Chronic Stress-Dependent Activation of Somatostatin Neurons in the Nucleus Accumbens Facilitates Maladaptive Eating Behaviors

by

Elizabeth Liu

B.S.E. in Chemical Engineering
University of Michigan (2003)

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Author ..... Signature redacted
Department of Brain and Cognitive Sciences
January 29, 2016

Certified by ..... Signature redacted
Ki Goosens
Assistant Professor of Brain and Cognitive Sciences
Thesis Supervisor

Accepted by ..... Signature redacted .....
Matthew A. Wilson
Sherman Fairchild Professor of Neuroscience and Picower Scholar
Director of Graduate Education for Brain and Cognitive Sciences

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Abstract

Stressors are known to impact eating behaviors. However, recapitulating the intricate interplay between chronic stress and aberrant human eating patterns in an animal model remains a challenge. Notably, binge eating, a diagnostic feature associated with many types of eating abnormalities, particularly pertains to the binge eating disorder. To more closely investigate the etiology underlying eating behavior-associated maladaptation, the present study provides a novel and ethologically relevant animal model based on predatory odor stress. My data show that chronic stress in female mice selectively increases consumption of highly palatable, but not the regular, diet, when it is presented during a limited time following stress exposure. In addition, the nucleus accumbens (NAc), a key component in the neural circuitry of reward, is also an established neural substrate susceptible to the effects of stress. Given the cellular complexity in NAc, identifying the neuronal subtypes that are selectively involved in chronic stress-elicited physiological and behavioral alterations will provide grounds for further understanding in the underlying cellular changes. Because deficits in the somatostatin (SOM) neurons have been implicated in mice exhibiting traits of anxiety and depression, this neuron subtype may play an important role in modulating negative behavioral emotionality. Here I report an abundance of somatostatin neurons, majority of which are located in the rostral-ventral region of the NAc and are activated by chronic stress exposure. Together, these results provide the first line of evidence in linking chronic stress and the somatostatin neurons within the NAc to binge eating. Further fluorescent labeling quantification and cell-type-specific optogenetic manipulation will be needed to further delineate the role of SOM neurons in orchestrating the inhibitory components of stress-modulated reward circuitry.

Thesis Supervisor: Ki Goosens
Title: Assistant Professor of Brain and Cognitive Sciences

Acknowledgement

I want to dedicate special thanks to my folks. My Mama and Baba, whose unfaltering faith in me and unconditional love and support are a constant source of my strength. Gramama, although no longer with us, has always been my inner compass and role model. The love of my life, Mr. Y.M. the Incredible; without a doubt, this work could not have been completed without you. Thank you for being the beacon and rock in my life. Finally, G.G., who has always been there watching over me. I know you would have been proud. This one is for you.

Specific Aims

Prolonged exposure to stressful experiences can lead to a variety of maladaptive behavioral and physiological changes [1]. Moreover, stress and negative affect are commonly associated with increased susceptibility for eating disorders [2,3]. A core symptom for several eating disorders is characterized by recurrent episodes of binge eating [4]. Research using animal models of stress has begun to yield physiological and biochemical insights underlying aberrant eating behaviors [5-7]. Nonetheless, stress does not generate a uniform effect on the animals' responses toward food, which vary greatly, in part, depending on the type of stressor used. Such inconsistency warrants an experimental design for a more ethologically natural model, preferably one that incorporates innate anxiety into emotional stress-elicited eating.

Furthermore, it is widely recognized that chronic stress-induction procedures profoundly affect motivated behaviors [3,7]. Notably, the mesolimbic system, including the nucleus accumbens (NAc), contributes significantly to basic motivational processes [8-12]. The NAc is composed of heterogeneous cell types, including the somatostatin (SOM) neurons. Chronic inhibition of SOM cells in the frontal cortex produces anxiety and depression-like behaviors in mice [13]. Despite implications that SOM neurons may play a role in mediating behavioral emotionality, SOM neuronal subtype in the NAc remains largely unexplored. Using cell-type-specific Cre-dependent recombination technologies, this study aims to elucidate the functional role of SOM neurons within the NAc in a modified binge-eating animal model that is manifested by protracted stress and anxiety.

Aim 1: Establish and characterize a binge-eating animal model driven by chronic predator stress. Prior to the induction of stress, wild type (WT) female mice will be randomly assigned to one of two groups: stressed (S) or non-stressed (NS). On successive days of stress, the S group will be presented with the predator odor stressor, whereas the NS group will be placed in a cage with clean bedding. Immediately following stress sessions, both groups of animals will have limited access to food pellets containing high fat and high sucrose (HFHS). To examine the resulting behavioral and physiological outcomes, measurements such as the amount of HFHS intake, daily body weight and standard chow (STD) consumption will be recorded. The animals will also be subjected to multiple behavioral assays, as well as a corticosterone test to confirm the state of stress-dependent affect. Presumably "primed" by stress-associated changes, such as elevated anxiety levels, I hypothesize that animals from the S group show increased HFHS intake compared to the NS group.

Aim 2: Investigate the role of SOM neuronal activation within the NAc in stress experience-dependent bingeing behavior. Two transgenic mouse lines, the SOM-Cre and tdTomato (tdT) fluorescent reporter, will be crossed to generate SOM-tdT mice. Examining the SOM-specific fluorescent labeling in those mouse brains will allow one to identify specific locations of the SOM neuronal ensembles within functionally distinct regions of the NAc. A group of SOM-tdT animals will either be subjected to predator stress or clean cage exposure as described in Aim 1 and sacrificed 1 hr after the final stressor or NS context exposure session. The tdT expression within the NAc from these animals will then be quantified in combination with a neuronal activation marker, c-Fos. Because the NAc has been established as a key mediator of appetitive behavior and a neural substrate for stress [14], activity of the SOM neurons in this region are well-situated to be affected by stress. The expected outcome is an increased co-localization between c-Fos and SOM labeling in the S group. Furthermore, Cre-dependent ArchT-GFP or GFP virus will be injected and optic fibers will be implanted in the NAc of the SOM-Cre mouse. After 1 month of recovery, the mice will be divided into S and NS groups within mice that received either ArchT or GFP infusion. Green light will then be delivered to every mouse undergoing S or NS exposures. I hypothesize that SOM-specific inhibition in the ArchT-S group will generate the most pronounced reduction in HFHS bingeing behavior compared to mice from the GFP-N, GFP-S, or ArchT-N groups.

Background and Significance

Stressful experiences have been long established as a potent trigger for a range of behavioral abnormalities. Although acute stress is generally considered adaptive, chronic stress, particularly when coupled with a perceived lack of control, contribute to affective and emotional deficits and cause physiological and behavioral changes [1]. Importantly, human eating behaviors are highly susceptible to the effects of stress and anxiety [3,15,16]. In a retrospective human study, findings from Oliver and Wardle (1999) reported approximately 40% of college participants exhibited hyperphagia under stress. More importantly, the same study showed the majority of the respondents (73%) reported increased frequency in snacking when under stress, with greater consumption in energy-dense foods, such as sweets and chocolate (70%), than the less calorie-compact bread (29%). Parallel observations were found by Zellner *et al.* (2006), where a shift toward choosing higher calorie or highly palatable foods like M&Ms increases under stress.

Given these earlier findings, it is not surprising that a growing number of human and animal studies suggest an association between stress and the development of eating disorders. Multiple clinical findings provide evidences linking stress, including adverse life events and physical or emotional abuse, with the onset and expression of eating disorders [17-19]. Binge eating disorder, which affects approximately 5% of the general population as well as 2% of the population that suffers from bulimia, is the most common form of eating disorder [20]. Patients afflicted with binge eating disorder are often overweight or obese, and have an increased cardiovascular disease and type II diabetes symptoms, presenting multiple long-term adverse public health concerns [20,21]. Interestingly, a core symptom for several eating disorders, such as binge eating disorder, *bulimia nervosa*, and binge/purge subtype of *anorexia nervosa*, is recurrent episodes of binge eating [4]. Specifically, binge eating involves a rapid and excessive food consumption that is not propelled by hunger [4].

