Effects of Physiological Need States on Affect and Reward Sensitivity

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THESIS

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TABLE OF CONTENTS

LIST OF TABLES

TABLE PAGE

LIST OF FIGURES

FIGURE PAGE

LIST OF ABBREVIATIONS OR NOMENCLATURE

5TG 5-thio-d-glucose

ACE angiotensin-converting enzyme

AgRP agouti-related peptide

ARC arcuate nucleus of the hypothalamus

BNST bed nucleus of the stria terminalis

BSR brain stimulation reward

ceA central amygdala

CNS central nervous system

CRH corticosterone-releasing hormone

DA dopamine

FR food restricted

GOAT ghrelin *O*-acyltransferase

ICSS intracranial self-stimulation

ICV intracerebroventricular

IP intraperitoneal

LH lateral hypothalamus

mePO median preoptic area

MFB medial forebrain bundle

NAc nucleus accumbens

NPY neuropeptide Y

NTS nucleus of the solitary tract

OVLT vascular organ of the lamina terminalis

PVN paraventricular nucleus

SC subcutaneous

SFO subfornical area

VMH ventromedial hypothalamus

VTA ventral tegmental area

SUMMARY

It is well known that hunger alters reward sensitivity for food, which then enhances goal-directed, motivated behavior to find food. However, the mechanisms by which hunger, and other physiological need states, such as thirst, act to motivate behavior remain incompletely understood, specifically when it comes to rewards that do not act to fill the need state. The present study sought to determine the manner in which physiological signals of hunger and thirst alter reward sensitivity and motivated behavior. We attempted to capture reward sensitivity using a well-validated (Carlezon Jr & Chartoff, 2007) intracranial self-stimulation (ICSS) paradigm to determine the theoretical threshold for ICSS. We then determined how specific 'hunger' or 'thirst' mimetics altered ICSS threshold using a within-subjects design.

We first attempted to confirm that our 'hunger' and 'thirst' mimetics induced feeding or drinking. We administered intracerebroventricular (ICV) infusions of ghrelin, neuropeptide Y (NPY), 5-thio-d-glucose (5TG), Angiotensin II, and vehicle (saline) and measured chow and water intake. We found that, while only NPY administration caused a significant increase in food intake, ghrelin and NPY also showed a trend toward an increase in feeding compared to saline.

Next, we used threshold for brain stimulation reward (BSR) during ICSS to examine reward sensitivity before and after administration of these same compounds. We found that infusions of ghrelin, NPY, and 5TG all increased threshold for brain stimulation reward when compared to both vehicle infusions and the animals threshold values from the day preceding infusions.

Finally, in order to compare these manipulations to natural hunger and thirst, we deprived animals of food or water for a 24-hour period and compared their thresholds for BSR. We found that neither 24-hour food nor water restriction had a significant effect on threshold BSR. While

others have shown a decrease in threshold for BSR following food and water restriction (see Table 3), their parameters for stimulation differed greatly and most targeted the medial forebrain bundle (MFB) or lateral hypothalamus (LH) as their stimulation site, whereas this study stimulated the ventral tegmental area (VTA). It is therefore possible that stimulation to the VTA is less sensitive to natural hunger than the MFB and LH.

It has been demonstrated by others that manipulations associated with negative affective states, such as withdrawal from drugs of abuse, cause an increase in threshold for BSR and those associated with positive affective states, such as cocaine use, cause a decrease in threshold for BSR (Barr, Markou, & Phillips, 2002; Carlezon Jr & Wise, 1996). Thus, the increase in BSR threshold following infusions of 'hunger' mimetics suggests that activation of these specific neural pathways may be aversive and generate a negative affective state. Further, artificial stimulation of these physiological pathways may not mirror natural hunger and thirst states. Future work will aim to identify the specific neural substrates that underlie changes in reward sensitivity and affective state during physiological need states.

I. INTRODUCTION

A. Physiological need states motivate behavior

Throughout evolution, hunger has been an important signal that works to motivate animals to seek food and is highly conserved across species. While the ways in which hunger causes action toward food have been somewhat well studied, hungers' effect on reward sensitivity, in general, and sensitivity for non-food rewards, specifically, remains incompletely understood. Previous theories, such as drive reduction theory and incentive motivation have sought to make sense of the ways in which physiological need states motivate behavior and alter affect, though largely in theoretical or abstract terms (Berridge & Robinson, 1998; Crespi, 1942; Dickinson & Balleine, 1994; Hull, 1943). However, recent work has attempted to identify and characterize the specific mechanisms by which need states alter reward sensitivity and suggests the generation of a negative affective state as a candidate (Betley et al., 2015; Chen, Lin, Kuo, & Knight, 2015; Seeley & Berridge, 2015). For example, recently Betley et al. showed that animals would work to avoid activation of agouti-related peptide (AgRP) neurons, a population of neurons located in the arcuate nucleus of the hypothalamus (ARC) primarily involved in increased feeding behavior (Betley et al., 2015; Ollmann et al., 1997; Shutter et al., 1997). This suggests that perhaps hunger works to motivate behavior by generation of a negative affective state that animals work to alleviate. This work opens the door to further investigation of the process by which need states alter reward sensitivity.

It is critical to understand the complex relationship between reward and homeostatic brain pathways in the healthy brain in order to move towards combating the pathologies that arise when these systems go awry. For example, highly palatable foods, which have been shown to cause massive increases in reward signaling, and their widespread availability has likely

contributed to the obesity epidemic (Bassareo & Di Chiara, 1997, 1999). The present study aims to continue to fill in the details of the effect of need states, such as hunger, on affective state. The study utilized intracranial self-stimulation (ICSS) as a means to assess the reward sensitivity of an animal, which others have shown to be an index of, or at least altered in, different affective states. Experimental manipulations included a variety of peptide and drug injections to induce hunger (assessed separately via food intake) and thirst (assessed separately via drinking).

