiments including the examination of local DBB circuits between cholinergic and glutamatergic neurons, the genetic interaction between DBB Glu+ neurons and the ob gene and the effect of DBB Glu+ neurons in motivation feeding will be accomplished in the next year. Overall, the results of the supported work will provide insights on how basal forebrain ACh+ and Glu+ neurons are connected, how Glu+ neurons in the basal forebrain influence feeding behavior and related behaviors and identify the downstream targets that mediate basal forebrain Glu+ neurons in regulating eating and related behaviors.

15. SUBJECT TERMS
Basal forebrain, eating disorder, glutamate, acetylcholine, lateral hypothalamus, optogenetics, in vivo Ca2+ imaging, metabolism

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INTRODUCTION

Abnormal eating habits that drive excessive food seeking- or avoidance can manifest as health-threatening and socially impactful eating disorders. To date, studies investigating the neural contribution to eating habits and body weight control have focused largely on neuropeptidergic signaling in the hypothalamus, a key brain region involved in feeding behavior. Although signaling from extra-hypothalamic brain regions has also been implicated in regulating nutrient metabolism, appetite, and satiety, it is unclear which brain regions and pathways are involved. The scope of this research includes 3 main aims: 1) to determine how basal forebrain ACh+ and Glu+ neurons are connected; 2) to determine how Glu+ neurons in the basal forebrain influence feeding behavior; and 3) to identify downstream targets of basal forebrain Glu+ neurons that govern feeding behavior. Specific results from this reporting period have identified that selective activation of DBB Glu+ neurons severely reduced feeding, and importantly, targeted activation of DBB Glu+ fibers in the lateral hypothalamus (LH) also reduced feeding, suggesting that the LH as a major downstream target that mediates DBB Glu+ neuron feeding effects. However, specific inhibition of DBB Glu+ fibers in LH failed to increase feeding, suggesting a sufficient but not necessary role for LH neurons in feeding behavior. The remaining planned experiments include the examination of local DBB circuits between cholinergic and glutamatergic neurons, the genetic interaction between DBB Glu+ neurons and the ob gene, and the effect of DBB Glu+ neurons in motivational feeding will be accomplished in the next year. Overall, the results of the supported work will provide insights on how basal forebrain ACh+ and Glu+ neurons are connected, how Glu+ neurons in the basal forebrain influence feeding and related behaviors, and identify the downstream targets that mediate basal forebrain Glu+ neurons in regulating eating and related behaviors.

KEYWORDS

Basal forebrain, eating disorder, glutamate, acetylcholine, lateral hypothalamus, optogenetics, in vivo Ca2+ imaging, metabolism

ACCOMPLISHMENTS

Major goals

Major goal 1: identify subtypes of basal forebrain neurons responding to eating; SOW completion date and site: 1-12 months in Site 1 (Dr. Arenkiel). This goal has been accomplished 1-12 months.

Major goal 2: map local inputs onto basal forebrain Glu+ neurons; SOW completion data and site: 3-12 months in Site 2 (Dr. Tong). This goal has been delayed because of unavailability of SAD rabies virus during the pandemic. We have generated the required animal models. However, we found some issues with the use of the SAD rabies virus in local circuit tracing. We are trying alternative ways to overcome the issues. This goal will be accomplished in the next 6 months.

Major goal 3: Profile Ach receptor expression in basal forebrain Glu+ neurons; SOW completion data and site: 1-12 months in Site 1 (Dr. Arenkiel). This goal has been mostly accomplished. Initial expression analysis has been conducted using FISH, but we are still confirming basal forebrain cell types of expression. This has been delayed due to the pandemic, but is currently nearly completed. We have also added the detailed profiling of ACh receptor subtypes in DBB Glu+ neuron targets.

Major goal 4: Decipher the nature of neurotransmitter between basal forebrain Ach+ neurons and Glu+ neurons; SOW completion date and site: 3-12 months in Site 1 (Dr. Arenkiel). This goal has been accomplished 3-12 months and included in the last report.

Major goal 5: Image basal forebrain Glu+ neurons *in vivo* responses to eating; SOW completion data and site: 6-20 months in Site 1 (Dr. Arenkiel). This goal has been accomplished and included in the last report.

Major goal 6: Modulate basal forebrain Glu+ neuron activity and assess impact on feeding and physiology; SOW completion date and site: 6-24 months and Site 2 (Dr. Tong). This goal has been accomplished 6-24 months and included in the last report.

