AWARD NUMBER: W81XWH-19-1-0016

TITLE: The Role of Lateral Hypothalamus Orexin Glucose-Inhibited Neurons in Bing

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CONTRACTING ORGANIZATION: Rutgers, The State University of New Jersey

REPORT DATE: September 2022

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
September 2022	Annual	08/15/2021 to 08/14/2022

4. TITLE AND SUBTITLE

The Role of Lateral Hypothalamus Orexin Glucose-Inhibited Neurons in Binge-Eating Disorder

5a. CONTRACT NUMBER
W81XWH-19-1-0016

5b. GRANT NUMBER
PR180398P1

5c. PROGRAM ELEMENT NUMBER

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Repeated patterns of intermittent calorie restriction followed by re-feeding of highly palatable foods can contribute to binge pathologies in some at risk populations. For this project we use a murine of model of dietary binge eating that investigates the interaction of intermittent calorie restriction (i.e., hypoglycemia bouts) and rapid consumption of highly palatable food (i.e., sweetened fat; SF) on lateral hypothalamus (LH) orexin neurons. Mice with repeated access to SF without the repeated calorie restriction do not demonstrate a binge phenotype. Behavioral data indicates that using chemogenetic activation of LH orexin neurons we determined that mice with SF access only developed a binge phenotype. In vitro electrophysiology experiments demonstrated intermittent calorie restriction increases glutamate transmission on LH orexin target dopamine-containing in the ventral tegmental area (VTA). This increased VTA glutamine current could be a mechanism to drive binge eating following repeated calorie restriction. Our research is designed to investigate how binge eating pathology is different from normal homeostatic eating, as it pertains to the addictive nature of highly palatable rewarding foods. Our goal is to assist in developing clinically effective treatment strategies for improving the quality of life for military service members and veterans afflicted with eating disorders.

15. SUBJECT TERMS

binge eating, bulimia nervosa, eating disorders, weight restriction, orexin, feeding

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	14	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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1. INTRODUCTION: Military service members are at risk for developing eating pathologies. Disordered eating, in particular binge eating, can arise from establishing unhealthy dietary habits and maintaining lower than normal body weight. Intermittent feeding behaviors, such as caloric restriction and rapid consumption of highly palatable calorie dense foods dysregulate the neural controls of feeding. The purpose of this grant is to uncover the role of a distinct population of lateral hypothalamic (LH) orexin neurons, which are activated during calorie restriction or low blood glucose (i.e., glucose inhibited; GI). Engagement of LH orexin GI neurons are believed to be involved in dietary habits that establish binge-like eating behaviors. In particular, LH orexin-GI neurons send projections to ventral tegmental (VTA) dopamine neurons that are involved in reward and reinforcing behaviors. Our overall hypothesis is that intermittent caloric restriction alters the activity and glucose sensitivity of LH orexin-GI neurons to enhance the glutamatergic activity (i.e., AMPA/NMDA receptor ratio) of VTA dopamine neurons. These experiments will use a murine model of dietary-induced binge eating to further understand the neural circuitry involved in the maintenance of binge eating disorder and necessary for developing clinically effective treatment strategies for improving the quality of life for military service members and veterans afflicted with eating disorders.

2. KEYWORDS: binge eating, bulimia nervosa, eating disorders, weight restriction, orexin, feeding

3. ACCOMPLISHMENTS: What were the major goals of the project?

There are 3 major goals of this project

Goal #1. Determine whether the development of binge eating is associated with enhanced glutamatergic transmission onto VTA DA neurons. (Major Tasks 1 & 2)

Goal #2. Determine whether the development of binge eating alters the glucose sensitivity of LHA orexin neurons. (Major Task 3)

Goal #3. Determine whether inhibiting orexin neurons during caloric restriction suppresses binge-like eating behavior (Major Task 4)

What was accomplished under these goals?

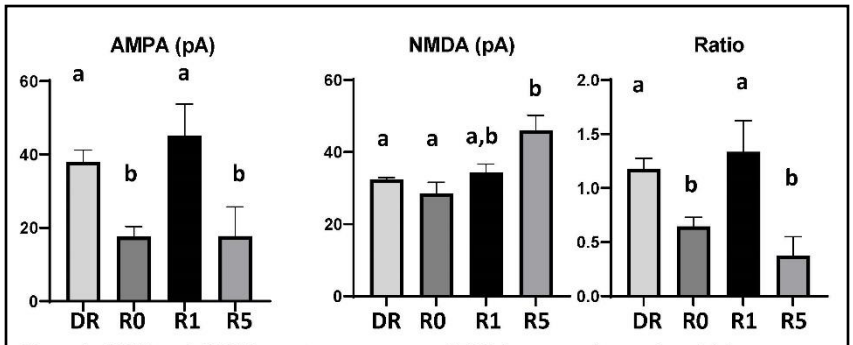

Figure 1. AMPA and NMDA receptor currents on VTA DA neurons from mice which were not diet restricted (R0), restricted once (R1), exposed to 5 intermittent restriction events (R5; binge protocol) or subjected to 2 weeks of constant diet restriction (DR)