In animal models of eating disorders, the effects of chronic stress on food intake has been studied extensively. To replicate features of human binge eating conditions, most animal studies involve the use of physical stressors, food restriction, or a combination of both [5,22,23], and often focus on the actual amount of food consumption following a stress manipulation [24]. Nevertheless, conclusions drawn from these animal studies often vary considerably from one another and also differ from clinical observations [25]. One study comparing chronic physical stress (footshock) with emotional (witness) stress in rats revealed that physical stress reduced consumption and preference for saccharin drink, whereas emotional stress increased saccharin preference and consumption compared to water [26]. Taken together, such disparate results occur largely due to variation in the type, intensity, and duration of the stressors applied, highlighting the challenges in recapitulating the complexity of human experiences. In the present study, a more naturalistic predatory stress paradigm will be established. Exposure of rodents to natural predators or to their odors has been shown to induce anxiety-like states [27]. Because generalized anxiety is a part of a range of stress manifestations, it is conceivable that a more naturalistic animal model for stress and anxiety can be used to generate stress-associated binge eating behavior in mice.

Much of the literature on binge-like feeding neural correlate in the brain focuses on the reward circuit, specifically the mesolimbic dopaminergic pathway [28]. According to the pioneering work of Bart Hoebel, neuronal alterations underlying hedonic, reward-based (non-homeostatic) feeding, often involves excessive consumption of highly palatable food, may produce similar physiological changes in the brain reward circuit as in drug addiction [28-30]. Namely, administration of drugs of abuse in rats led to increased extracellular dopamine (DA) release in the NAc [31]. Similar dysregulation has been observed in sugar-addicted [30] and high-fat binge eating [32] animals. Moreover, based on the consumption and taste-reactivity measures of palatability in rodents exhibiting sugar addiction, several groups have reported that mu-opioid receptor binding is significantly enhanced in the NAc shell, among other brain regions, in

mediating hedonic processing or reward "liking" [8,33-35]. Conversely, the administration of the opioid antagonist, naloxone, precipitates signs of withdrawal [36], indicating that excessive sugar intake can alter the endogenous opioid system specifically in the NAc. Collectively, these neurochemical results strongly posit a role for the NAc in mediating binge-eating of the calorie-dense food.

Interestingly, the reward circuitry involving the NAc can be shaped by aversive experiences as well [7,37,38]. Using the social defeat paradigm, findings from Berton *et al* (2006) demonstrates a robust and long-term elevation in the brain-derived neurotrophic factor (BDNF) levels within the NAc region in the brains of the socially-defeated mice. Additional work by Lim *et al*. (2012) revealed that, in chronically-stressed animals, a melanocortin 4 receptor (MC4R) knock-down specifically in the NAc resulted in the prevention of stress-induced reduction in sucrose preference, indicating potential interaction between NAc, stress, and feeding dysregulation. To further parse the common neural substrates involved in stress-potentiated maladaptive food behavior, it is important to dissect the role of specific neuronal cell-types located in the NAc. Even though the majority of the NAc is composed of medium spiny γ-aminobutyric acid (GABA)ergic neurons (MSN), approximately 5% of this structure contains other GABAergic cell types, including cholinergic, parvalbumin, calretinin, and the SOM-interneurons, that are classified on the basis of distinct morphology, protein content, and electrophysiological properties [39,40]. The SOM cells have been shown to regulate various aspects of physiological and behavioral stress responses [13,41,42]. However, little is known about the functional role of the SOM neuronal population located in the NAc. It is conceivable that stress-potentiated hedonic feeding likely involves the NAc, and further investigation in this circuit, particular at a cell-type-specific level, may yield important insights in the etiology of the binge eating disorder.

Research Plan

Innovation

Increasing evidence in humans and animals suggests that chronic stress and negative affect state may present potent triggers to the onset of eating disorder endophenotypes [43,44]. In addition, the use of chronic stress in affecting the animal's eating behavioral patterns has gained traction over the years. However, major limitations arise from the current animal models. First, binge eating can manifest without the need for prior food deprivation [45]. Second, the stressors used do not produce consistent eating patterns across studies. Third, the most commonly used physical stressors are rarely encountered by the animals in nature. As a result, it potentially confounds the interpretations of the type of emotional affect generated. To overcome the drawbacks outlined, the model proposed here will utilize predatory odor to potentiate binge-like stress eating in mice. This stressor has been established as an ethologically relevant threat in rodents and, in large part, produces anxiogenic effects without eliciting depression-like symptoms, which might more closely reproduce the loss-of-control emotional component often observed in human individuals with bingeing conditions [27]. Additionally, food is provided *ad libitum* based on the current design, avoiding potential complications in interpretation when two stressors are used. Together with an experimental focus on female animals' feeding behaviors, the observations based on the present animal model can be utilized to gain insights into the mechanism of the onset of stress-potentiated binge eating and subsequent findings may provide a foundation for intervention work in female individuals with binge eating disorder.

Another goal of the present study is to identify and characterize the cellular substrate within the NAc that may potentially contribute to the neuronal processes underlying stress-elicited behavioral plasticity that particularly pertains to binge feeding behavior. The use of

multiple means, including neurochemical markers, has revealed a heterogeneous cellular composition in the NAc. However, due to a paucity of research focusing on the investigation of the SOM neurons located in the NAc, whether and how this particular cell type might play a role in the aversive experience-driven appetitive behavior await further investigation. By taking advantage of the genetically engineered SOM-Cre mice and delivering Cre-dependent AAV virus carrying archaerhodopsin TP009 (ArchT) specifically into the NAc region of the mouse brain, I will be able to directly manipulate the SOM neurons within NAc as the animals are behaving under the predatory stress. The resulting observations will provide valuable insights into the functional role of the SOM cells in the NAc for regulating stress-driven feeding behavioral dysfunction observed in an animal model of binge eating.

Approach

Aim 1: Establish and characterize a binge-eating animal model driven by chronic predator stress

Accumulating data have implicated chronic stress as a precipitating factor in major eating disorders, of which binge eating is a core symptom [46]. Interestingly, Zellner *et al.* (2006) reported a shift in food choice from healthy, low fat foods to highly palatable, yet fat- and sugar-enriched foods within the subjects under stress. Thus, stress not only facilitates hyperphagia, but it may also lead to an over-consumption of high caloric foods upon availability [47]. Additional studies have shown a correlative relationship between stress/negative affect and increased intake of high fat and high sugar food among women compared to men [48,49]. These findings provide some basic evidence that gender differences, among other factors, may also contribute to the development of stress-induced eating.

Much of the animal work used for modeling the effects of stress on food choice and eating behavior suggests that various forms of stress can produce diverse, often conflicting,

feeding behaviors [24,50,51]. For example, findings from Pecoraro *et al.* (2004) revealed that when rats underwent chronic restraint stress, they exhibited increased comfort food ingestion. In contrast, Patterson *et al.* (2010) demonstrated that WT mice subjected to chronic variable stress decreased both their weight gain and caloric intake, which might serve as a more appropriate model for depression. In addition, most commonly employed physical stressors, including restraint, foot shock, chronic variable stress, and forced swim, do not resemble the animal's natural threats, whereas the use of social defeat stress is not amenable to the study of female bingeing conditions. Hence modeling human stress-induced alterations in food intake using animals has not been straightforward.

Interestingly, caloric restriction coupled with physical stressors as opposed to imposing stress alone has been shown to yield consistently elevated palatable or non-palatable food consumptions in female animals [6]. One caveat is that limited food access presents an added layer of (metabolic) stressor. More importantly, not all individuals suffering from binge eating have a history of caloric restriction [52,53]. In fact most humans with binge eating conditions do not overeat because of physical hunger or metabolic needs [4,54]. Rather, binge eating is often accompanied by emotional distress and loss of control, indicative of the contribution of social circumstances [4,55]. Furthermore, in individuals with binge eating disorder, elevated perceived stress and increased incidence of life stressors have been demonstrated to precede the onset of binge eating [46,56].

Taken together, these studies impart the need for an animal model to more closely emulate key aspects of human, particularly female, stress-associated binge eating conditions. To this end, I propose to develop and test an animal bingeing model involving predator stress. Natural contexts such as the predatory stimuli are ecologically relevant for the animal's survival and recognition of such stimuli involves the activation of the animal's innate mechanisms [57,58]. It has been proposed that behaviors exhibited to the presence of a predator reflect fear responses, whereas the behaviors evoked by a cat odor reflect anxiety [59]. Similar conclusions

were drawn to a rat-exposed mouse [58]. Moreover, rats have been observed, in nature as well as in a lab setting, to kill and consume mice, hence they are considered as valid natural predators [60,61] to mice.