B. Intracranial Self-Stimulation: a behavioral assay to index reward sensitivity

While it is relatively easy to ask humans about their affective state, basic science relies on non-human models to investigate underlying mechanisms. Since discerning affective state in non-human animals is not as simple as asking a question, behavioral paradigms have been developed in order to probe affective state. Intracranial self-stimulation has been developed over decades to serve such a purpose by using reward sensitivity as a proxy for affective state. Drs. James Olds and Peter Milner discovered, in the now classic experiment, that animals would return to an area where they had received brain stimulation and eventually trained them to lever press to self-administer stimulation (Olds & Milner, 1954). These experiments led directly to the foundation of brain stimulation reward (BSR) and intracranial self-stimulation.

Brain stimulation reward is a useful tool to assess behavior for many reasons. It eliminates several confounds that are introduced by food reward studies, which is crucial when investigating motivated behavior. Firstly, as an animal consumes more and more food throughout a behavioral testing session, they will eventually become satiated, find the food much less reinforcing, and will eventually cease to work for food. Whereas, left alone, animals will work for brain stimulation reward for hours (Wise, 2002). This means that experiments can be run over a much longer time course, which allows for testing of more long-term manipulations.

Secondly, brain stimulation reward sensitivity has been shown to be very stable within an individual animal over days to weeks (Carlezon Jr. & Chartoff, 2007). Lastly, and crucially for the present work, food-restriction, which alters the motivational state of the animal, is totally avoided. Food reward is also inappropriate for the present study because gustatory systems, mastication, and any nutritive component of food reward would all create their own feeding or satiety signals, which would greatly cloud any results.

While many studies using ICSS stimulate in areas like the lateral hypothalamus (LH) and medial forebrain bundle (MFB), others stimulate in the ventral tegmental area (VTA; Corbett & Wise, 1980; Negus & Miller, 2014). The VTA is the site of many dopamine (DA) cell bodies and therefore ICSS in the VTA directly excites DA neurons (Fiorino, Coury, Fibiger, & Phillips, 1993; Ranaldi, 2014). Further, it has been shown that DA receptors, located in the nucleus accumbens (NAc), are necessary for ICSS behavior and that animals will press for DA stimulation specifically, using optogenetics (Steinberg et al., 2014). By stimulating in the VTA, the site of these DA neurons, we can directly activate the reward system and potentially avoid the confound of direct activation of the homeostatic systems which are more directly tied to other common stimulation sites.

Animals will press at greater rates for higher frequencies of BSR and less for lower frequencies. Below a certain frequency of BSR, animals will cease to make operant responses for stimulation, suggesting stimulation below a certain threshold is no longer reinforcing enough to support lever-pressing behavior. Using this threshold, it has been demonstrated that ICSS can be used as a means to assess reward sensitivity (Carlezon Jr. & Chartoff, 2007; Hoebel & Teitelbaum, 1962). Animals are initially trained to lever press for BSR at a stable frequency. Once they have learned this association, the frequencies of BSR are reduced throughout a session

in a logistic function and the threshold value can be determined. The threshold stabilizes for an individual animal across multiple sessions of the rate-frequency paradigm and is a proxy for the animal's sensitivity to BSR. Threshold under normal conditions can then be compared to threshold following experimental manipulations to assess the change in reward sensitivity. Studies have shown that contexts which are associated with negative mood in humans, such as withdrawal from drugs of abuse, cause an increase in the threshold for BSR (Barr, Markou, & Phillips, 2002; Markou, Hauger, & Koob, 1992; Markou & Koob, 1991). Conversely, agents that are associated with positive mood in humans, like psychostimulant use, cause a decrease in the threshold for BSR (Brunton, Lazo, & Parker, 2010; Carlezon Jr. & Wise, 1996; Stewart, de Wit, & Eikelboom, 1984; Tomasiewicz, Mague, Cohen, & Carlezon Jr., 2006; Wise, 1996). Using this information, the present study aims to provide support for the association of a hunger states with either a positive or negative affective state. Therefore, ICSS is a very useful tool for examining affective state in animals by proxy of reward sensitivity to BSR and was utilized to further understand affective state in a physiological need state.

C. The many pathways to inducing need states: hunger

Hunger is generated in the body through a number of very complex physiological processes. It is imperative that our body can signal to us when we are in need of more fuel and nutrients for survival. There are a multitude of neuromodulators that signal hunger and satiety throughout the neuraxis. Consequently, there are many pathways to induce a hunger-like state in animals. First, to look at specific pieces of this highly integrated system, three different compounds were infused that are known to promote feeding behavior in animals. Ghrelin, neuropeptide Y (NPY), and 5-thio-d-glucose (5TG) promote feeding behavior in animals through differing mechanisms of action and therefore probe a hunger-like state generated by activation of

the ARC as well as through the activation of glucose-sensors. Then, the study activated the entire system by fasting animals to induce hunger. This study can therefore compare the effects of the activation of this homeostatic network through differing mechanisms on reward sensitivity.