Major Task 1, Subtask 1, month 1. This is subtask is completed. (Routh, NJMS, Rutgers, Newark,NJ)

Major Task 1, Subtask 2, months 2-7. Measure the AMPA/NMDA receptor ratio after 1 and 5 episodes of restriction (Figure 1). The original subtask is completed. As mentioned in previous progress reports, we were significantly delayed in this subtask. The delay was primarily due to COVID-19 personnel restrictions in conjunction with having to cease animal breeding and cut our colonies to 50%. Further, an experienced electrophysiologist left the lab and the challenge of training a new individual when we were not able to allow staff to be in the lab full time until late 2020 was substantial. The staffing issue was exacerbated by COVID-19 infections with long-term sequelae among key staff. However, despite these issues we have made significant progress and have completed the originally planned subtask. However, our very interesting findings indicated that we must add one more study group (no restriction; R0).

Our original hypothesis was that glutamate plasticity, as measured by the AMPA/NMDA receptor current ratio, would increase from the first overnight restriction episode (R1) to the fifth (R5) reaching a threshold for triggering binge behavior presumably between R4 and R5. Interestingly, the AMPA/NMDA ratio decreased between R1 and R5 due to decreased AMPA and increased NMDA receptor currents. This is in stark contrast to our related study with chronic diet restriction and weight loss, in which AMPA but not NMDA receptor currents increased. We added the R0 group (no restriction control) to

determine whether R5 reversed the effects of a single restriction episode. Our findings are shown in Figure 1 and described below.

As we previously published, one bout of overnight food restriction (R1) significantly increased AMPA receptor mediated currents on VTA DA neurons compared to ad lib fed controls (R0) leading to an increase in the AMPA/NMDA receptor current ratio. However, after the 5 episodes of intermittent overnight food restriction associated with the initiation of binge eating in rodents (R5) we found that AMPA receptor currents and the AMPA/NMDA current ratio actually decreased back to levels seen with the ad lib fed mice. While NMDA receptor currents were unchanged after one restriction (R1) compared to ad lib fed mice, R5 significantly increased NMDA receptor currents compared to ad lib fed. We then compared these results to a related study examining the effects of diet-restriction sufficient to decrease body weight to 85% of control for a period of two weeks (DR). Interestingly, it appears that chronic dieting and intermittent restriction associated with binge eating have distinct effects on glutamatergic signaling on VTA DA neurons. Chronic dieting leads to a persistent increase in AMPA receptor currents, with no change in NMDA receptor currents. In contrast, the intermittent restriction which leads to binge eating behavior reverses the effects of food restriction on AMPA receptor currents, while increasing NMDA receptor currents. The significance of this difference may be related to cognitive processes involved in sensory experience and learning (Kopp et al, Neuropharmacology 2007). Another intriguing consequence of increased NMDA vs AMPA receptor currents is that the former is associated with the change from burst to tonic firing in VTA DA neurons. Burst firing may play a role in the value judgement regarding salience of environmental stimuli according to Zaharov et al., 2016. Thus, our findings suggest that intermittent restriction that increases NMDA current could potentially affect decision making about palatable food and lead to bingeing. This may contrast with a simple drive to increase intake of palatable food in response to weight loss. To complete this subtask, we only need to increase the N value for the R0 group, thus this subtask is nearly complete. (Routh, NJMS, Rutgers, Newark,NJ)

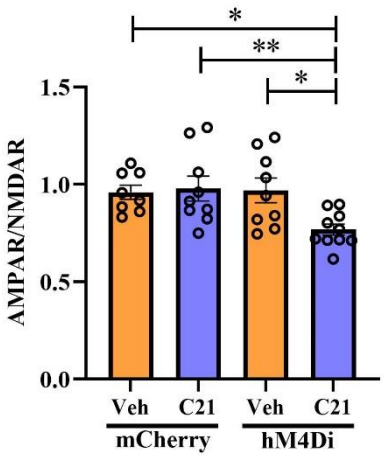

Figure 2. Orexin inhibition blocks the increase in AMPA/NMDA receptor current ratio seen with diet restriction. The inhibitory cre-dependent DREADD hM4Di or mCherry control was injected bilaterally into the LHA of mice expressing cre-recombinase in orexin neurons (orexin-cre mice). All mice were diet restricted to 85% of their original body weight and maintained at this weight for 1 week. As indicated, mice received either vehicle (veh) or the DREADD agonist C21 in their drinking water for the entire duration of diet restriction. DREADD inhibition of orexin neuron reduced the AMPA/NMDA receptor current ratio to that of non-restricted controls (see also figure 1).