In the present study, predatory stress was induced by exposing female mice to a separate cage containing rat bedding. I hypothesized that a variety of predatory sensory stimuli, including odor (rat urine), tactile (rat feces), and visual (sight of rat feces) inputs, would induce elevated anxiety levels in these mice and result in overindulgence of the HFHS food. Of note, the HFHS food used was a chow formulated to model a typical high fat (20% vs. 7% in STD) and high sucrose (34% vs. 1% in STD) Western diet. Specifically female wild-type mice were handled for two to three days and baseline body weight, as well as total STD food consumption were recorded at 5:00 PM daily. During handling, less than 0.30 g of HFHS chow would be provided for food acclimation purposes. In this protocol, the animals were never food-deprived; binge-eating was elicited in mice by combining predatory odor stress and limited access to HFHS pellets in satiated mice. The animals from the S group were subjected to four consecutive days of predatory stress (15 min per day), whereas the animals from the N group were exposed to an equal-length of non-stress-inducing, neutral environment consisted of a clean cage with new bedding materials.

Immediately following the stress or no-stress experience, all animals were presented with HFHS pellets and their consumption in palatable food was recorded each hour for two hours. At the end of the HFHS presentation, the animals were placed back under the STD lab chow. Additionally, a parallel experiment was conducted, in which the same paradigm applied, with the exception that no HFHS food was given during the stress period. All animals within this group received only STD chow during the daily 2 hr post-stress period. This experimental design was used to control for any potential feedback influences from the HFHS intake that might confound subsequent anxiety behavioral readout. The animals fed only STD food throughout the study were subjected to measures of anxiety, including open field test (OF) and

elevated plus maze (EPM) one day after the last stress day. Tail suspension test (TST) was also administered to test whether predator stress might potentiate depression-like symptoms. Finally a separate group of animals will undergo the stress paradigm (with STD only) as described and blood will be drawn and plasma corticosterone levels will be tested using enzyme-linked immunosorbent assay. Unlike previous two experiments, this group of animals will not be subjected to any behavioral testing to prevent any fluctuations in corticosterone levels. The S group will presumably show increased corticosterone level compared to the N group due to stress induction.

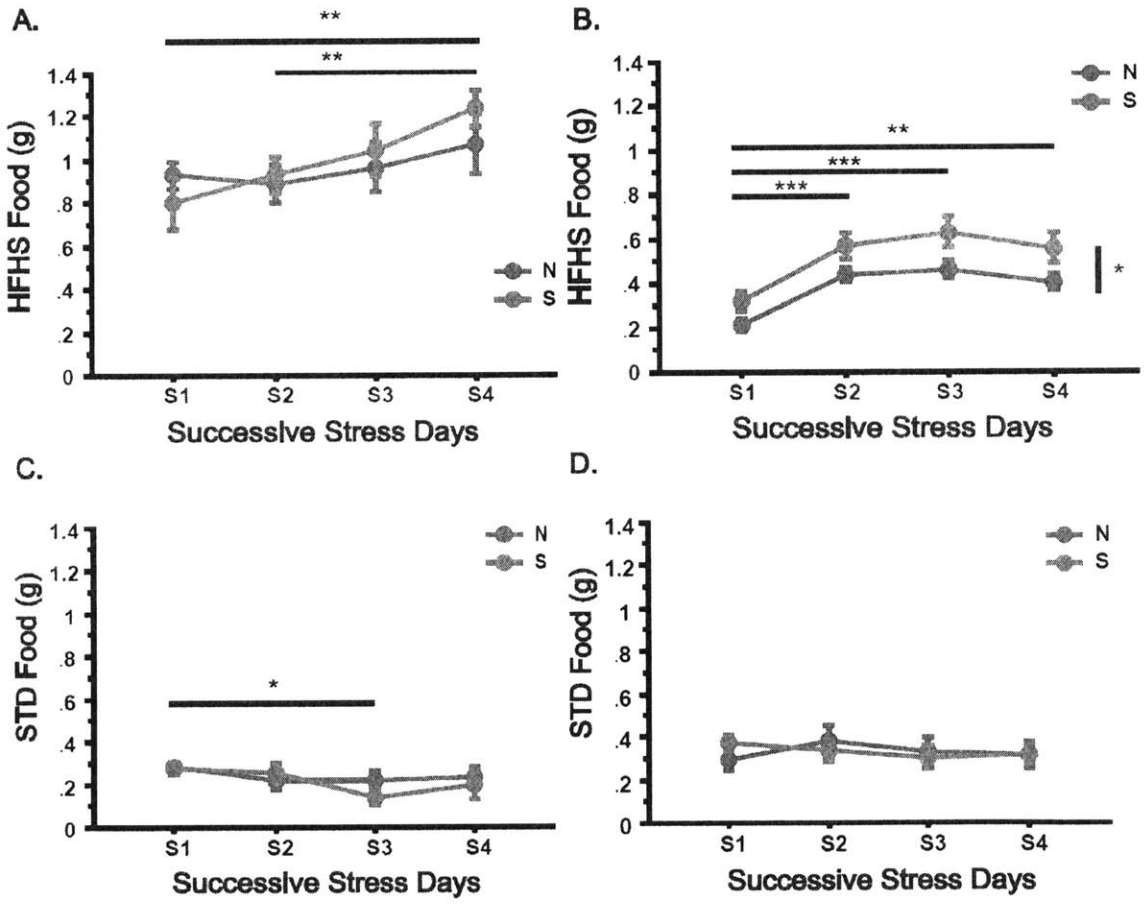

Figure 1. Effects of predatory stress (S, stressed group; N, non-stressed group) on food intake within the first two hours following stress on successive stressed days. (A) High-fat-high-sucrose (HFHS) food consumption during the first post-stress hour. Main day effect: $F(3,24) = 5.642$, $p<0.01$; n=4. (B) HFHS food consumption during the second post-stress hour. Main stress effect: $F(1,24) = 11.824$, $p<0.05$, and main day effect: $F(3,24) = 14.997$, $p<0.0001$; n=4. (C) Standard (STD) food consumption during the first post-stress hour. Main day effect: $F(3,48) = 3.661$, $p<0.05$; n=6-8. (D) STD food consumption during the second post-stress hour; n=6-8. Error bars denote s.e.m.; Two-way ANOVA and Bonferroni/Dunn tests; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

Preliminary results from the group with HFHS access are compared with those from the group exposed only to STD food (Fig. 1). As predicted, when fed only STD diet, stress was not able to potentiate any over-eating behaviors, as there was no added reward value encoded in the non-palatable food, hence the animals were not motivated to over-eat. Both the N and S subgroups consumed comparable amount of food when only STD chow was available. On the other hand, both subgroups under the stress-HFHS regiment already ate visibly more than the STD-only group (Fig. 2). This is, perhaps, unsurprising due to the elevated reward value encoded by the HFHS content compared to the STD chow. However, when comparing just the two subgroups with post-stress access to HFHS, the animals that underwent predatory odor stress showed a marked increase in HFHS consumption compared to the N subgroup.