Major goal 7: Inhibition of basal forebrain Glu+ neurons and impact on feeding; SOW completion date and site: 12-28 months and both Sites 1 and 2 (Drs. Arenkiel and Tong). This goal has been accomplished 12-36month and included in this report.

Major goal 8: Modulate basal forebrain Glu+ neuron activity and assess feeding; SOW completion date and site: 12-28 months and Site 2 (Dr. Tong). This goal has been accomplished and included in this report.

Major goal 9: Evaluate roles for basal forebrain Glu+ neurons mediating the action of ACh+ neurons in feeding regulation and body weight; SOW complete date and site: 12-36 months and Site 2 (Dr. Tong). This goal has been delayed due to issues encountered in animal models. We anticipate completion in the coming months.

Major goal 10: Genetic rescue of obesity; SOW completion date and site: 6-36 months and Site 1 (Dr. Arenkiel). This goal has been delayed due to breeding issues. Mice are currently available, and actively being investigated. We anticipate completion in the coming months.

Major goal 11: Evaluate downstream targets of basal forebrain Glu+ neurons that govern feeding behavior; SOW completion date and site: 18-36 months and both Sites 1 and 2 (Drs. Arenkiel and Tong). This goal has been accomplished 18-36 months and reported in this report.

Major goal 12: Decipher basal forebrain Glu+ neuron to LH neuron projection in feeding motivation; SOW completion date and site: 12-30 months and Site 2 (Dr. Tong). This goal has been accomplished 50%, and we have established behavioral protocol for nose poke for food. We anticipate completion of analysis in the coming months.

Major goal 13: Determine whether LH Glu+ neurons are directly downstream of basal forebrain Glu+ neurons; SOW completion date and stie: 6-18 months and Site 1 (Dr. Arenkiel). This goal has been accomplished (6-18 months).

Major goal 14: Determine whether LH Glu+ neurons mediate the effect of basal forebrain Glu+ neurons in feeding regulation; SOW completion date and site: 6-36 months and Site 2 (Dr. Tong). This goal has been accomplished 6-36 months and included in this report.

Accomplishment during the reporting period:

In the previous reports, we have accomplished major goals 1, 4, 5, and 6. During the reporting period, we have accomplished major goals 7, 8, 11. 13 and 14, and also made significant progress in major goals 2, 3, 9, 10 and 12.

Major goal 2 (Dr. Tong): Here the goal has been to determine whether ChAT neurons in the basal forebrain send monosynaptic projections to Glu+ neurons. We plan to use a monosynaptic pseudotyped rabies viral tracing approach to trace monosynaptic upstream neurons. Toward this, we have prepared *Vglut2-Cre* models, in which AAV-Flex-TVA-mCherry and AAV-Flex-G viral vectors will be injected into the basal forebrains of these mice.

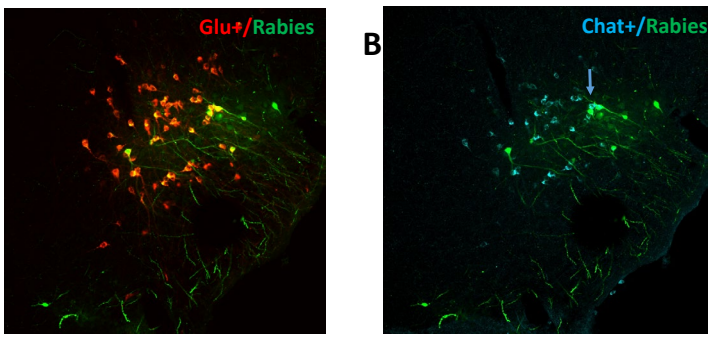

Fig. 1

One week later, SADdeltaG-GFP virus will be injected to the same location. Two weeks later, mice will be perfused to document the viral expression. However, due to the pandemic, the viral core for the pseudotyped rabies virus SADdeltaG-GFP had been shut down and the experiments had to be delayed from year 1 to year 2. We have accomplished this experiment in year 2 (Fig. 1, arrow identifies ChAT+ neurons positive for rabies viral expression). However, we only found a limited number of neurons that are presynaptically traced. The low efficiency of this pseudorabies monosynaptic viral tracing has been reported previously. We just learned a new method based on the tracing ability of heavy chain of tetanus toxin for much more effective tracing from neuromodulatory cell types, and we will use this method to confirm the pseudotyped rabies virus tracing. We have already obtained the new heavy chain viral vectors and the experiments are under way and we project we will accomplish these experiments in 36-48 months.