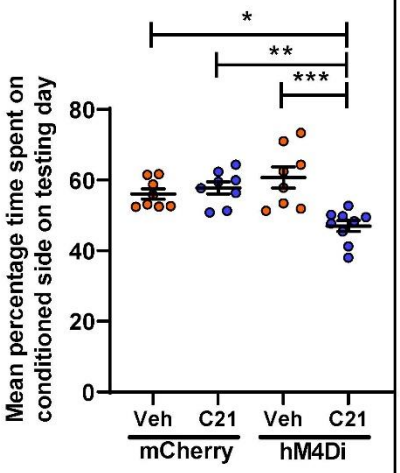

Figure 3. DREADD inhibition of orexin neurons reduced the motivation to seek palatable food after food restriction and weight loss. Mice were treated as described for Figure 2. DREADD inhibition of orexin neurons reduced the time the mice spent on the side of the conditioned place preference box associated with rewarding food (Froot Loops).

Major Task 2, Subtask 1, months 8- 29. Perform the chemogenetic experiments with Designer Receptors Exclusively Activated by Designer Drugs (DREADD) inhibition of orexin neurons and VTA DA glutamate plasticity, which require stereotaxic surgery. For this subtask we needed to hire a technician in the Routh laboratory to learn and perform surgeries; however, we could not hire during the COVID-19 restrictions. We were able to recruit an individual while under restrictions who was willing to wait and this individual is now in place. Due to COVID-19 restrictions, the training veterinarians were not allowed to conduct in person training and/or validation of skills in aseptic technique and suturing (required by the IACUC for surgeries at Rutgers). This technician has now been trained by a graduate student using DREADDs for a diet restriction project and has been approved by Rutgers veterinarians to begin these studies. Thus, we are proceeding with these experiments. (Routh, NJMS, Rutgers, Newark, NJ).

The necessity of these (DREADD) studies is highlighted by our finding above that the binge restriction paradigm produces different results than those seen with chronic dieting. The graduate student investigating diet restriction has shown that orexin mediates the increase in the AMPA/NMDA receptor current ratio during chronic dieting

and that inhibition of orexin neurons reduces the motivation to seek palatable food (Figures 2 and 3). These data also demonstrate that we are able to use DREADD modulation of orexin neurons to affect glutamate signaling in the VTA and feeding behavior. It is possible that orexin-independent mechanisms mediate the reduction in AMPA current and/or the increase in NMDA after the binge restriction protocol. For example, overnight food restriction is considered a stressor and corticotropin releasing factor (CRF) is implicated in binge feeding behavior. Both orexin and CRF increase NMDA receptor currents on VTA DA neurons (Borgland et al Brain Research 2010). Thus, it is critical to determine whether orexin mediates the effects of the binge restriction protocol on AMPA and NMDA receptor currents.

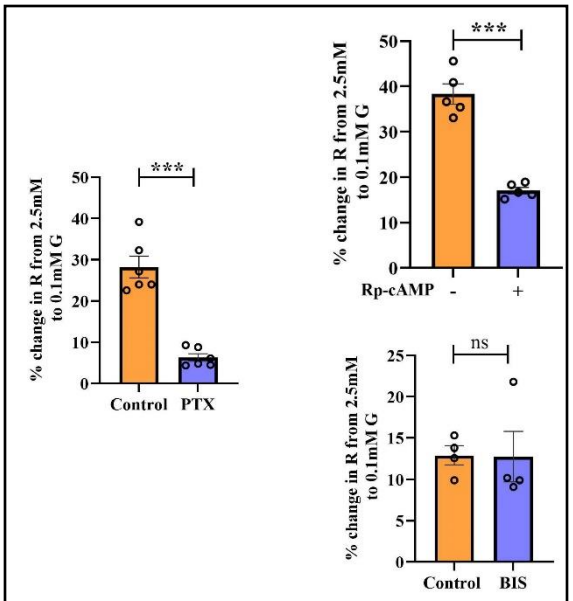

Figure 4. Protein kinase A (PKA) mediates glucose sensing in orexin neurons. Orexin glucose-inhibited neurons were evaluated using electrophysiology in brain slices from mice expressing GFP in their orexin neurons. Pertussis toxin (PTX; blocks G inhibitory protein Gi/o) and Rp-cAMP (blocks PKA) block the increase in input resistance (IR) in orexin neurons as glucose (G) is lowered from 2.5 to 0.1 mM. The PKC inhibitory BIS is without effect.

Direct effects of stress related projections from the bed nucleus of the stria terminalis (BNST) on orexin neurons may also modulate glucose sensitivity and thus, orexin impact on VTA DA neurons (Giardino et al., Nature Neuroscience 2018). Preliminary studies in our lab show that activation of orexin neurons by low glucose is blocked by pertussis toxin (blocks Gi/o) and Rp-cAMP (blocks protein kinase A; PKA). This suggests that the adenylyl cyclase (AC)-cAMP-PKA pathway mediates glucose sensing (Figure 4). The CRF receptor is also linked to PKA (Kageyama et al., Peptides 2009, Kurada et al., Plos One 2014). Thus, this signaling pathway may be a site of intersection between glucose sensing and stress regulated pathways which triggers binge eating behaviors. The interaction between stress and intermittent diet restriction is of particular relevance to the development of eating disorders among military personnel. Thus, once the planned studies are finished, our future direction is to explore this interaction between CRF, glucose sensing in orexin neurons, VTA DA glutamate plasticity and binge eating behavior.