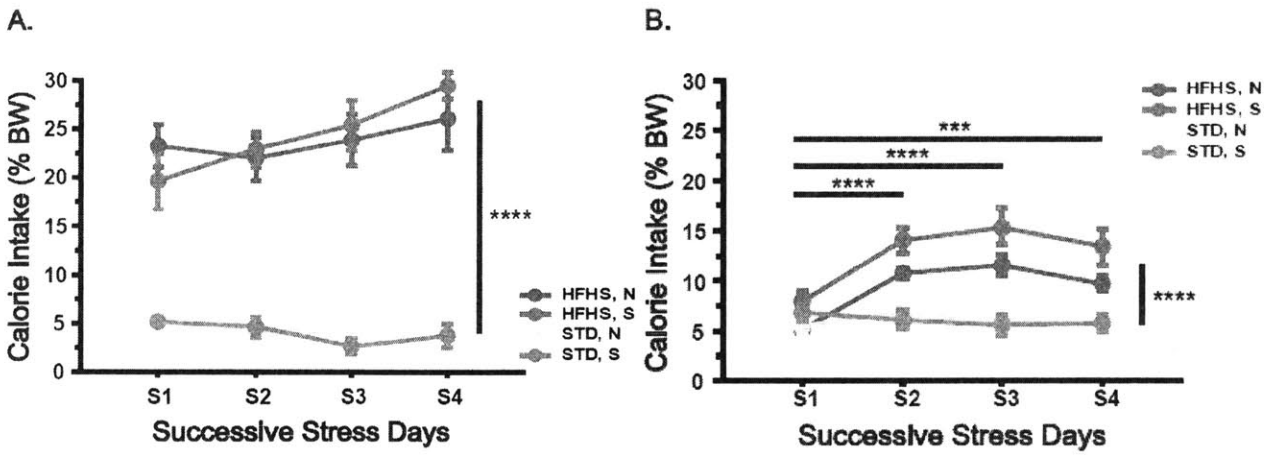

Figure 2. Effects of predatory stress (S, stressed group; N, non-stressed group) on calorie intake [normalized to body weight (BW)] within the first two hours following stress on successive stressed days. (A) High-fat-high-sucrose (HFHS) or standard (STD) calories consumed as a percentage of body weight during the first post-stress hour. Main food group effect: $F(1,72) = 277.131$, $p<0.0001$; n=4-8. (B) HFHS or STD calories consumed as a percentage of body weight during the second post-stress hour. Main food group effect: $F(1,72) = 28.462$, $p<0.0001$ and main day effect: $F(3,72) = 11.130$, $p<0.0001$; n=4-8. Error bars denote s.e.m.; Two-way ANOVA and Bonferroni/Dunn tests; ***$P<0.001$, ****$P<0.0001$.

Upon closer examination, the HFHS-fed S subgroup showed a significant and sustained elevation in the amount of calories ingested across stress days. Here the caloric intake was normalized to body weight. Collectively, these data indicate that predator stress can serve as a

robust driver for the animals to develop binge-eating-like phenotype. Interestingly, the stressed animals were bingeing not simply to meet metabolic demands (mice were kept satiated) or merely due to the palatability-induced hyperphagia (a more generalized feeding process). Thus, it is conceivable that stress is able to increase the "liking" of the HFHS food either by priming the reward circuit or enhancing the hedonic value of the HFHS food. In all, these results are highly suggestive of the loss-of-control bingeing episodes typically exhibited by individuals with binge eating disorder

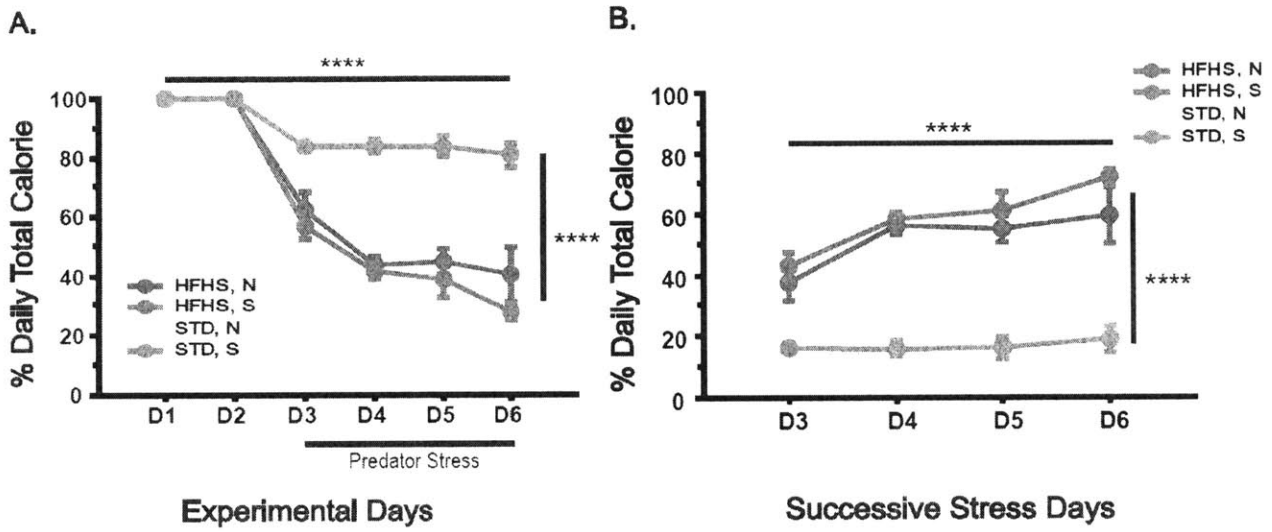

Figure 3. Effects of predatory stress (S, stressed group; N, non-stressed group) on calorie intake derived specifically from standard (STD) food consumption (expressed in terms of percentage of daily total calorie intake). (A) High-fat-high-sucrose chow (HFHS)-exposed or STD chow-exposed subgroups' peri-stress (excluding the 2-hr post-stress period) STD food calorie intake, expressed as a percentage of the daily total calories consumed. Main food group effect: $F(1,108) = 127.242$, $p<0.0001$; main day effect: $F(5,108) = 199.730$, $p<0.0001$; day x food effect: $F(5,108) = 60.390$, $p<0.0001$; n=4-8. (B) HFHS-exposed or STD-exposed subgroups' HFHS or STD, respectively, food calorie intake during the 2-hr post-stress period, expressed as a percentage of the daily total calories consumed. Main food group effect: $F(1,72) = 127.242$, $p<0.0001$; main day effect: $F(3,72) = 20.691$, $p<0.0001$; day x food effect: $F(3,72) = 12.313$, $p<0.0001$; n=4-8. Error bars denote s.e.m.; Two-way ANOVA test; ***$P<0.001$, ****$P<0.0001$.

Findings from Rada *et al* (2005), comparing rats with intermittent access to just chow or rats that tasted sugar only twice, revealed that a blunted DA response develops as the food loses its novelty. It is tempting to postulate based on the present findings that bingeing on HFHS might produce a neurological response that is quite different from that of consuming HFHS without bingeing (as demonstrated in the N group under the HFHS condition). In fact, stress

seems to contribute to a gradual shift in food preference, though not significantly different from the N group), from the daily consumption of STD food to the HFHS food only accessible post-stress (Fig. 3). These observations are consistent with the clinical observations by Zellner *et al.* (2006), in which stressed college students bypassed healthy food in favor of the calorie-dense snacks. In addition, the body weight for N and S groups from either HFHS- or STD-experiments did not differ during the span of the study, despite a significant weight gain over time (Fig. 4). Notably overeating can occur independent of obesity [62]. This particular animal stress model allows for the identification of variables contributing specifically to overeating without introducing an increased body weight, as increased body mass index and obesity are well known to also impart a deleterious effect on physiology. Finally, the tail suspension test analysis revealed a significantly less amount of time spent in immobilization in mice from the S group compared to the N group (Fig. 5). This might be an indication that predator stress potentiates more anxiety- than depressive-like traits, and that the S group devoted more energy on finding escape routes. Validation with the OF or EPM tests are needed to further confirm this speculation

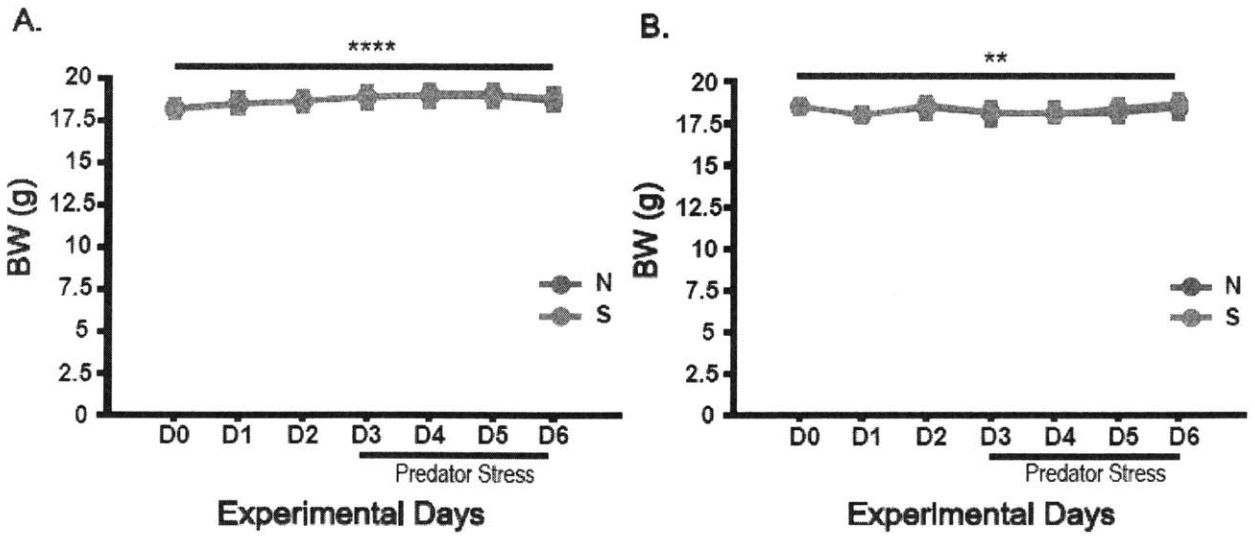

Figure 4. Effects of predatory stress (S, stressed group; N, non-stressed group) on daily body weight (BW) throughout experiment. (A) Standard (STD) food group's BW over time. Main day effect: $F(6,84) = 11.936$, $p<0.0001$; n=6-8. (B) High-fat-high-sucrose (HFHS) food group's BW over time; Main day effect: $F(6,42) = 4.503$, $p<0.01$; n=4. Error bars denote s.e.m.; Two-way ANOVA test; $^{**}P<0.01$, $^{****}P<0.0001$.