Ghrelin is an important and well-studied peptide that is known to induce feeding, even in sated animals, when injected either peripherally and centrally (Nakazato et al., 2001). Ghrelin, commonly referred to as the "hunger hormone," is released from endocrine cells lining the stomach and is then converted to its active form via the enzyme ghrelin *O*-acyltransferase (GOAT; Kojima et al., 1999; Sakata et al., 2002; Yang, Brown, Liang, Grishin, & Goldstein, 2008). It is then released into the bloodstream and peaks just before the start of a meal (Cummings, Frayo, Marmonier, Aubert, & Chapelot, 2004). Ghrelin, which comes from the phrase "growth hormone release-inducing," was discovered by Bowers and colleagues from obese mouse models (Bowers, 1998). Ghrelin travels from the gut to the brain, primarily to the ARC (Dickson & Luckman, 1997). Here, ghrelin excites NPY/AgRP neurons (see paragraph below) which then signal upstream to the paraventricular nucleus (PVN) and LH and promote feeding behavior (Cowley et al., 2003; Willesen, Kristensen, & Rømer, 1999). It has also been shown that ghrelin acts in the LH to enhance DA release and stimulate feeding via activation of orexin neurons (Cone, McCutcheon, & Roitman, 2014; Mason, Wang, & Zigman, 2014; K. P. Skibicka, Hansson, Alvarez-Crespo, Friberg, & Dickson, 2011). Ghrelin receptors are also found in a number of other places throughout the brain, some of which have important implications for affect as well as promoting feeding, including the nucleus of the solitary tract (NTS), hippocampus, amygdala, and the VTA (Alvarez-Crespo et al., 2012; Zigman, Jones, Lee, Saper, & Elmquist, 2006). Circulating ghrelin levels are positively correlated with subjective hunger ratings in humans, as well as measures of stress and anxiety in animals (Alvarez-Crespo et al.,

2012; Chuang & Zigman, 2010; Cummings et al., 2004). While ghrelin seems to play a role in both hunger pathways and mood and anxiety, it remains unknown whether ghrelin may be acting as one interface between these two systems in the brain. Further, ghrelin has been demonstrated to increase the firing rate of DA neurons in the VTA, increase preference for highly palatable food, and increase lever pressing for sucrose (Abizaid et al., 2006; Egecioglu et al., 2010; Skibicka, Shirazi, Hansson, & Dickson, 2012). Together, these findings suggest that using intracerebroventricular (ICV) ghrelin administration as a proxy for hunger may provide insight into the effects of hunger on affective state. There is also specific evidence that suggests ghrelin has a role in the processing of BSR in ICSS. Genetically modified rats without ghrelin receptors required much higher stimulation frequencies to support lever pressing behavior than their wildtype counterparts (Wellman et al., 2012). The present study aims to expand on this by using a within-subjects design, since there can be individual variability in stimulation threshold, in genotypically normal rats**.**

NPY is another important peptide in the hunger pathway. It was discovered in 1980's and its neurons are located throughout the central nervous system (CNS; Allen et al., 1983). However, the co-expressing AgRP/NPY neurons of the ARC in particular play a critical role in hunger and food intake. Administration of NPY has been shown to produce robust feeding effects and inhibition of NPY neurons can cause hypophagia (Clark, Kalra, & Crowley, 1984; Kanatani et al., 1998; Stanley, Kyrkouli, Lampert, & Leibowitz, 1986). NPY receptors are also found in the NAc and the VTA (Korotkova, Brown, Sergeeva, Ponomarenko, & Haas, 2006; van den Heuvel et al., 2015). Interestingly, infusion of NPY into the VTA has been shown to increase chow intake in rats but does not increase lever pressing for sucrose, as is seen with ghrelin (Jewett, Cleary, Levine, Schaal, & Thompson, 1995). NPY has also been demonstrated to have

extra-feeding behavioral implications, such as the discovery that NPY causes a direct release of corticosterone-releasing hormone (CRH), which creates a stress response, and has been shown to suppress sex behavior in both male and female rats (Haas & George, 1989; Kalra, Clark, Sahu, & Dube, 1988). Again, this suggests that NPY administration may have an impact on affective state in addition to its feeding effects. One study showed that NPY did not prevent an increase in BSR threshold associated with nicotine withdrawal, and in fact elevated BSR threshold in control rats on its own (Rylkova et al., 2008). Conversely, another study showed no effect of NPY on BSR threshold following administration in various hypothalamic sites (Fulton, Woodside, & Shizgal, 2002). These seemingly contradictory results give further evidence for the necessity of a more investigation into the effects of NPY on reward sensitivity.

While both ghrelin and NPY act centrally in the arcuate nucleus to exert their effects on feeding behavior, 5TG induces feeding through different mechanisms, which do not occur naturally in the body. 5TG is an antimetabolic glucose analogue that prevents cells from properly utilizing glucose, therefore signaling a lack of energy stores, even when ample glucose is available. It was first synthesized in 1973 by substituting the oxygen in D-glucose for sulfur in order to study its effects on insulin secretion (Hellman, Lernmark, Sehlin, Täljedal, & Whistler, 1973). Later, it was noticed that administration of 5TG caused between a three to five-fold increase in blood glucose and increased feeding behavior, both of which are dependent on noradrenergic cell bodies of the hindbrain (Hudson & Ritter, 2004; Ritter & Slusser, 1980; Ritter, Slusser, & Stone, 1981). 5TG also has been shown to inhibit growth of cancer cells and produce temporary sterility in males (Bushway & Whistler, 1975; Zysk, Bushway, Whistler, & Carlton, 1974). Interestingly, 5TG administration had no effect on performance in a maze test for food reward, which is in contrast to what might be predicted if 5TG is increasing motivation for

food (Bushway, Whistler, & Myers, 1977). Until this point, no studies have examined the effects of 5TG on BSR. 5TG, NPY, and ghrelin all utilize different pathways to stimulate feeding behavior and allow for investigation of affective state when an animal is seeking food.