<u>Major Task 3, Subtask 1, months 30-35</u>, This subtask has been delayed for the above reasons; however it is scheduled to begin in early 2023. (Routh, NJMS, Rutgers, Newark, NJ). Notably, once COVID-19 restrictions were lifted we have been able to increase our colony of mice that express green fluorescent protein in their orexin neurons (orexin-GFP mice). These mice are critical for this task and our colony is now sufficient to support these experiments.

<u>Major Task 4, Subtask 1, months 1-34</u>. There are some minor experiments needed to be completed for controls and to validate the viral injections with immunofluorescence for publication. However, this task will be completed in the next few months. For this task there were two sets of chemogenetic DREADD experiments reliant on adeno-associated viral vectors containing Gq excitatory construct (pAAV- hSyn-DIO-hM3D-mCherry) or Gi inhibitory construct (pAAV- hSyn-DIO-hM4D-mCherry) or) from Addgene (Watertown, MA). At PND 35 (5 weeks of age) hM3D-Gq (excitatory) were stereotaxically injected into the LH of OREXIN:CRE mice fated for the Binge (B) and Naive (N) groups to mimic caloric restriction by excitation of orexin neurons. In contrast, hM4D-Gi (inhibitory) were stereotaxically injected into the LH of OREXIN:CRE mice fated for the Restrict Binge (RB) and Restrict (R) groups to allow for inhibition of orexin neurons during caloric restriction. After 1-week recovery time, each group followed their respective feeding protocols. DREADDS agonist compound 21 (C21) injections (0.3 mg/kg) were given intraperitoneal (IP) once per day 30 minutes prior to access to sweetened fat (vegetable shortening blended with 10% sucrose) and/or chow pellets during the sweetened fat access ("binge") day only (RB, R, B) or on the comparable day for the control feeding group (N). Controls from each group received saline injections. ***<u>Excitatory DREADDS.</u>*** **<u>Binge Intake.</u>** For B and N, there was an overall group effect [$F(1, 52) = 184$, $p < 0.0005$], group X treatment effect [$F(1, 52) = 5.1$, $p < 0.05$], and binge session x treatment effect [$F(4, 208) = 3.1$, $p < 0.05$]. Post-hoc Tukey's HSD revealed Binge group receiving C21 had greater intake 30 min at binge session 5 compared with Binge group receiving saline ($p < 0.05$), see Figure 5A. *<u>There were no overall sex differences, but there were sex-specific effects</u>*. For *<u>females</u>*, there was a

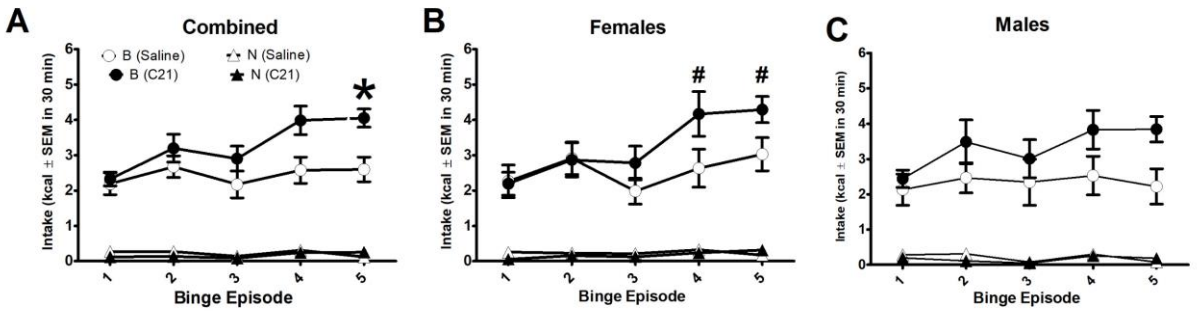

Figure 5. Excitatory (hM3D Gq) DREADDS activation in feeding groups *without restriction*, Binge (B) and Naive (N). B (saline) = 7 females/8 males, B (C21) = 7 females/8 males, N (saline) = 7 females/8 males, N (C21) =8 females/7 males. * indicates p< 0.05 from B (saline) at Binge 5, **#** indicates p < 0.005 from Binge 1.