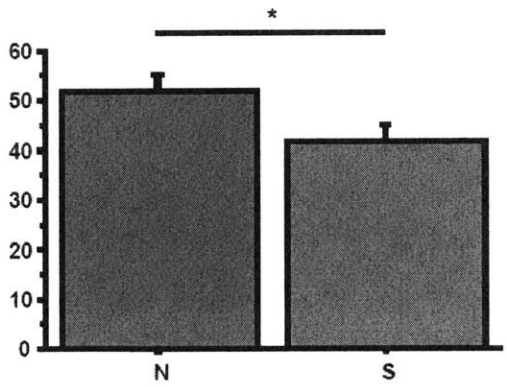

Figure. 5. Effects of predatory stress (S, stressed group; N, non-stressed group) on the percent of time the animals spent in immobilization during tail suspension test. Main stress effect: $F(1,294) = 4.813$; $p<0.05$; n=4. Two-way ANOVA; $^{*}P < 0.05$.

Aim 2: Investigate the role of SOM neuronal activation within the NAc in stress experience-dependent bingeing behavior

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The NAc is a heterogeneous brain region known to be an important mediator for both reward-driven behavior and aversive experiences [7,30,37]. Regarding food intake in particular, repeated stimulation of the reward pathway through highly palatable food may lead to neuronal maladaptation that eventually increases the compulsive nature of binge-eating [63]. Work from Hoebel's group demonstrated via microdialysis studies that excessive intake of palatable foods, similar to drug addiction, produces bouts of DA neurotransmitter release in the NAc [29,30]. In addition, pharmacological studies targeting the opioid, $GABA_B$, and dopamine D2 receptors specifically in the NAc may also affect palatable food liking, suggesting a complex interplay of neurotransmitter systems converges onto the NAc in determining the animal's response to food [34,64]. Collectively, these data lend support for a role of the NAc in animal models of binge eating.

In addition to the presentation of palatable food, stress has also been found to impact the NAc. Following repeated social defeat stress, an increased BDNF levels were observed in the NAc of stress-susceptible mice [37]. A follow-up study from the same group demonstrated that

the stress-related peptide, corticotropin-releasing factor (CRF), signaling in the NAc was necessary for the social defeat stress-induced BDNF increase, as intra-NAc administration of the CRF antagonist was able to block this effect [7]. Moreover, work from Lim *et al.* (2012) applied 8 d of chronic restraint stress to the animals, which was found to cause a reduction in sucrose preference, an increased immobility, and changed physiology in the dopamine D1 receptor. It was shown that knocking down the MC4R in the NAc selectively prevented these stress-induced effects; interestingly, incubation of the brain slices from naïve animals with MC4R ligand recapitulated the D1-MSN physiological changes occurred under stress without altering that of D2-MSN. These results uncovered a mechanism through which a feeding-related peptide can affect stress-induced sucrose preference changes by altering a specific cell-type in the NAc. Taken together, these data support a role of specific cell types in the NAc to mediate stress-associated feeding aberrance.

Although SOM deficits are implicated in disorders with stress and mood disturbances [13], such that ablation of SOM cells in the frontal cortex increases anxiety levels in adult mice, there is a paucity of research on the functional significance of this particular NAc neural subtype in the etiology of stress eating. I will approach this question by performing the following experimental steps. First, the SOM-Cre-expressing mouse line will be crossed with a fluorescent reporter line such as TdT for genetic cell targeting. Selective expression of the TdT will presumably be restricted in the SOM cells as a result. Only female SOM-TdT mice will be used in the predator stress paradigm as outlined in Aim 1. Similar to the no-HFHS experimental procedure in Aim 1, these animals will not be exposed to the HFHS diet throughout the study and free access to STD food will be provided except during the 15-minute stress period on a daily basis. Moreover, four groups of animals are required by this experimental design, namely 4-d S, 4-d N, 1-d S, and 1-d N, in which the 4-d S group will be subjected to 4-d repeated stress, while 1-d acute stress will be applied to the 1-d S group. Subsequently, the mouse brains will be perfused 1-1.5 hr immediately following the final stress or NS context exposure and then sectioned for

immunohistochemistry. Subsequently, confocal images of selective brain sections with SOM fluorescence (594 laser line; R) as well as c-Fos (488 laser line; G) and DAPI (405 laser line; B) immunofluorescence will be quantified using ImageJ.

This experiment serves to confirm the presence of SOM cells in the NAc region as well as to survey chronic predator stress-induced SOM-neuronal c-Fos expression profile. Because the SOM cells within the NAc are expected to be susceptible to chronic stress manipulations, I expect to observe a differential amount of R, G, and B channel co-labeling compared to R and B double-labeling between SOM cells from 4-d S group compared to the 4-d N group. The measurement will indicate the percentage of SOM cells that are activated by stress, with the 4-d S group hypothesized to show a higher ratio of R, G, B co-labeling than the 4-d N group. Moreover, to ensure the expected difference stems from the chronicity of the stress treatment, similar quantification will be performed for the 1-d groups, for which no significant amount of overlapping fluorescence is expected of either the S or N subgroups.

My preliminary data (Fig. 6) are composed of images focusing on several major regions belonging to the limbic system, including the basolateral amygdala, central nucleus of amygdala, (posterior-ventral) medial nucleus of amygdala, CA1 and dentate gyrus regions of hippocampus, as well as the NAc. Surprisingly, the NAc contains an abundance of SOM neurons compared to other brain regions, with the exception of CeA, which is enriched in SOM cells. Notably, the SOM cells are concentrated in the shell part of the NAc. Chronic predator stress indeed led to marked activation in the NAc SOM population (Fig. 7). However, it is surprising that the difference between triple-labeled cells is hardly discernible in the brain of a chronically stressed mouse compared to the NS mouse. Further concrete quantification of the images obtained from a higher magnification using a 40x objective and an increase in sample size will be performed for clarification. Alternatively, immunofluorescent staining for FosB, a more sensitive neuronal activation marker able to detect compounded neural activation, will be compared across the 4-d and 1-d groups in order to isolate the chronic stress effects.