Major goal 3 (Dr. Arenkiel): Here the goal has been to analyze ACh receptors in basal forebrain Glu+ neurons. Toward this, RNA probes have been synthesized, tested for efficacy, and initial expression analysis has been completed. Due to the shutdown we are now resuming further experimentation to validate the cell type-specific patterns of AChR expression. We have obtained single cell FISH results, and new approaches towards single cell sequencing of basal forebrain material are currently underway.

Major goal 5 (Dr. Arenkiel): Here the goal is to determine the *in vivo* responses of basal forebrain Glu+ neurons to fed, fasting, and food-related odors. We have prepared *Vglut2-Cre* mice, delivered AAV-Flex-GCamp6m virus, and successfully implanted GRIN lenses into these animals. We have observed that GCamp6m signaling in the forebrain region responded to various external signals and found that, interestingly, these neurons responded to different food-related odors, but not to the mineral oil controls. These responses were rapid and reversable, suggesting a direct relevance of these neurons to feeding. This goal has been accomplished and reported in the last report.

Major goal 6 (Dr. Tong): Here the goal has been to determine feeding and body weight impacts in mice with basal forebrain Glu+ neurons expressing NaChBac. In the last report, we have prepared *Vglut2-Cre* mice and delivered AAV-Flex-NaChBac virus or control GFP virus to the basal forebrain. We found that NaChBac injected mice exhibited rapid body weight reduction, due to dramatic reduction of feeding.

Other experiments in this major goal are to test whether reduced food intake and associated reduced body weight can be rescued by forced feeding with food gavage. We found that by liquid food gavage, the survival rate of the animal with NaChBac expression was significantly improved, but not fully rescued. This goal has been accomplished and discussed in the last report.

Major goal 7 (Drs. Arenkiel and Tong): Here we sought to determine how loss-of-function manipulations in basal forebrain Glu+ neurons affects feeding (Dr. Arenkiel) and energy expenditure (Dr. Tong) by eliminating neurotransmitter release via expression of tetanus toxin light chain (TeNT). AAV-Flex-TeNT-p2A-GFP virus has been delivered to the forebrains of *Vglut2-Cre* mice, and we monitored feeding and body weight. However, we did not observe obvious differences in feeding, body weight, or O2 consumption, and/or locomotion, which has been summarized in the last report. Verification of injection and expression, and confirmation of TeNT achieving loss of Glu release has been completed.

Major goal 8 (Dr. Tong): Here we sought to determine effects on feeding via manipulation of forebrain Glu+ neurons activated with ChR2 or inhibited with eNpHR3.0. Towards this, we delivered AAV-Flex-ChR2-GFP virus to the forebrains of *Vglut2-Cre* mice, and at the same time implanted optic fibers. We found that delivery of blue light significantly reduced fasting-induced refeeding (Fig. 2), suggesting an importance of forebrain Glu+ neurons in feeding control. However, when we delivered AAV-Flex-eNpHR3.0 virus to the same neurons with light stimulation, we failed to observe significant effects on fasting refeeding. All the injections have been verified.

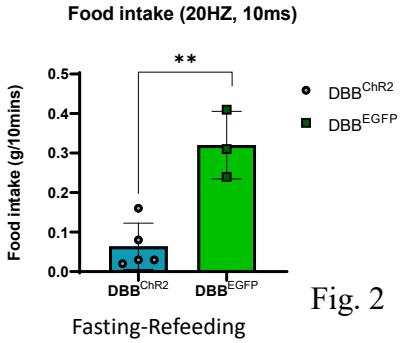

Fig. 2

Major goal 9 (Dr. Tong): Here the goal has been to determine whether basal forebrain Glu+ neurons mediate the action of ACh+ neurons in feeding and body weight. Towards this, we have crossed *ChAT-Cre* mice with *Vglut2-Flp* mice to obtain *ChAT-Cre::Vglut2-Flp* models, in which we delivered AAV-Flex-ChR2-GFP and AAV-fDIO-hM4Di-mCherry, or control AAV-fDIO-mCherry viruses to the forebrain. However, we found an issue with Vglut2-Flp mice in that this line does not seem to express Flp in Vglut2 neurons in the DBB area. We are identifying issues and will try alternative ways to overcome this issue.