group effect [F (1, 25) = 108.8, p <.00005], a binge session effect [F (4, 100) = 8.0, p< 0.00001), and a binge session x treatment effect [F (4, 100) =2.7, p < 0.05]. Post-hoc testing indicated that for the female Binge C21 group, Binge 4 and 5 were greater than Binge 1 (p < 0.005 for both), see Figure 5B. For <u>males</u>, there was only a group effect [F (1, 27) = 81.0, p< 0.00005], see Figure 5C. **<u>Inhibitory *DREADDS*.</u> <u>Binge Intake.</u>** For RB and R, there was an overall group effect [F (1, 48) = 471, p < 0.0005], overall dose effect [F (1, 48) = 32.4, p< 0.0005], and overall group x dose effect [F (1, 48) = 38.9, p< 0.0005]. Post-hoc Tukey's HSD indicated that C21 treated RB group had less 30 min binge intake than the saline treated RB group (p < 0.005), see Figure 6A. There were not any intake differences in the R groups treated with either C21 or saline. There were no sex differences nor were there sex-specific effects. In females, there was a group effect [F (1, 22) = 200, p < 0.00005], treatment effect [F (1, 22) = 11.9, p < 0.005], and treatment x group effect [F (1, 22) = 11.7, p< 0.05]. In males, there was a group effect [F (1, 26) = 277.1, p < 0.0005], treatment effect [F (1, 26) = 22.1, p< 0.005], and group X dose effect [F (1, 26) = 31.6, p < 0.005]. Similar to the post-hoc testing when sex was a factor in the analysis, when male and females were analyzed

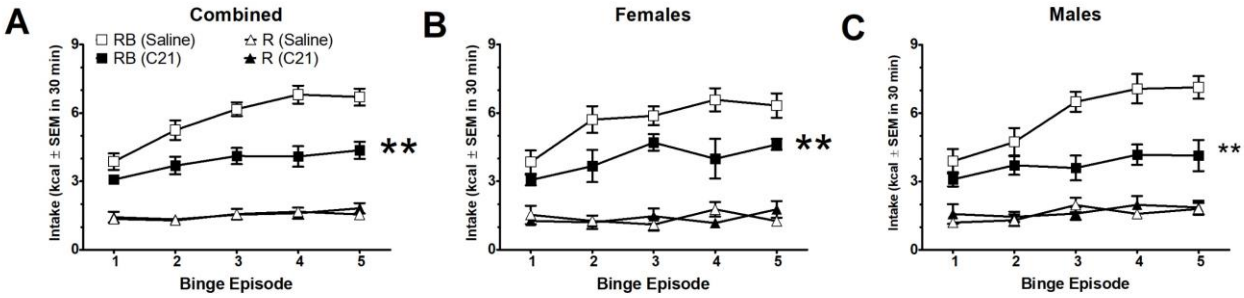

Figure 6. Inhibitory (hM4D Gi) DREADDS activation in feeding groups with restriction, Restrict Binge (RB) and Restrict (R). RB (saline) = 8 females/7 males, RB (C21) = 7 females/7 males, R (saline) = 7 females/8 males, R (C21) = 7 females/* males. ** indicates p< 0.005 from RB (C21) from RB (saline).

separated, post-hoc Tukey's HSD indicated that C21 treated RB group had less 30 min binge intake than the saline treated RB group (p < 0.005) and there were no differences with treatment in the R group, see Figure 6B and 6C.

Notably, one major difference between the excitatory and inhibitory DREADDS experiments was a time-dependent difference. Specifically, *repeated* C21 administration was needed to increase binge intake with excitatory DREADSS (i.e., binge session X treatment effect), whereas no time dependent effect was needed to decrease binge intake with inhibitory DREADSS. Together this suggest that repeated activation of LH orexin neurons is needed to produce binge-like eating. We next began to determine how chemogenetic manipulations influenced the individual differences in binge propensity. From the median sweetened fat intake, mice with the upper intake were classified as <u>*Binge Prone*</u>, whereas mice in the lower intake were classified as <u>*Binge Resistant*</u>. Before the start of the dietary induced binge eating protocol and C21 or saline,

mice were given a preexposure to the sweetened fat and classified as Binge Prone or Resistant. ***Excitatory DREADDS.*** **Binge propensity.** The underlying hypothesis was that excitation of LH orexin pathway *without a history of caloric restriction* would *increase* binge propensity. For the B group treated with either saline or C21, there was only an overall treatment effect [F (1, 22) = 6.68, p < 0.05] with C21 treated mice consuming more sweetened fat at Binge 5 (p < 0.05), regardless of pre-exposure prone/resistant classification. There were no overall sex differences, but there were sex-specific effects. For *females*, there were no significant effects, but for *males* there was a treatment effect [F (1, 12) = 5.0, p < 0.05]. B males treated with C21 had greater sweetened fat intake at binge 5 (p < 0. 05) regardless of prone/resistant classification. At binge 5, mice were reclassified as prone/resistant, for females there were 4 prone out of 7 (57%) in the C21 treated group, whereas there were 3 prone out of 7 (43%) in the saline treated group, see Figure 7A. For males, there 5 prone out of 8 (62.5%) in the C21 treated group, and 3 prone out of 8 (37.5%) in the saline treated group, see Figure 7B.