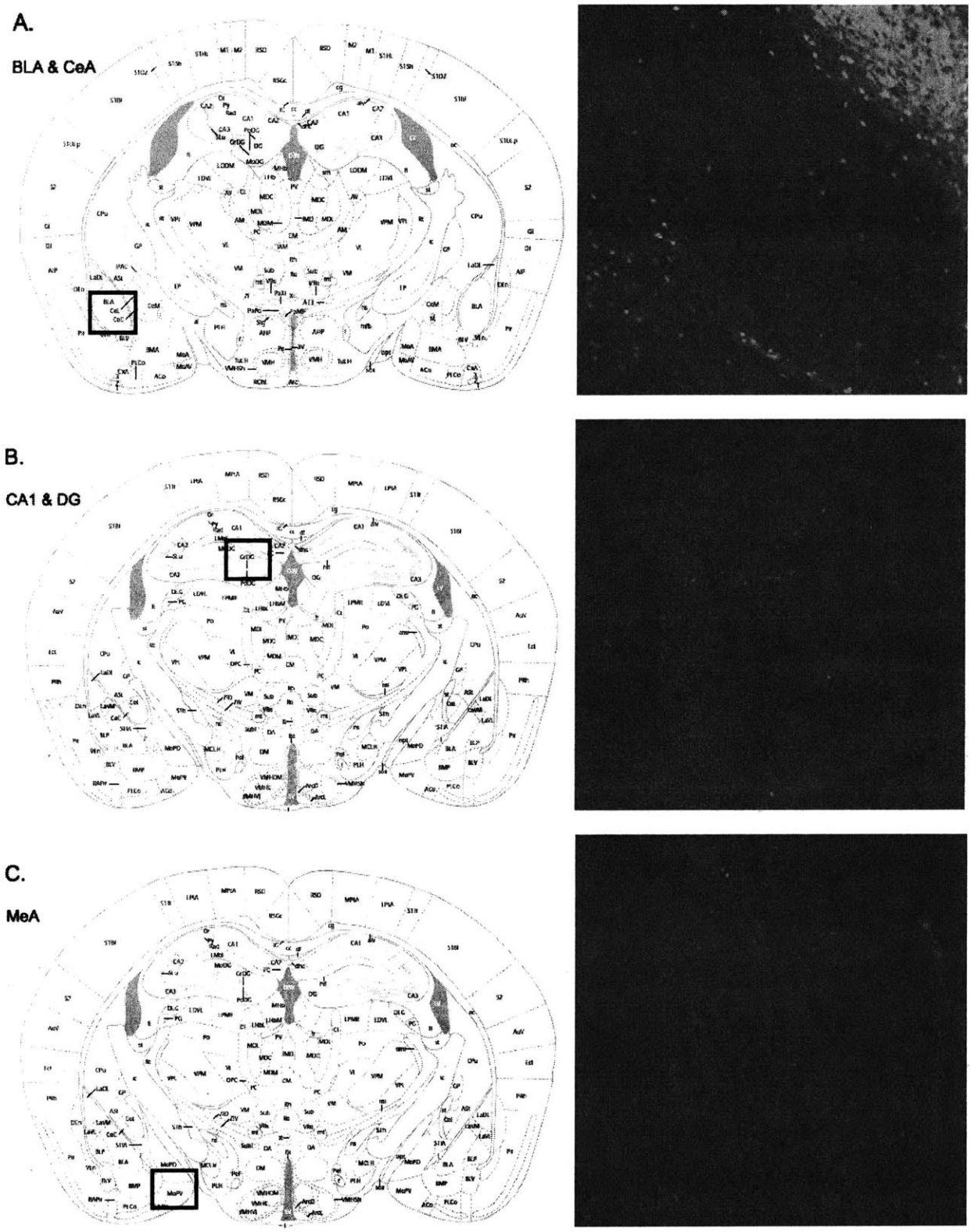

Figure. 6. Confocal images (10x objective; coronal sections) showing somatostatin (red) and DAPI (blue) fluorescence co-localization in a range of limbic system structures. (A) BLA (basolateral amygdala) and CeA (central amygdala). (B) CA1 and dentate gyrus (DG) regions of hippocampus. (C) MeA (medial nucleus of amygdala).

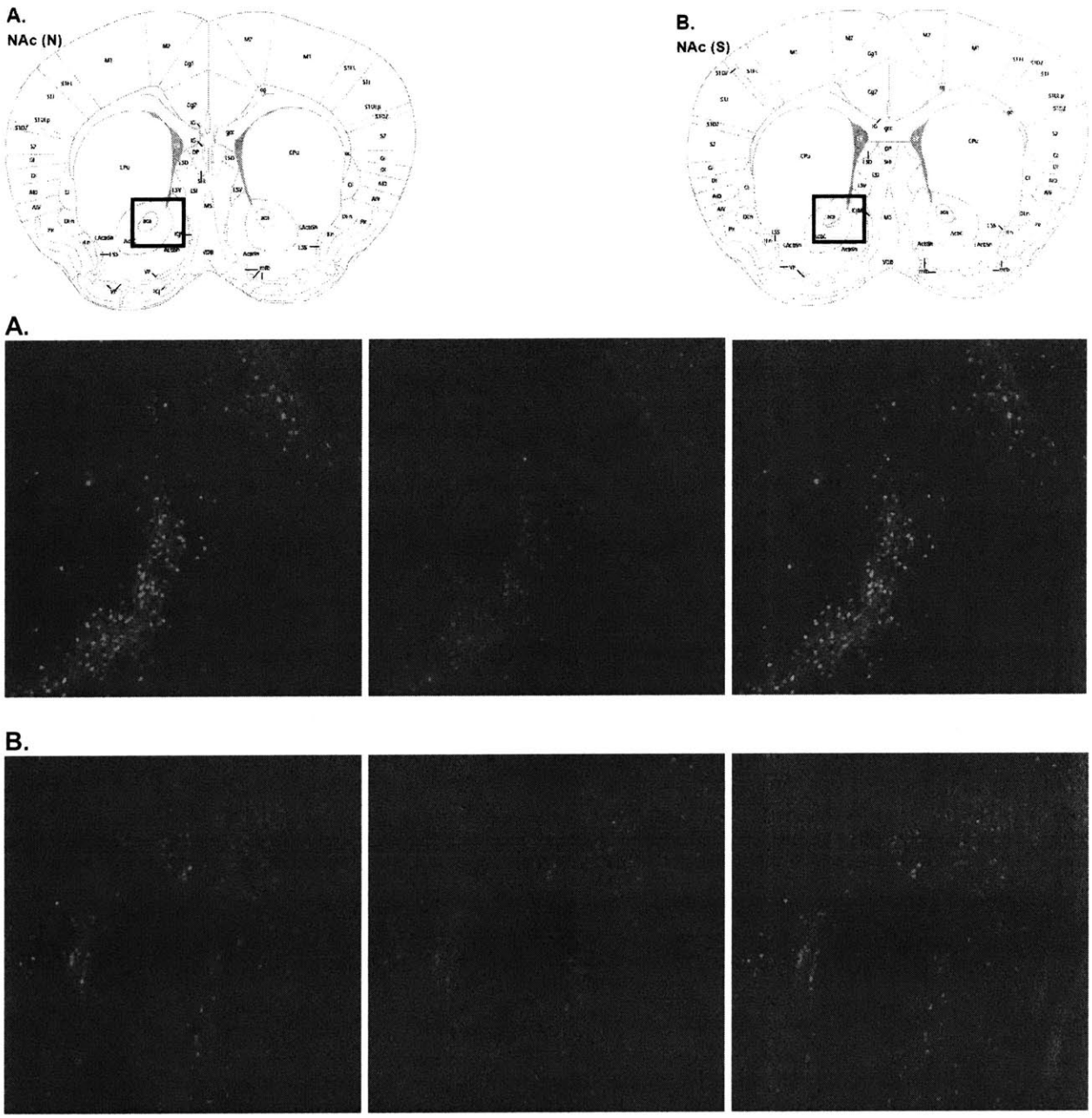

Figure. 7. Confocal images (10x objective; coronal sections) showing somatostatin (red), c-Fos (green), and red-, green- and DAPI (blue)-merged fluorescence in the ventral part of NAc (nucleus accumbens) following exposure to a (A) non-stress-inducing (N) or (B) predator stress-inducing (S) environment.

Finally, long-term behavioral and physiological consequences of manipulating NAc SOM neurons in behaving animals remain to be investigated. To this end, I will couple the use of FLEX (Cre recombinase-dependent viruses using loxP flanked doubled inverted open reading frames)-ArchT-GFP or control FLEX-GFP viruses with SOM-Cre transgenic mouse line.

Specifically I will express either FLEX-ArchT-GFP (Arch) or FLEX-GFP (GFP) control adeno-associated virus (AAV) viruses into the ventral rostral part of the NAc via microinjection. The viral injection surgery will be performed at the same time as optic fiber implant. Because peak expression of AAV normally requires at least 3 weeks, the animals will be allowed to recover for 1 month prior to the induction of chronic predator stress. Subsequently, the animals transduced with either ArchT or GFP control virus will be further divided into 4-d S or 4-d N subgroups. Across stress days, regardless of whether stress is applied, green laser light (532 nm) will be delivered while the animals encounter either a stressful environment (S group) or an innocuous environment (NS group). The light delivery will last for the entire duration of stress or non-stress context exposure for four days. Behavioral measurements such as the amount of HFHS intake during stress, daily body weight and STD food intake will be recorded and compared across groups. Lastly, half of each group will be subjected to OF, EPM, and TST behavioral testing one day after the final stress exposure, while the brains from the other half of the animals will be perfused and sectioned to confirm the sites of viral infusion and optic fiber implant. Immunostaining for c-Fos will be performed to confirm a decreased level of neuronal activation specifically in ArchT virus- as opposed to the GFP virus-infected SOM cells.