Major goal 10 (Dr. Arenkiel): Here the goal has been to determine whether hypophagia by basal forebrain Glu+ neuron activation can rescue the ob/ob obesity. *Vglut2-Cre*::ob/ob mice have been generated and the AAV-Flex-NaChBac-GFP or control AAV-Flex-GFP virus has been delivered to the basal forebrain region of some of these mice. Experiments are planned to measure weekly body weight and feeding of these mice. The breeding of ob/ob mice has been slow and we have not yet accumulated enough numbers of mice to complete this experiment. We project that we will accomplish this experiment in the next 6-12 months.

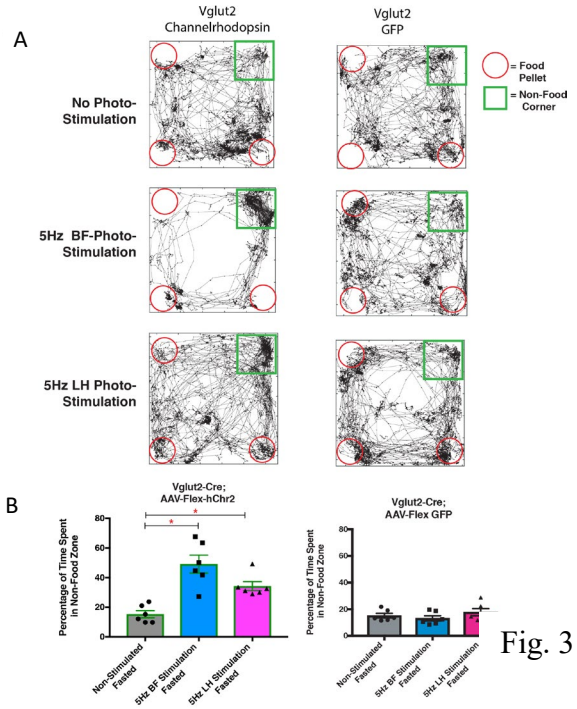

Fig. 3

Major goal 11 (Drs. Arenkiel and Tong): Here the goal has been to determine whether the LH functions as a key downstream target to mediate hypophagia (Dr. Arenkiel) or aversion (Dr. Tong) induced by forebrain Glu+ neuron activation. Stereotaxic surgery has been performed to deliver AAV-Flex-ChR2 virus to the forebrains of *Vglut2-Cre* mice and at the same time, optic fibers implantation at the LH. Our results showed that stimulation of fibers in LH elicited aversion associated with food odor (Fig.3A), which represents a major part of the total aversion induced by the stimulation of DBB Glut+ neurons (Fig. 3B), suggesting a major role of LH neurons in mediating DBB neuron function in aversion associated with food odor.

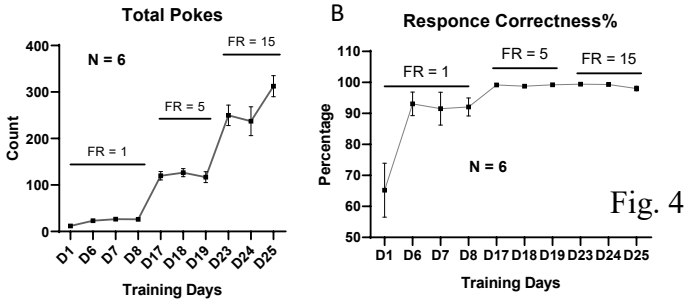

Fig. 4

Major goal 12 (Dr. Tong): the goal here has been to determine whether inhibition of basal forebrain Glu+ neuron projections to the LH promotes motivation to eat. Bilateral delivery of AAV-Flex-eNpHR3.0 virus has been made to the basal forebrain regions of *Vglut2-Cre* mice and optic fibers have also been implanted targeting the LH. These mice are scheduled to receive training in a nose poke chamber and then we will test whether light delivery will impact correct nose pokes and latency to nose poke. We have established the animal nose poke protocol in wild type animals (Figs. 4A and 4B) and we will perform the experiments in the experimental group in the coming months.

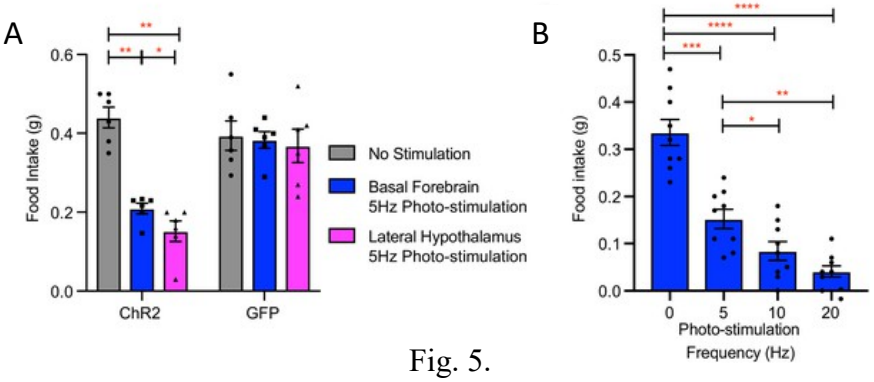

Fig. 5.