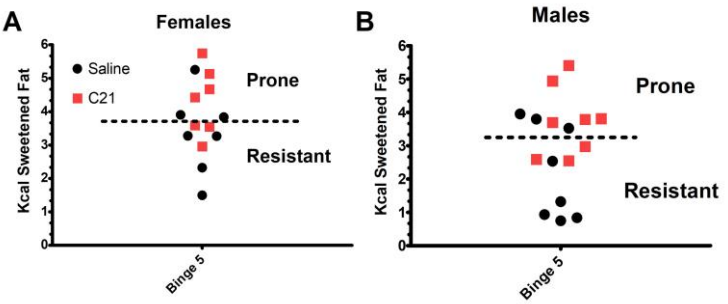

Figure 7. Characterization of binge propensity of excitatory (hM3D Gq) DREADDS activation in Binge group. Dotted line represents median split of intake of binge food, sweetened fat.

Inhibitory DREADDS. **Binge propensity.** The underlying hypothesis was that inhibition of LH orexin pathway *with a history of caloric restriction* would *decrease* binge propensity. For the RB group treated with either saline or C21, there was a treatment effect [F (1, 20) = 28.5, p < 0.005] with C21 treated mice consuming less sweetened fat at Binge 5 (p < 0.05) and an overall propensity effect [F (1, 20) = 5.19, p < 0.05]. There were no overall sex differences, but there were sex-specific effects. For females, there was a treatment effect [F (1, 10) = 10.0, p< 0.05] with C21 treatment reducing sweetened fat intake at binge 5 (p < 0.05). For males, there was a treatment effect [F (1, 10) = 19.6, p< 0.005] with C21 treated mice consuming less sweetened fat at Binge 5. Remarkably, there was an overall propensity effect [F (1, 10) = 12.7, p < 0.005] with mice classified as initially as binge prone at pre-exposure consuming *less* sweetened fat at binge 5 (p < 0.005). At binge 5, mice were reclassified as prone/resistant, for females there was 1 prone out of 7 (14%) in the C21 treated group, whereas there were 6 prone out of 7 (87%) in the saline treated group, see Figure 8A. For males, there was 2 prone out of 7 (28%) in the C21 treated group, and 5 prone out of 7 (72%) in the saline treated group, see Figure 8B. Taken together, these data suggest that modulating the LH orexin pathway can influence the initial propensity to binge eating.

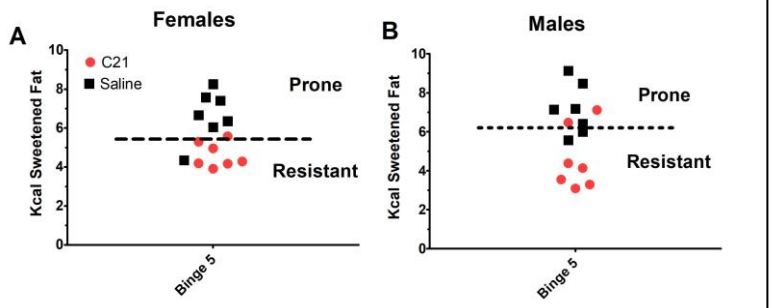

Figure 8. Characterization of binge propensity of excitatory (hM4D Gi) DREADDS activation in Restrict Binge group. Dotted line represents median split of intake of binge food, sweetened fat

Major Task 4, Subtask 2, month 1-34. We gave begun these experiments, we have initial data examining the influence of activation of the LH orexin and the effectiveness of sibutramine feeding suppression. Previously, we demonstrated that sibutramine feeding suppression in our dietary-induced binge eating paradigm (Sachdeo et al, Frontiers in Psychology, 2019). We observed that mice exposed to the repeated episodes of calorie restriction and SF access were resistant to sibutramine feeding suppression. The purpose of these experiments was to determine whether activation or inhibition of the LH orexin system influence the differential feeding suppression of sibutramine. These experiments are ongoing. In the Bello lab, a laboratory technician, Lori Scarpa, and a graduate student, Gregory Berger, are fully committed to this project. Assistance in the Bello lab has also been provided by Dr. Lihong Hao, Research Associate. Dr. Hao received her Ph.D. under research direction of Dr. Routh and bring a complementary skill set to strengthen the collaborative partnership between the Bello and Routh lab and provide assistance for Major Task 3.