Under the previous postulation, which posits that prolonged stress might lead to the activation of the SOM cells contained in the NAc, and selectively inhibiting this neural cell type will presumably mitigate stress-induced bingeing effect at the behavioral level. In comparison, both of the N subgroups (GFP or ArchT) will still display a high intake of HFHS pellets, due to food palatability. Moreover, the S group infected with the GFP virus is expected to exhibit the highest HFHS intake yet of the four groups. Finally, the rescued behavioral phenotype (from bingeing to non-bingeing) expected of the ArchT-S group should also accompany reduced anxiety levels, testable via OF, as well as an ameliorated corticosterone level. By directly silencing the SOM neuron ensembles residing in the NAc, it is conceivable that the axons coursing through the structure are also silenced, producing a non-cell-type-specific optical

inhibition. One way to circumvent this problem is to first trace the projections from these SOM cells by examining the expression of the FLEX-AAV-GFP virus, and then to stimulate the axon terminals by placing the optic fibers at a selected downstream feeding-related target structure, such as the ventral pallidum.

Significance of Proposed Study

The work proposed here will provide a preliminary characterization of the SOM neurons residing in the NAc at the cellular and functional levels. Moreover, by providing a more naturalistic animal stress model, many aberrant eating conditions in individuals with eating disorders can now be more closely studied. Because one in three people in the U.S.A. suffer from binge eating [65], finding key cellular substrates for stress-facilitated dysfunction in the reward neural circuit is likely to have implications for distinct cellular basis to stress-eating vulnerability, as well as to provide better therapeutic targets in the future.

Bibliography

1 McEwen, B. S. Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. *Annals of the New York Academy of Sciences* **1032**, 1-7, doi:10.1196/annals.1314.001 (2004).

2 Cattanach, L., Malley, R. & Rodin, J. Psychologic and physiologic reactivity to stressors in eating disordered individuals. *Psychosomatic medicine* **50**, 591-599 (1988).

3 Zellner, D. A. *et al.* Food selection changes under stress. *Physiology & behavior* **87**, 789-793, doi:10.1016/j.physbeh.2006.01.014 (2006).

4 APA, A. P. A. *Diagnostic and statistical manual of mental disorders (5th ed.).* (American Psychiatric Association, 2013).

5 Avena, N. M., Bocarsly, M. E., Rada, P., Kim, A. & Hoebel, B. G. After daily bingeing on a sucrose solution, food deprivation induces anxiety and accumbens dopamine/acetylcholine imbalance. *Physiology & behavior* **94**, 309-315, doi:10.1016/j.physbeh.2008.01.008 (2008).

6 Boggiano, M. M. & Chandler, P. C. Binge eating in rats produced by combining dieting with stress. *Current protocols in neuroscience / editorial board, Jacqueline N. Crawley ... [et al.]* **Chapter 9**, Unit9.23A, doi:10.1002/0471142301.ns0923as36 (2006).

7 Krishnan, V. *et al.* Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell* **131**, 391-404, doi:10.1016/j.cell.2007.09.018 (2007).

8 Castro, D. C. & Berridge, K. C. Opioid hedonic hotspot in nucleus accumbens shell: mu, delta, and kappa maps for enhancement of sweetness "liking" and "wanting". *The Journal of neuroscience : the official journal of the Society for Neuroscience* **34**, 4239-4250, doi:10.1523/jneurosci.4458-13.2014 (2014).

9 Di Chiara, G., Loddo, P. & Tanda, G. Reciprocal changes in prefrontal and limbic dopamine responsiveness to aversive and rewarding stimuli after chronic mild stress: implications for the psychobiology of depression. *Biological psychiatry* **46**, 1624-1633 (1999).

10 Namburi, P. *et al.* A circuit mechanism for differentiating positive and negative associations. *Nature* **520**, 675-678, doi:10.1038/nature14366 (2015).

11 Stuber, G. D. *et al.* Excitatory transmission from the amygdala to nucleus accumbens facilitates reward seeking. *Nature* **475**, 377-380 (2011).

12 Urstadt, K. R., Coop, S. H., Banuelos, B. D. & Stanley, B. G. Behaviorally specific versus non-specific suppression of accumbens shell-mediated feeding by ipsilateral versus bilateral inhibition of the lateral hypothalamus. *Behavioural brain research* **257**, 230-241, doi:10.1016/j.bbr.2013.09.048 (2013).

13 Lin, L. C. & Sibille, E. Somatostatin, neuronal vulnerability and behavioral emotionality. *Molecular psychiatry* **20**, 377-387, doi:10.1038/mp.2014.184 (2015).

14 Walsh, J. J. *et al.* Stress and CRF gate neural activation of BDNF in the mesolimbic reward pathway. *Nat Neurosci* **17**, 27-29, doi:10.1038/nn.3591 (2014).

15 Oliver, G. & Wardle, J. Perceived effects of stress on food choice. *Physiology & behavior* **66**, 511-515 (1999).

16 Willenbring, M. L., Levine, A. S. & Morley, J. E. Stress induced eating and food preference in humans: A pilot study. *International Journal of Eating Disorders* **5**, 855-864, doi:10.1002/1098-108X(198607)5:5<855::AID-EAT2260050507>3.0.CO;2-O (1986).

17 Block, J. P., He, Y., Zaslavsky, A. M., Ding, L. & Ayanian, J. Z. Psychosocial stress and change in weight among US adults. *American journal of epidemiology* **170**, 181-192 (2009).

18 Fowler-Brown, A. G. *et al.* Psychosocial stress and 13-year BMI change among blacks: the Pitt County Study. *Obesity (Silver Spring, Md.)* **17**, 2106-2109, doi:10.1038/oby.2009.130 (2009).

19 Kent, A. & Waller, G. Childhood emotional abuse and eating psychopathology. *Clinical psychology review* **20**, 887-903 (2000).

20 Hudson, J. I., Hiripi, E., Pope, H. G., Jr. & Kessler, R. C. The prevalence and correlates of eating disorders in the National Comorbidity Survey Replication. *Biological psychiatry* **61**, 348-358, doi:10.1016/j.biopsych.2006.03.040 (2007).

21 Stunkard, A. J. Eating patterns and obesity. *Psychiatric Quarterly* **33**, 284-295, doi:10.1007/bf01575455 (1959).

22 Boggiano, M. M. *et al.* Combined dieting and stress evoke exaggerated responses to opioids in binge-eating rats. *Behavioral neuroscience* **119**, 1207-1214, doi:10.1037/0735-7044.119.5.1207 (2005).

23 Teegarden, S. L. & Bale, T. L. Effects of stress on dietary preference and intake are dependent on access and stress sensitivity. *Physiology & behavior* **93**, 713-723 (2008).

24 Patterson, Z. R., Ducharme, R., Anisman, H. & Abizaid, A. Altered metabolic and neurochemical responses to chronic unpredictable stressors in ghrelin receptor-deficient mice. *The European journal of neuroscience* **32**, 632-639, doi:10.1111/j.1460-9568.2010.07310.x (2010).

25 Greeno, C. G. & Wing, R. R. Stress-induced eating. *Psychological Bulletin* **115** (1994).

26 Pijlman, F. T., Wolterink, G. & Van Ree, J. M. Physical and emotional stress have differential effects on preference for saccharine and open field behaviour in rats. *Behavioural brain research* **139**, 131-138 (2003).

27 Adamec, R., Walling, S. & Burton, P. Long-lasting, selective, anxiogenic effects of feline predator stress in mice. *Physiology & behavior* **83**, 401-410, doi:10.1016/j.physbeh.2004.08.029 (2004).

28 Avena, N. M., Rada, P. & Hoebel, B. G. Evidence for sugar addiction: behavioral and neurochemical effects of intermittent, excessive sugar intake. *Neuroscience and biobehavioral reviews* **32**, 20-39, doi:10.1016/j.neubiorev.2007.04.019 (2008).