D. Additional need states: thirst and the signals involved

Hunger is, of course, not the only physiological need state to consider. Thirst is another powerful physiological state that is carefully coordinated through a series of feedback loops between the kidneys, vascular system, and brain. Baroreceptors in the blood vessels mechanically sense the amount of pressure being put on their walls, which is a direct measure of blood volume. During hypovolemia, the baroreceptors send an inhibitory signal to the NTS, which then triggers the release of vasopressin from the posterior pituitary gland. Vasopressin, also commonly referred to as antidiuretic hormone, increases blood pressure and reabsorption of water by the kidneys (du Vigneaud, Ressler, & Trippett, 1953; Oliver & Schäfer, 1895). The release of vasopressin is also stimulated by osmoreceptors, which detect any shrinking or swelling of cells in the vascular organ of the lamina terminalis (OVLT; Bourque & Oliet, 1997). At the same time, renin is released from the kidneys, which converts angiotensinogen from the liver into angiotensin I, which is then converted into its active form, angiotensin II by angiotensin-converting enzyme (ACE; Houssay & Fasciolo, 1937; Page & Helmer, 1940; Tigerstedt & Bergman, 1898). Angiotensin II is able to enter the brain in the OVLT and subfornical organ (SFO), which sit beside the hypothalamus. The SFO sends an excitatory signal to the median preoptic area (MePO), the PVN, and the LH, which stimulate drinking behavior to bring the system back to homeostasis (Fitzsimons, 1998; Leib et al., 2016). Both intracranial and peripheral angiotensin II administration has been shown to induce drinking behavior in rats (Epstein, Fitzsimons, & Rolls, 1970; Fitzsimons & Simons, 1969). Indeed, rats trained to lever

press for water will press up to 64 times for merely 0.1 ml of water after intracranial infusion of angiotensin II, demonstrating just how salient this effect is on increasing motivation to drink (Rolls, Jones, & Fallows, 1972). cFos studies, which serve as marker of recent neuronal activity, have shown activation following angiotensin II administration in many brain areas including NTS, PVN, bed nucleus of the stria terminalis (BNST), and the central nucleus of the amygdala (ceA; McKinley, Badoer, & Oldfield, 1992; Oldfield, Badoer, Hards, & McKinley, 1994). Interestingly, one study suggested that stimulation of angiotensin II receptors inhibited DA synthesis (Nakaoka et al., 2015). Again, suggesting that there may be recruitment of brain areas involved in affective state in addition to the more traditional purely homeostatic functions.

E. Interactions between hunger and reward centers

While much is known about the impact of hormones that relate physiological state on brain homeostatic circuitry, there is a gap in knowledge in addressing the interaction between these homeostatic processes and reward processes. The two systems have shown to be highly anatomically linked, for example there are projections from LH and ARC to both NAc and VTA, but the functional relationship of these connections needs to be further studied (Cassidy & Tong, 2017).

It is well demonstrated that hunger increases the value of both food rewards and non-food rewards (Lockie & Andrews, 2013; Sharma, Fernandes, & Fulton, 2012; Xu, Schwarz, & Wyer, 2015). Specifically, studies have shown an increase in drug seeking and self-administration of drugs of abuse, which do nothing to alleviate the animals' hunger (Cabeza de Vaca & Carr, 1998; Carr, 2002; Carroll, France, & Meisch, 1981; Pothos, Creese, & Hoebel, 1995; Shalev, 2012). Some studies have also shown a decrease in threshold, or a facilitation, of BSR when

animals are deprived of food though the food restriction paradigm and stimulating electrode sites have varied greatly (Blundell & Herberg, 1968; Frutiger, 1986). Various studies have shown animals will self-deprive themselves of food, water, sodium, and warmth in favor of BSR (Carlisle & Snyder, 1970; Eckert & Lewis, 1967; Falk, 1961; R. A. Frank, Preshaw, Stutz, & Valenstein, 1982; Mogenson & Morgan, 1967; Routtenberg & Lindy, 1965; Spies, 1965). However, these studies have shown that the degree to which the animals will choose BSR to the level of starvation, freezing, etc., varied greatly based on a number of factors, such as stimulation site, stimulation parameters, and the animals state at the start of the experiment and more recent work has shown that perhaps BSR does not outcompete some rewards at all (Conover & Shizgal, 1994; Conover, Woodside, & Shizgal, 1994; R. Frank & Stutz, 1984). It has been proposed that BSR out competes other rewards either due to activation of homeostatic pathways that alleviate the drive or because BSR more directly, and thus more effectively, actives the reward pathways in the brain. While it is clear that the homeostatic and reward systems in the brain are inextricably linked, more work needs to be done to discover the nature of this relationship.

The following study examined the effects of different hunger peptides/drugs on food and water intake and then on the sensitivity of BSR. The study utilizes ICSS to measure sensitivity to BSR before and following ICV infusion of various peptides/drugs that are known to affect homeostatic condition and thus utilizes a within subjects design. Stimulating electrodes for ICSS are placed in the VTA, as opposed to MFB or LH, in an attempt to more effectively isolate the activation of dopamine neurons and parse out any homeostatic effects due to peptides or drugs from possible effects of stimulation of hypothalamic nuclei. Various infusions were administered to the lateral ventricle during ICSS sessions. Ghrelin and NPY-sensitive sites that modulate food intake have focused on midbrain and hypothalamic sites, whereas 5TG is thought to exert its

main food-promoting action through brainstem circuitry (Cowley et al., 2003; Ritter et al., 1981; Williams et al., 2001). Angiotensin II was administered to examine the effects of thirst. While previous studies dating back as far as the early 1960s have investigated the effects of food and water deprivation on ICSS and found a facilitation of BSR, or a decrease in threshold, none have investigated this effect when stimulating in the VTA (Blundell & Herberg, 1968; Deutsch & Di Cara, 1967; Hoebel, 1969; Hoebel & Teitelbaum, 1962; Olds, 1958). Therefore, this study also examined the effects of acute food and water deprivation on sensitivity for BSR. This also allows us to directly compare the effects of any changes induced by activation of a specific pathway or cell population with the infusions, to actual hunger and thirst. Change in reward sensitivity to VTA stimulation after infusion of peptides/drugs which induce feeding and drinking behavior should better inform the affective state that is induced by the physiological need state.