Related work from other projects: In the Bello lab we have examined the possibility that disruption in the neural process by which sensory information is integrated and transmitted to motor output. Sensorimotor gating disruptions have been noted in several psychiatric and neurodegenerative disorders. However, the involvement of sensorimotor gating processes in eating disorders have not been well characterized. Our objective was to examine the sensorimotor gating of the acoustic startle response following dietary-induced binge eating and high-fat diet (HFD) induced weight gain in male C57B/6J mice. Acute nisoxetine (0.5 and 5 mg/kg) and GBR 12783 (1.6 and 16 mg/kg) were administered alone or in combination to assess norepinephrine and dopamine alterations, respectively. Male mice with repeated bouts of calorie restriction (Restrict) and with limited access to a sweetened fat food (Binge) demonstrated an escalation of intake over 2.5 weeks under standard chow conditions. Restrict Binge (RB) mice had a reduced startle response to the startle pulse (110 dB) compared with the Naïve control group at 5 mg/kg nisoxetine. There was an overall effect of nisoxetine (0.5 and 5 mg/kg) to in-creased percent inhibition at prepulse (74 dB), %PP74. Under HFD conditions, the RB group did not demonstrate a binge-like eating phenotype. The RB group on HFD had a higher response to 74 dB with nisoxetine (5.0 mg/kg) compared with a combinational dose of nisoxetine (5.0 mg/kg) and GBR 12783 (1.6 mg/kg). These findings suggest that dietary conditions that promote binge-like eating can influence the central noradrenergic and dopaminergic controls of the acoustic startle response and potentially influence sensorimotor gating. Additional work has been done to examine how a rodent model of post-traumatic stress disorder (PTSD) can be modelled in rodents and the outcomes on binge-like eating. In the Routh laboratory, a graduate student working on a related project which evaluates chronic food restriction and weight loss on glutamatergic signaling in the VTA has made observations relevant to this project. In 2018 (Teegala et al), he found that after an overnight 24 hour fast, the amplitude of excitatory currents (AMPA and NMDA combined) increased compared to control only when exposed to glucose levels seen in the brain during fasting (0.7 mM) but not when exposed to glucose levels seen in the brain during the fed state (2.5 mM). This was associated with a slight (~30%) but significant increase in the AMPA/NMDA receptor current ratio (an index of in vivo glutamate plasticity). He has now found that diet restriction to 85% body weight and maintenance of this weight for 1 week significantly increased the amplitude of total glutamate currents in 2.5 mM (fed) glucose (control: 46.1 +/- 7 pA vs weight loss: 70.8 +/- 5 pA; P=0.014). Moreover, the AMPA/NMDA receptor current ratio was increased 100% (control: 0.68+/-0.11 vs weight loss: 1.5 +/- 0.2 pA; P=0.017). Interestingly, this was entirely due to changes in the AMPA receptor mediated currents (control: 18.28 +/- 3.7pA vs weight loss 41.5 +/-4.2 pA; P = 0.002) with no change in the NMDA receptor mediated current (control 28 +/-4.2 pA vs weight loss 29 +/- 2.3 pA; P = 0.37). These data indicate first that changes in glutamate signaling are maintained over an extended time course. More importantly, the increase that we saw after one overnight fast were not representative of the maximum output of the system. Weight loss substantially enhanced glutamate signaling in the VTA. This suggests that similar changes will not only be maintained but enhanced after multiple episodes of food restriction. Interestingly, weight loss was associated with increased AMPA signaling whereas intermittent restriction increased NMDA but not AMPA receptor mediated circuits. Further comparison of these differences should provide interesting information regarding the triggers for binge eating behavior specifically as opposed to a simple drive to increase caloric intake following weight loss.

What opportunities for training and professional development has the project provided?

Lab personnel involved with this project have the opportunity to learn and expand their technical skills. These are related to *in vivo* survival surgeries in mice, in vitro electrophysiology, and chemogenetic platforms. As we generate more data for this project, personnel will have professional opportunities to present findings at national and international scientific conferences, invited talks, and publication in peer-reviewed journals.

How were the results disseminated to communities of interest?

At this stage, preliminary results of the project have been disseminated as departmental seminars open to the public. These seminars were in person and well attended (~100 participants). Gregory Berger, a graduate student in the Bello lab, presented a seminar at the Graduate Research in Interdisciplinary Biosciences Conference on Rutgers New Brunswick Campus entitled “Chemogenetic Manipulation of Orexin Neuronal Activity in Binge-like Eating” on April 12, 2022, which updated the progress on the Major Task 4, subtask 1 experiments. Several undergraduate students from the Bello lab presented different aspects of this project at forums open to the public. Suraj Teegala, a graduate student in the Routh lab

presented a data talk entitled “The role of lateral hypothalamic area orexin –glucose inhibited neurons in reward-based feeding” at the Society for the Study of Ingestive Behavior (SSIB) in Porto Portugal in July of 2022, which updated the progress on the Major Task 1, subtask 2 experiments. As noted in our previous progress report, as results are generated the findings will be presented at national and international scientific conferences (i.e., Society for Neuroscience, Society for the Study of Ingestive Behavior, etc., Endocrine workshop at Rutgers), invited talks, and publication in peer-reviewed journals. Scientific conference over the last 2 years has been virtual, which has made attendance and dissemination of research findings challenging. However, as scientific conferences move to “in person” or hybrid forums, we look forward to presenting and disseminating our findings to a wider audience as we did in Porto for the SSIB meeting.

What do you plan to do during the next reporting period to accomplish the goals?