29 Avena, N. M., Rada, P., Moise, N. & Hoebel, B. G. Sucrose sham feeding on a binge schedule releases accumbens dopamine repeatedly and eliminates the acetylcholine satiety response. *Neuroscience* **139**, 813-820, doi:10.1016/j.neuroscience.2005.12.037 (2006).

30 Rada, P., Avena, N. M. & Hoebel, B. G. Daily bingeing on sugar repeatedly releases dopamine in the accumbens shell. *Neuroscience* **134**, 737-744, doi:10.1016/j.neuroscience.2005.04.043 (2005).

31 Di Chiara, G. & Imperato, A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proceedings of the National Academy of Sciences* **85**, 5274-5278 (1988).

32 Liang, N. C., Hajnal, A. & Norgren, R. Sham feeding corn oil increases accumbens dopamine in the rat. *American journal of physiology. Regulatory, integrative and comparative physiology* **291**, R1236-1239, doi:10.1152/ajpregu.00226.2006 (2006).

33 Barbano, M. F. & Cador, M. Opioids for hedonic experience and dopamine to get ready for it. *Psychopharmacology* **191**, 497-506, doi:10.1007/s00213-006-0521-1 (2007).

34 Pecina, S. & Berridge, K. C. Opioid site in nucleus accumbens shell mediates eating and hedonic 'liking' for food: map based on microinjection Fos plumes. *Brain research* **863**, 71-86 (2000).

35 Smith, K. S. & Berridge, K. C. Opioid limbic circuit for reward: interaction between hedonic hotspots of nucleus accumbens and ventral pallidum. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **27**, 1594-1605, doi:10.1523/jneurosci.4205-06.2007 (2007).

36 Colantuoni, C. *et al.* Evidence that intermittent, excessive sugar intake causes endogenous opioid dependence. *Obesity research* **10**, 478-488, doi:10.1038/oby.2002.66 (2002).

37 Berton, O. *et al.* Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science (New York, N.Y.)* **311**, 864-868, doi:10.1126/science.1120972 (2006).

38 Lim, B. K., Huang, K. W., Grueter, B. A., Rothwell, P. E. & Malenka, R. C. Anhedonia requires MC4R-mediated synaptic adaptations in nucleus accumbens. *Nature* **487**, 183-189, doi:10.1038/nature11160 (2012).

39 Gerfen, C. R. The neostriatal mosaic: multiple levels of compartmental organization in the basal ganglia. *Annual review of neuroscience* **15**, 285-320, doi:10.1146/annurev.ne.15.030192.001441 (1992).

40 Kawaguchi, Y., Wilson, C. J., Augood, S. J. & Emson, P. C. Striatal interneurones: chemical, physiological and morphological characterization. *Trends in neurosciences* **18**, 527-535 (1995).

41 Palkovits, M., Tapia-Arancibia, L., Kordon, C. & Epelbaum, J. Somatostatin connections between the hypothalamus and the limbic system of the rat brain. *Brain research* **250**, 223-228 (1982).

42 Wolff, S. B. E. *et al.* Amygdala interneuron subtypes control fear learning through disinhibition. *Nature* **509**, 453-458, doi:10.1038/nature13258 (2014).

43 Dallman, M. F. Stress-induced obesity and the emotional nervous system. *Trends in endocrinology and metabolism: TEM* **21**, 159-165, doi:10.1016/j.tem.2009.10.004 (2010).

44 Nishitani, N., Sakakibara, H. & Akiyama, I. Eating behavior related to obesity and job stress in male Japanese workers. *Nutrition (Burbank, Los Angeles County, Calif.)* **25**, 45-50, doi:10.1016/j.nut.2008.07.008 (2009).

45 Calvez, J. *et al.* Role of relaxin-3/RXFP3 system in stress-induced binge-like eating in female rats. *Neuropharmacology* **102**, 207-215, doi:10.1016/j.neuropharm.2015.11.014 (2016).

46 Striegel-Moore, R. H. *et al.* Risk factors for binge-eating disorders: an exploratory study. *The International journal of eating disorders* **40**, 481-487, doi:10.1002/eat.20400 (2007).

47 Gluck, M. E., Geliebter, A., Hung, J. & Yahav, E. Cortisol, hunger, and desire to binge eat following a cold stress test in obese women with binge eating disorder. *Psychosomatic medicine* **66**, 876-881, doi:10.1097/01.psy.0000143637.63508.47 (2004).

48 Grunberg, N. E. & Straub, R. O. The role of gender and taste class in the effects of stress on eating. *Health psychology : official journal of the Division of Health Psychology, American Psychological Association* **11**, 97-100 (1992).

49 Michaud, C. *et al.* Relationships between a critical life event and eating behaviour in high-school students. *Stress Medicine* **6**, 57-64, doi:10.1002/smi.2460060112 (1990).

50 Patterson, Z. R., Khazall, R., Mackay, H., Anisman, H. & Abizaid, A. Central ghrelin signaling mediates the metabolic response of C57BL/6 male mice to chronic social defeat stress. *Endocrinology* **154**, 1080-1091, doi:10.1210/en.2012-1834 (2013).

51 Pecoraro, N., Reyes, F., Gomez, F., Bhargava, A. & Dallman, M. F. Chronic stress promotes palatable feeding, which reduces signs of stress: feedforward and feedback effects of chronic stress. *Endocrinology* **145**, 3754-3762, doi:10.1210/en.2004-0305 (2004).

52 Abbott, D. W. *et al.* Onset of binge eating and dieting in overweight women: implications for etiology, associated features and treatment. *Journal of psychosomatic research* **44**, 367-374 (1998).

53 Reas, D. L. & Grilo, C. M. Timing and sequence of the onset of overweight, dieting, and binge eating in overweight patients with binge eating disorder. *The International journal of eating disorders* **40**, 165-170, doi:10.1002/eat.20353 (2007).

54 Waters, A., Hill, A. & Waller, G. Internal and external antecedents of binge eating episodes in a group of women with bulimia nervosa. *The International journal of eating disorders* **29**, 17-22 (2001).

55 Latner, J. D. & Clyne, C. The diagnostic validity of the criteria for binge eating disorder. *The International journal of eating disorders* **41**, 1-14, doi:10.1002/eat.20465 (2008).

56 Pike, K. M. *et al.* Antecedent life events of binge-eating disorder. *Psychiatry research* **142**, 19-29, doi:10.1016/j.psychres.2005.10.006 (2006).

57 Hubbard, D. T. *et al.* Development of defensive behavior and conditioning to cat odor in the rat. *Physiology & behavior* **80**, 525-530, doi:http://dx.doi.org/10.1016/j.physbeh.2003.10.006 (2004).

58 Yang, M. *et al.* The rat exposure test: a model of mouse defensive behaviors. *Physiology & behavior* **81**, 465-473, doi:10.1016/j.physbeh.2004.02.010 (2004).

59 Brain, P. F., Kusumorini, N. & Benton, D. 'Anxiety' in laboratory rodents: a brief review of some recent behavioural developments. *Behavioural processes* **25**, 71-80, doi:10.1016/0376-6357(91)90010-w (1991).

60 Karli, P. The Norway Rat's Killing Response to the White Mouse: An Experimental Analysis. *Behaviour* **10**, 81-103 (1956).

61 O'Boyle, M. Rats and mice together: the predatory nature of the rat's mouse-killing response. *Psychol Bull* **81**, 261-269 (1974).

62 Boggiano, M. M. *et al.* High intake of palatable food predicts binge-eating independent of susceptibility to obesity: an animal model of lean vs obese binge-eating and obesity with and without binge-eating. *International journal of obesity (2005)* **31**, 1357-1367, doi:10.1038/sj.ijo.0803614 (2007).

63 Volkow, N. D. & Wise, R. A. How can drug addiction help us understand obesity? *Nat Neurosci* **8**, 555-560 (2005).

64 Rao, R. E., Wojnicki, F. H., Coupland, J., Ghosh, S. & Corwin, R. L. Baclofen, raclopride, and naltrexone differentially reduce solid fat emulsion intake under limited access conditions. *Pharmacology, biochemistry, and behavior* **89**, 581-590, doi:10.1016/j.pbb.2008.02.013 (2008).

65 Ogden, C. L., Yanovski, S. Z., Carroll, M. D. & Flegal, K. M. The epidemiology of obesity. *Gastroenterology* **132**, 2087-2102, doi:10.1053/j.gastro.2007.03.052 (2007).