II. GENERAL METHODS

A. Subjects

32 male Sprague-Dawley rats obtained from Charles River Laboratories were used for the described research. Rats were housed in shoebox cages and given *ad libitum* access to food and water unless otherwise stated as part of an experiment. They were kept in a temperature and humidity controlled vivarium on 12hr light-dark cycle from 7:00am to 7:00pm.

B. Surgery

All animals were anesthetized using a mixture of ketamine hydrochloride (100 mg/kg, intraperitoneal [i.p.]) and xylazine hydrochloride (10 mg/kg, i.p.) and depth of anesthesia was monitored by periodically pinching the rat's toe and observing a withdrawal reflex. Once anesthetized, the site of incision was cleared of hair and sterilized with betadine. Rats were then placed in a stereotaxic frame (Kopf Instruments, Tujunga, California). Measurements were made relative to bregma using the rat brain atlas (Paxinos & Watson, 1998). Animals for the peptide related experiments were implanted with a 22-gauge infusion cannula (PlasticsOne) in the lateral ventricle (AP: -0.8, ML: +1.7, DV: -3.6), and animals for ICSS experiments received a bipolar stimulating electrode in the ventral tegmental area (AP: -5.2, ML: +0.8, DV: -8.4). Stainless steel screws were implanted in the skull and dental acrylic was used to secure cannula. Animals were given Meloxicam (0.2 mg/ml, subcutaneous [s.c.]) following surgery and were allowed to recover to pre-surgery weighs before being returned to the vivarium. Animals were monitored for a minimum of ten days post-operatively.

C. Drug Infusions

Animals received ICV infusions of angiotensin II, 5TG, NPY, ghrelin, and saline (see doses below) in a random order based on a latin square design. Following ICSS, animals were placed immediately back into their home cages and food consumption, calculated as change in weight of cage tops, and water intake, calculated as change in weight of water bottle, were measured for a one hour period. Animals for measuring peptide induced feeding and drinking effects received infusions and were placed immediately back in their home cages where food and water intake were measured in the same manner for a period of one and a half hours. Latency to begin eating and drinking was also measured in a subset of these animals. Timers were started as soon as animals were placed back in their home cages and were stopped when their mouths came in contact with chow and the water sipper. If they had not begun eating or drinking within a ten-minute period, the timer was stopped and a latency of 600s was recorded. Infusions were separated by two days of free feeding and drinking with no infusions.

D. Intracranial Self-Stimulation

1. Training

Animals for ICSS experiments were initially trained to lever press for BSR using standard Med Associates software and operant chambers, stimulators and software (Med Associates, Inc., Fairfax, VT). The amount of current was adjusted to the lowest value that would sustain lever pressing behavior to a criteria of 40 presses per minute for three consecutive days (Carlezon Jr. & Chartoff, 2007). Rats were then moved to a rate-frequency behavioral paradigm until BSR threshold values were consistent within ten percent for a minimum of three days. During rate-frequency training, rats received

'blocks' of stimulation frequencies. A block is defined as an initial five non-contingent pulses of stimulation at a given frequency, which serve to inform the animal what stimulation frequency was available. A tone played each time the animal received stimulation. A lever then extends and the cue light above it was on for 50 seconds, during which the animal can press as many times as they want. After this time, the lever retracts and there is a five second time out period before the next block begins. Blocks are always presented in descending stimulation frequency (141, 126, 112, 100, 89, 79, 71, 63, 56, 50, 45, 40, 35, 32, and 28 Hz) and there are 15 blocks that make up each 'pass' (Figure 1A). Each animal received five passes each day of training and data was analyzed to determine "theta," or the theoretical threshold frequency for stimulation, below which animals would not press for stimulation (Figure 1C). Once animals had an average daily theta value within 15% variability for three consecutive days, there were considered stable. Maximum rate of lever pressing during each pass was also captured.

2. <u>Testing: ICV Infusions</u>

Once rats had stable BSR threshold values, they could be tested. Rats received a drug free session on the days immediately preceding and following the test day. Rats completed three initial passes; the first of these passes was excluded, since it typically is far more variable, and the remaining two were used to calculate the pre-infusion theta, a direct measure of BSR threshold (Carlezon Jr. & Chartoff, 2007). The animals were then removed from the operant chamber and received an intracranial infusion of the drug or vehicle being tested. They were then placed back into the operant chamber and completed six additional passes. Immediately following the completion of the session, animals were

placed back into their home cages and food consumption, calculated as change in weight of cage tops, and water intake, calculated as change in weight of water bottle, were measured for a one hour period. As previously stated, animals were given a drug-free session the following day, and a return to a stable BSR threshold value was required before they could be used for another test session (Figure 1D).

3. **Testing: Food and Water Deprivation**

Once animals had a stable BSR threshold value, they could also be tested. Animals were deprived of either food or water, with the order determined using a latin square, for a 24-hour period immediately preceding testing. Therefore, a "day-of" average theta could not be utilized to calculate percent change in theta. Pre-treatment theta for this experiment was calculated by averaging the theta value for each pass on the previous three days of rate-frequency training. Animals completed six rate-frequency passes at exactly 24 hours. Following testing, animals were returned to their home cages and resumed ad libitum feeding and drinking. Animal weights were monitored to confirm food or water deprivation.

E. Drugs

Animals were given angiotensin II (Sigma Aldrich, 100 ng/µl; Buggy & Fisher, 1976), 5TG (Sigma Aldrich, 200 µg in 2 µl; R. C. Ritter et al., 1981), NPY (Tocris, 5 µg/µl; Bertholomey, Henderson, Badia-Elder, & Stewart, 2011), ghrelin (Tocris, 1 µg/µl; Cone, McCutcheon, & Roitman, 2014) or vehicle (0.9% Sodium Chloride) into the lateral ventricle. Dosage information is listed in Table I.