Major goal 13 (Dr. Arenkiel): Here the goal has been to determine whether LH Glu+ neurons are direct downstream

targets of basal forebrain Glu+ neurons. Towards this, AAV-Flex-ChR2-GFP virus has been delivered to the basal forebrain, while AAV-Flex-GFP virus has been delivered to the LH of *Vglut2-Cre* mice. We have finished the feeding experiments and as shown in Fig. 5, where local photostimulation in the LH potently inhibited feeding, the extent of which is similar to that observed with direct activation of basal forebrain Glu+ neurons (Fig. 5A). Notably, the effect on feeding inhibition was dependent on laser frequency (Fig. 5B). The studies are now wrapping up, with verification of accurate viral delivery and induction of c-Fos in the LH neurons after photostimulation have been completed.

Major goal 14 (Dr. Tong): Here the goal has been to determine whether LH Glu+ neurons mediate the effect of basal forebrain Glu+ neurons in feeding. *Vglut2-Cre* mice were prepared and AAV-Flex-ChR2 virus has been delivered to the basal forebrain, while at the same time AAV-Flex-hM4Di-mCherry was delivered to the LH. Initial experiments on verification of viral delivery have been confirmed. Our results show that inhibition of LH neurons failed to reduce food inhibition effects by DDB Vglut2 neuron activation, and we reasoned that the results observed might be due to activation of collaterals in other downstream neurons is sufficient to reduce feeding. We are currently testing this idea by examining whether DBB projections to the lateral habenula reduce feeding.

Training and professional development

Dr. Zhiying Jiang (postdoc) and Ms. Jing Cai (graduate student) (Site 2, Dr. Tong) are currently involved in this project, and are supported by this funding. Both of them are required to attend our departmental and center seminar series. The departmental seminar series invites well-known scientists across the US to present their new findings and the center seminar series will mainly presented by postdocs and students from each lab to share their research. Thus, both will have the opportunities to be exposed to research outside the campus and to present their own ongoing research. In addition, Ms. Cai will have planned activity and training classed within the graduate school, including required committee meetings in which Dr. Arenkiel is a committee member. We also have a regular lab meeting in which all lab members are required to present their own research, as well present a journal club on the papers from peers. Importantly, both trainees have regular weekly meetings with me. Through these various platforms, both trainees have ample exposure to extended mentoring and research exposure. With the pandemic, almost all seminar series with the UT and TMC have implemented a virtual format, in which they regularly attend. Unfortunately, with the impact of the pandemic, it is not easy for us to go to local/national meetings and we are trying to participate virtual meetings organized by various research foundations and other institutions, in order to get better exposure to peers. Of note, Ms. Cai and Dr. Tong have just attended this year's SfN meeting in November 2022 at San Diego

Lab personnel in Arenkiel lab include Ms. Mayuri Patel and Ms. Peyshyuan Chin. Both are current graduate students focused on this project as part of their thesis work. Mayuri and Peyshyuan have received direct training in *in vivo* imaging, electrophysiological recordings, and behavioral analysis, and have also been learning imaging and *in vivo* electrophysiological recording methods. They are utilizing these skills towards the proposed studies. All are making excellent progress towards the stated project goals, and have maintained timely committee meeting progress reports. As another training opportunity, both Ms. Patel and Ms. Chin have attended this year's SFN meeting. Also, as partial contributors, Mr. Benjamin Belfort and Mr. Suyang Bao (two new students to the laboratory) are serving as support and collaborators to the individuals focusing 100% effort towards the project. Although practical research experimentation has been paused during the funding period due to the pandemic, the listed personnel were able to continue analysis of existing data, and reinitiate new experimentation. Additionally, the Arenkiel Lab members have been actively involved with a program called the McNair Teaching Fellows program, where they interface with high school teachers and their classrooms in North Carolina to host lectures and virtual lab tours on their projects. This has been highly beneficial towards them gaining practical teaching experience and to share current scientific findings associated with their work.