We have completed a draft of systematic review article of the current literature of rodent models for the pharmacological treatments for binge eating disorder (BED) and bulimia nervosa (BN). We had anticipated to submit this review last funding cycle, but realized, as previously written, the review did not address a major issue with the use of rodent models for eating disorders. Specifically, there is a major incongruency between the outcomes in rodent models and clinical trials with human subjects. Rodent models measure binge *size* to determine effectiveness of pharmacological intervention, whereas clinically efficacy trials measure binge *frequency*. Moreover, there are no FDA industry guidelines for determining the effectiveness of pharmacotherapy for binge eating in BED or BN. Our forthcoming review addresses this major discrepancy and we also included in the review a relevance for at-risk military populations. To date, the Routh laboratory has trained an electrophysiologist and has successfully recruited an animal behaviorist. The mouse colonies have been incrementally increased since the Rutgers return to research, according to breeding restrictions the University imposed based on COVID-19 and are now at optimal level. Major Task 1 is nearly completed and we have demonstrated the ability to perform the studies in Major Task 2, which we have begun. Major Task 3 is routine for our laboratory and we now have the mouse colonies fully online. For Major Task 4, subtask 1 since all the major experiments are completed, we plan to finish up the linger experiments and submit a manuscript for publications in November/December 2022. For Major Task 4, subtask 2, we are in the process of completing these experiments. Based on our current progress, we have established a strong foundation to continue to make progress on the subtasks on these projects as described in the Statement of Work.

4. Impact

As data are generated, we will report distinctive contributions, major accomplishments, innovations, successes, and/or any change in practice as a result of the project.

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. Changes/Problems

Changes in approach and reasons for change.

Despite the COVID-19 restrictions and associated setbacks, there are no changes in our approach. Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them.

COVID-19 restrictions have significantly delayed progress due to closures and restrictions on breeding our mouse colonies. Other problems were noted elsewhere and have been resolved.

Changes that had a significant impact on expenditures.

There are some lingering issues regarding not being able to hire or work during the COVID-19 restrictions, thus we have some funds remaining for the upcoming no-cost extension to pay salaries to complete the final studies and publish the results. As previously stated, we do not anticipate this delay in these expenditures will significantly impact complete the goals of the project, especially in light of our exciting new data which strongly support our hypothesis.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report

Significant changes in use or care of human subjects

Not Applicable

Significant changes in use or care of vertebrate animals.

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. Products

Nothing to Report

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?

What individuals have worked on the project?

Name:	Nicholas T. Bello, Ph.D.
Project Role:	Investigator (Collaborating)
Researcher Identifier (e.g. ORCID ID):	0000-001-5300-5604
Nearest person months worked	6 months
Contribution to Project:	Oversees research on Major Tasks 4 (and assistance on Major Tasks 3)
Funding Support:	In addition to the current project, NIH R01 AT0008933

Name:	Lori Scarpa, M.S.
Project Role:	Laboratory Technician
Researcher Identifier (e.g. ORCID ID):	0000-0002-4421-1948
Nearest person months worked	12 months
Contribution to Project:	Small animal surgeries, viral injections, binge-eating protocols
Funding Support:	Current project only

Name:	Gregory Berger, B.S.
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	0000-0003-0537-4493
Nearest person months worked	12 months
Contribution to Project:	Small animal surgeries, binge-eating protocols
Funding Support:	Current project, Rutgers Department of Animal Sciences Teaching Assistantship

Name:	Lihong Hao, Ph.D.
Project Role:	Research Associate
Researcher Identifier (e.g. ORCID ID):	0000-0001-7795-6981
Nearest person months worked	8 months
Contribution to Project:	Small animal surgeries, immunohistochemistry, behavioral tests.

Funding Support:	Current project and NIH/NCCIH R01 AT0008933

Name:	Vanessa H. Routh, Ph.D.
Project Role:	Investigator (Collaborating)
Researcher Identifier (e.g. ORCID ID):	0000-0003-3644-970X
Nearest person months worked	6 months
Contribution to Project:	Oversees research on Major Tasks 1-3
Funding Support:	In addition to the current project, NIH R01 DK103676, 2 R01 GM097000 and JDRF 3 SRA 2017 488SB

Name:	Pallabi Sarkar, Ph.D.
Project Role:	Research Associate
Researcher Identifier (e.g. ORCID ID):	0000-0003-4837-0791
Nearest person months worked	6 months (mid-March to Aug 15 allowed to work 50%, currently allowed at 75%)
Contribution to Project:	Electrophysiologist on Major Tasks 1-3
Funding Support:	In addition to the current project, JDRF 3 SRA 2017 488SB

Name:	Dashiel Siegel
Project Role:	Technician
Researcher Identifier (e.g. ORCID ID):	

Nearest person months worked	2 months (hired in early June but not allowed to work until late June due to COVID-19 restrictions, late June to Aug 15 allowed to work 50%, currently allowed at 75%)
Contribution to Project:	Technical support on Major Tasks 1-3
Funding Support:	In addition to the current project, NIH R01 DK103676

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report

What other organizations were involved as partners?

Nothing to Report

8. Special Reporting Requirements

COLLABORATIVE AWARDS: This is a duplicate report for Initiating PI (Routh) and Partnering PI (Bello).

9. Appendices

Nothing Included