F. Histology

After testing concluded, animals were euthanized using CO_2 asphyxiation and decapitation and India ink was infused into the infusion cannula. Brains were drop fixed using formalin and sliced to visually confirm ink was in the ventricle. Tissue was stained with a cresyl violet Nissl stain and imaged (Figure 2).

G. Data Analysis

Theta was calculated for each individual pass by plotting the least-squares line of best fit across stimulation frequencies that elicited 20, 30, 40, 50, and 60% of the maximum response rate. This line is continued and the point at which it crosses the x-axis is considered the theoretical threshold frequency (theta), above which animals will press for stimulation (Figure 1B). Maximum rate was calculated by finding the maximum number of presses in any given block for each pass, and was captured to ensure that any effects of the ICV infusions were not due to decreased motor capabilities. Theta values and maximum rate were calculated using a rate-frequency curve analysis MatLab (Natick, MA) applet created by Dr. Matthew McMurray. The average of the second and third pre-infusion passes is used to calculate a percent change from pre-infusion for theta values and maximum rates of lever pressing for the six passes following drug infusion.

Data were analyzed using GraphPad Prism 5 (La Jolla, CA) software. A one-way ANOVA (analysis of variance) was run to examine the effect of drug infusions on food and water intake and was followed up with a Dunnett's multiple comparisons test with saline as the control. A two-way ANOVA was employed to examine the effects of drug infusion and pass on percent change in theta value and was followed with a Bonferroni multiple comparisons test. A one-way ANOVA was used to compare the effects of infusions on the average percent change in

theta across passes and was followed up with a Dunnett's multiple comparison test. Finally, to examine the effect of 24-hour food and water deprivation on average percent change in theta, theta values were normalized to the day prior to food or water deprivation. A repeated measure one-way ANOVA was then used to examine the effects of 24-hour food or water deprivation on normalized theta values and was followed up by a post hoc Dunnett's multiple comparison test.

III. RESULTS

For each rat, a minimum amount of current that supported ICSS was used. This current value differed across rats. Moreover, rats differed on their rates of responding at different frequencies. To address individual differences, that could accentuate variability in the raw data, we determined the theta and maximum rate of responding for each rat in each session untreated session. Data are hence expressed as percent change from pre-treatment theta values.

Mixed effects of infusions on food and water intake

Food intake was captured following ICV infusions of saline, angiotensin II, NPY, 5TG, and ghrelin and, though NPY, 5TG, and ghrelin all appeared to increase feeding, only NPY caused a significant increase in feeding ($F(4,57)= 24.22$, $p<0.001$). Post-hoc comparisons confirmed that there was a significant increase in food intake following administration of NPY (8.55 ± 1.2 g) compared to the administration of saline (0.75 ± 0.3 g; 95% $CI_{saline\text{-}NPY}$ [-10.18, -5.41]). As mentioned, ghrelin (2.5 ± 0.5 g) and 5TG (1.82 ± 0.3 g) appeared to show a trend of increased food intake but were not significant (Figure 3A).

Water intake was also measured following ICV administration of these drugs and no significant effects were found (Figure 3B; $F(4,56)= 3.31$, $p=0.017$). Although angiotensin II (6 ± 1.1 ml) appeared to cause an increase in drinking as compared to saline (3.5 ± 0.81 ml), it was not statistically significant. Infusions of NPY, ghrelin, and 5TG also failed to produce any significant increase in drinking as compared to saline.

Latency to begin eating and drinking were also measured in a subset of animals (n=3) in an attempt to further elucidate the results of the food and water intake. There was a significant main effect of infusion type on latency to begin eating or drinking ($F(4,13)= 6.09$, $p=0.012$) and

post hoc analysis showed that only NPY (273.0 ± 145.0 seconds) significantly decreased latency to begin feeding compared to saline (600.0 ± 0 seconds; 95% CI [98.5, 555.1]; Figure 4A). There was no significant effect of infusion type on latency to begin drinking ($F(4,14)= 0.47$, p=0.759; Figure 4B).

Infusions of feeding-related peptides attenuate sensitivity to BSR

In order to examine the effects of infusions of peptides, which classically stimulate feeding and drinking on reward sensitivity, we administered intracerebral ventricular infusions of various peptides, which cause feeding or drinking and measured threshold, or theta value for intracranial self-stimulation. We assessed reward sensitivity by comparing theta values for intracranial self-stimulation before and after administration of these peptides. All three of the drugs that induce feeding—ghrelin, NPY, and 5TG—increased theta values.

When examining the effects of the infusion and pass (also reflective of time passed since infusion) on the average percent change in theta values for BSR, we saw a main effect of infusion type ($F(4,20)= 53.58$, p<0.0001) and pass ($F(5,20)= 10.26$, p<0.0001; Figure 5A). Bonferroni post-tests were performed to follow up on these main effects and the results are displayed in Table 2. Ghrelin significantly increased theta relative to saline at all passes post-infusion, 5TG increased theta at passes two, three, and five, and NPY increased theta at passes one, two, and five (Table 2). There was no significant effect of drug infusion type on percent change of maximum rate of lever pressing ($F(4,31)= 1.51$, p=0.226; Figure 5B).

We also examined the cumulative effect of the infusions across passes and found a significant effect of infusion type on the percent change in theta ($F(4,31)= 2.981$, p=0.037; Figure 6A). Specifically, the percent change in theta following infusions of ghrelin (37.92 ±

14.4%) was significantly higher across passes than saline (2.54± 5.1%; 95% CI [-67.57, -3.193]). No other infusions caused a significant difference from saline. There was also no significant effect of infusion type on percent change in maximum rate across the testing session ($F(4,31)= 1.51$, p=0.226; Figure 6B).