Dissemination to community

Nothing to report

Plan for the next reporting period

We will execute the research plan outlined in SOW. There might be a slowdown in research progress because the required mouse colony reduction during the mandatory lockdown period of the pandemic. We will fully attempt to achieve the planned milestones, with some consideration of the delayed research plan.

IMPACT

Impact on development of principle disciplines

Nothing to report

Impact on other disciplines

Nothing to report

Impact on technology transfer

Nothing to report

Impact on society beyond science and technology

Nothing to report

CHANGES/PROBLEMS

It is not anticipated that there is a significant change in the approach or use of animals. However, due to the current pandemic, there might be a change in time to accomplish the planned and stated milestones. Unfortunately, during the initial lockdown, it was mandatory for us to significantly reduce mouse breeding/cages, which has caused a major delay in the availability of mouse study subjects. In addition, we are operating at a reduced personnel effort to achieve effective social distancing, which also delays the progress of research and associated research expenditure. Also due to school/daycare shut down with online course at home, the time spent on experiments on lab members with young kids has also been reduced. Despite facing these adversaries, we have managed to make the planned progress. The approved additional time will allow us to complete all proposed work.

PRODUCTS

During the course this funding period, we have published the following papers which indirectly benefited from this funding.

1. Anatomy and Function of Ventral Tegmental Area Glutamate Neurons. Cai J, Tong Q. Front Neural Circuits. 2022 May 20;16:867053. doi: 10.3389/fncir.2022.867053. eCollection 2022. PMID: 35669454. Acknowledgement of the federal support: Yes.

2. An estrogen-sensitive hypothalamus-midbrain neural circuit controls thermogenesis and physical activity. Ye H, Feng B, Wang C, Saito K, Yang Y, Ibrahimi L, Schaul S, Patel N, Saenz L, Luo P, Lai P, Torres V, Kota M, Dixit D, Cai X, Qu N, Hyseni I, Yu K, Jiang Y, Tong Q, Sun Z, Arenkiel BR, He Y, Xu P, Xu Y. Sci Adv. 2022 Jan 21;8(3): eabk0185. doi: 10.1126/sciadv.abk0185. Epub 2022 Jan 19.

PMID: 35044814. Acknowledgement of the federal support: Yes.

3. Hypothalamic CRH neurons: A crossroad between stress and metabolism. Jiang Z. and Tong Q. Current Opinion in Endocrine and Metabolic Research 2022, 26:100384. Acknowledgement of the federal support: Yes.

Nothing to report on products in other categories listed in the report instruction.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name	Benjamin Arenkiel
Project Role:	PI
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Nearest person month worked	12
Contribution to project	Supervision
Funding Support	This Award and NIH grants

Name	Qingchun Tong
Project Role:	PI
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Nearest person month worked	4
Contribution to project	Supervision
Funding Support	This Award and NIH grants

Name	Zhiying Jiang (Site 2)
Project Role:	Postdoctoral researcher
ORCID ID	0000-0002-3838-2791
Nearest person month worked	11
Contribution to project	Viral injections, feeding, body weight and recording
Funding Support	This Award

Name	Jing Cai (Site 2)
Project Role:	Graduate student
ORCID ID	Not available
Nearest person month worked	6
Contribution to project	Viral injections, body weight, feeding
Funding Support	This Award

Name	Mayuri Patel
Project Role:	Graduate student
ORCID ID	Not available
Nearest person month worked	12
Contribution to project	Circuit labeling, viral injections, and behavioral analysis.
Funding Support	This Award

Name	Peyshyuan Chin
Project Role:	Graduate student
ORCID ID	Not available
Nearest person month worked	12
Contribution to project	Circuit labeling, viral injections, and behavioral analysis.
Funding Support	This Award

Name	Benjamin Belfort
Project Role:	Graduate Student
ORCID ID	Not available
Nearest person month worked	3
Contribution to project	Imaging, viral labeling, circuit analysis
Funding Support	This Award

Name	Suyang Bao
Project Role:	Graduate student
ORCID ID	Not available
Nearest person month worked	6
Contribution to project	Circuit labeling, cellular analysis, and behavioral analysis.
Funding Support	This Award

There is no change in PI or key personnel during the reporting period.

Nothing to report on other organization involved as partners.

SPECIAL REPORTING REQUIREMENTS

Nothing to report

APPENDICES:

3 reprints of publications. Additional manuscripts are under review and will be reported in the next report.