Since animals had stable theta values for the three days preceding the test day, the day immediately before testing (termed baseline day 3) was used to normalize. After normalizing to baseline 3, we found a significant effect of infusions of 5TG, NPY, and ghrelin. There was no significant effect of saline administration on normalized theta values compared to pre-treatment ($F(3,35)= 0.62$, p=0.607; Figure 7A). Angiotensin II administration also showed no significant effect on normalized theta values ($F(3,27)= 2.41$, p=0.101; Figure 7B).

There was a significant effect of infusion of 5TG on normalized theta values compared to untreated days ($F(3,31)= 4.18$, p=0.018; Figure 7C). A Dunnett's Multiple Comparison Test showed that the average normalized theta value after 5TG (127.7 ± 14.2) was significantly greater than that of baseline 3 (100 ± 0, 95% CI [-51.9, -3.506]).

There was also a significant effect of infusion of NPY on normalized theta values compared to untreated days ($F(3,31)= 5.99$, p=0.004; Figure 7D). A Dunnett's Multiple Comparison Test showed that the average normalized theta value after NPY (129.2 ± 11.7) was significantly greater than that of baseline 3 (100 ± 0, 95% CI [-50.55, -7.764]).

Finally, there was also a significant effect of infusion of ghrelin on normalized theta values compared to untreated days ($F(3,27)= 14.03$, $p<0.0001$; Figure 7E). A Dunnett's Multiple Comparison Test showed that the average normalized theta value after NPY (131.9 ± 5.3) was significantly greater than that of baseline 3 (100 ± 0, 95% CI [-47.47, -16.33]).

Food and water intake were also measured for one and a half hours following the completion of ICSS on days where animals received infusions, the results of which are displayed in Figure 8. There was a significant main effect of infusion type on food intake ($F(4,39)= 9.89$, $p<0.0001$). A Dunnett's Multiple Comparison Test showed that this main effect was due to NPY infusions (6.33 ± 0.8 g) causing significantly more food intake than saline (2.22 ± 0.7 g; 95% CI [-7.028, -2.201]). There was no significant effect of drug infusion type on water intake following ICSS ($F(4,31)= 0.13$, $p=0.97$).

No significant effects of 24-hour food or water deprivation on sensitivity to BSR

Figure 9 shows the average percent change in theta (Figs. 9A and 9B) and maximum rate (Figs. 9C and 9D) presented for each pass (Figs. 9A & C) as well as averaged across passes (Figs. 9B & D) on test days after food and water deprivation. Theta values following food and water deprivation were normalized to baseline day 3 as described above (Figure 10). There was no significant effect of 24-hour food deprivation on normalized theta values compared to untreated days ($F(3,23)= 2.18$, $p=0.13$). There was also no significant effect of 24-hour water deprivation on normalized theta values compared to untreated days ($F(3,19)= 2.19$, $p=0.14$). The deprivation did cause a decrease in body weight for both food (-24.67 ±2.0 g) and water deprived (-25.25 ± 2.3 g) animals.

IV. DISCUSSION

The present study was conducted in order to further understand the link between homeostatic need states and reward processing, specifically of rewards that do not fill the need state. Intracranial self-stimulation was used as means to assay reward sensitivity following 24-hour food and water deprivation and infusions of various compounds that have previously been shown induce feeding and drinking behavior.

Our first goal was to verify the effects of ghrelin, NPY, 5TG, and Angiotensin II, which have been shown to cause an increase in food or water intake when administered ICV (Clark et al., 1984; Epstein et al., 1970; Levine & Morley, 1984; Ritter & Slusser, 1980; Toshinai et al., 2006), on food and water intake. We measured food and water intake for 90 minutes following infusions, mirroring the time during which an animal would be responding for BSR. While only NPY significantly increased food intake relative to saline, ghrelin and 5TG also clearly trended toward increased food intake. Similarly, while Angiotensin II administration did not cause a significant increase in water intake compared to saline, there was a trend toward increased drinking. In an attempt to further understand this pattern of results, latency to begin eating and drinking was measured in a cohort of animals. The pattern of results was very similar to the intake data in that only NPY caused a significant decrease in latency to begin eating, and therefore did not further elucidate the results. Food and water intake were measured in a somewhat crude manner for this experiment and animals were away from their colony room and had just experienced the stressor of handling during infusions. It is likely that if a more sensitive measure of food and water intake were used (e.g. feed- or lickometers), more robust changes would have been observed. Although we did not find the robust feeding effects of ghrelin and 5TG or drinking effects of Angiotensin II, we believed there was ample evidence from others to

continue with the experiment under the assumption that we were activating populations of neurons that cause feeding or drinking in other circumstances.

The present study next examined the effects of ICV infusions of ghrelin, NPY, 5TG, angiotensin II and saline on BSR. Ghrelin, 5TG, and NPY all significantly increased theta values, or attenuated BSR, compared to saline at various passes throughout the testing sessions. Numerous studies have shown that the threshold for BSR is also attenuated after manipulations which are associated with negative affective states in humans, such as administration of a kappa opioid receptor agonist, withdrawal from amphetamine, and acute and chronic stress paradigms (Carlezon Jr. et al., 2006; Cryan, Hoyer, & Markou, 2003; Donahue, Muschamp, Russo, Nestler, & Carlezon Jr., 2014; Moreau, Jenck, Martin, Mortas, & Haefely, 1992; Slattery, Markou, & Cryan, 2007; Todtenkopf, Marcus, Portoghese, & Carlezon Jr., 2004). When put in the context of previous literature, the finding that our manipulations also attenuated BSR, suggests that ICV infusions that increase feeding may be inducing a negative affective state. Indeed, the magnitude of change in threshold for BSR following the manipulations that cause a negative state in humans was typically between a 15-50% increase, which is consistent with our findings. Administration of angiotensin II had no effect on threshold for BSR, suggesting perhaps this circuitry does not interact as directly with reward systems in the brain. The fact that the manipulations, which induce feeding, attenuated BSR appears to contradict the findings of others in which food restriction caused the opposite effect on BSR threshold, since theoretically both create some state of hunger. Food restriction activates multiple systems throughout the body, whereas administration of ghrelin and NPY are thought to promote feeding, in part, by specifically activating AgRP neurons. Interestingly, selective excitation of AgRP neurons in animals conditions a place avoidance and posits that activation of AgRP neurons is inducing a

negative valence signal (Betley et al., 2015). Recent evidence suggests that, while AgRP neurons are highly active when animals are in a state of hunger, they shut off at the mere sight of food (Chen et al., 2015). Perhaps, by keeping these AgRP neurons turned on, an unnatural, aversive state is created which leads to the attenuation of BSR and also accounts for the findings of Betley et al, since they similarly overrode the shutting off of AgRP neurons (Chen & Knight, 2016; Seeley & Berridge, 2015). Interestingly, administration of 5TG, which does not act directly on these hypothalamic feeding circuits, also attenuated BSR to some degree. Although it is unlikely then that 5TG is artificially holding the tone of AgRP neurons on, it may also be creating some other unnatural overexcitation of neurons or the large increase in blood glucose which, in humans, such as individuals with diabetes, can cause a number of unpleasant symptoms and may be similarly aversive (Mayo Clinic, 2015; Ritter & Slusser, 1980).

Finally, we sought to compare the effect of activation of these specific neuron populations via infusions of peptides on reward sensitivity to the effect of food and water deprivation on reward sensitivity, as assayed by ICSS. Previous studies have shown a facilitation of BSR in response to food and water deprivation, however, these studies used hypothalamic stimulation sites such as the LH and MFB (Cabeza de Vaca & Carr, 1998; Frutiger, 1986; Hodos & Valenstein, 1960). The present experiment chose to stimulate in the VTA in attempt to separate out any effects that could be due to stimulation hypothalamic sites and isolate only the reward system (Ranaldi, 2014). Our results showed neither food nor water restriction led to a significant change in theta, which contradicts previous findings of facilitation of BSR in these conditions. Water restriction appeared to cause a slight attenuation of BSR, however several animals (n=2), ceased to make any operant responses for BSR after the first pass of rate-frequency—suggesting they had lost interest in the stimulation or had motor impairments, i.e.

lethargy. Upon deeper investigation of the literature, it seems that evidence for facilitation of BSR due to water restriction alone is perhaps not clearly established. The initial studies done by Olds and his colleagues do not test water restriction separately and, in fact, other studies show no effect and even attenuation of BSR following water restriction (Frutiger, 1986; Koolhaas, Mora, & Phillips, 1977).

More interesting, was the fact that, in the present experiment, 24-hour food deprivation as failed to produce facilitation of BSR. Yet, here once again, a greater scrutiny of literature shows that the results may not be conclusive as to the effect of food restriction on BSR. As is seen in Table 3, while many studies did show facilitation as a result of food deprivation, not all manipulations caused this effect. Studies vary greatly in terms of stimulation site and length of food deprivation.

Furthermore, to the author's knowledge, this is the first study to examine the effects of food and water deprivation on ICSS with the stimulating electrode located in the VTA. There is some evidence to suggest that stimulation sites may vary greatly in terms of the degree to which they are sensitive to food or water restriction (Fulton, Richard, Woodside, & Shizgal, 2004; Fulton et al., 2002). Though stimulation of the VTA supported behavior, it is possible that it is insensitive to food and water restriction. Further, there is also evidence of individual variation between animals in sensitivity to food restriction on BSR, which may explain why some individual animals did show a slight facilitation of BSR while others did not (Carr & Wolinsky, 1993).

Additionally, differences have been demonstrated in alteration of BSR due to length of food or water restriction. While this study utilized an acute 24-hour complete deprivation paradigm, others have shown greater effects following a chronic mild deprivation (Blundell & Herberg, 1968; Cabeza De Vaca, Holiman, & Carr, 1998; Hodos & Valenstein, 1960). Some studies have found that acute food deprivation does not show the same magnitude of facilitation of BSR as does chronic food deprivation (Fulton et al., 2004). It is possible that a longer period of deprivation is required to see facilitation of BSR when stimulating in the VTA.

Overall, this study continues to layer on to the complex and complicated relationship between homeostatic and reward pathways in the brain. A systematic study of different stimulation sites and parameters as well as food restriction parameters needs to be completed in order to put to rest the question of the effect of food restriction on BSR. While the natural response of homeostatic signals is becoming clearer and there is certainly an effect of manipulating these signals on reward sensitivity, the next step is to examine the exact neural signals that mediate this interaction. One possibility is that manipulation of these homeostatic signals operates to change sensitivity of dopamine neurons, and therefore change reward sensitivity. Some studies have already leant support to this idea demonstrating that administration of both NPY and ghrelin, as well as activation of other feeding-related neurons, altered the firing of DA neurons (Cassidy & Tong, 2017; Cone, Roitman, & Roitman, 2015; Dietrich et al., 2012; West & Roseberry, 2017). The further study of the interaction between these two systems in the brain is crucial in coming up with new treatments for issues like obesity and binge eating as well as many others that plague our society today.

V. ANIMAL CARE APPROVAL

All procedures proposed for this experimentation have been approved by the University of Illinois Animal Care Committee under protocol 15-004.

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Figure 1.

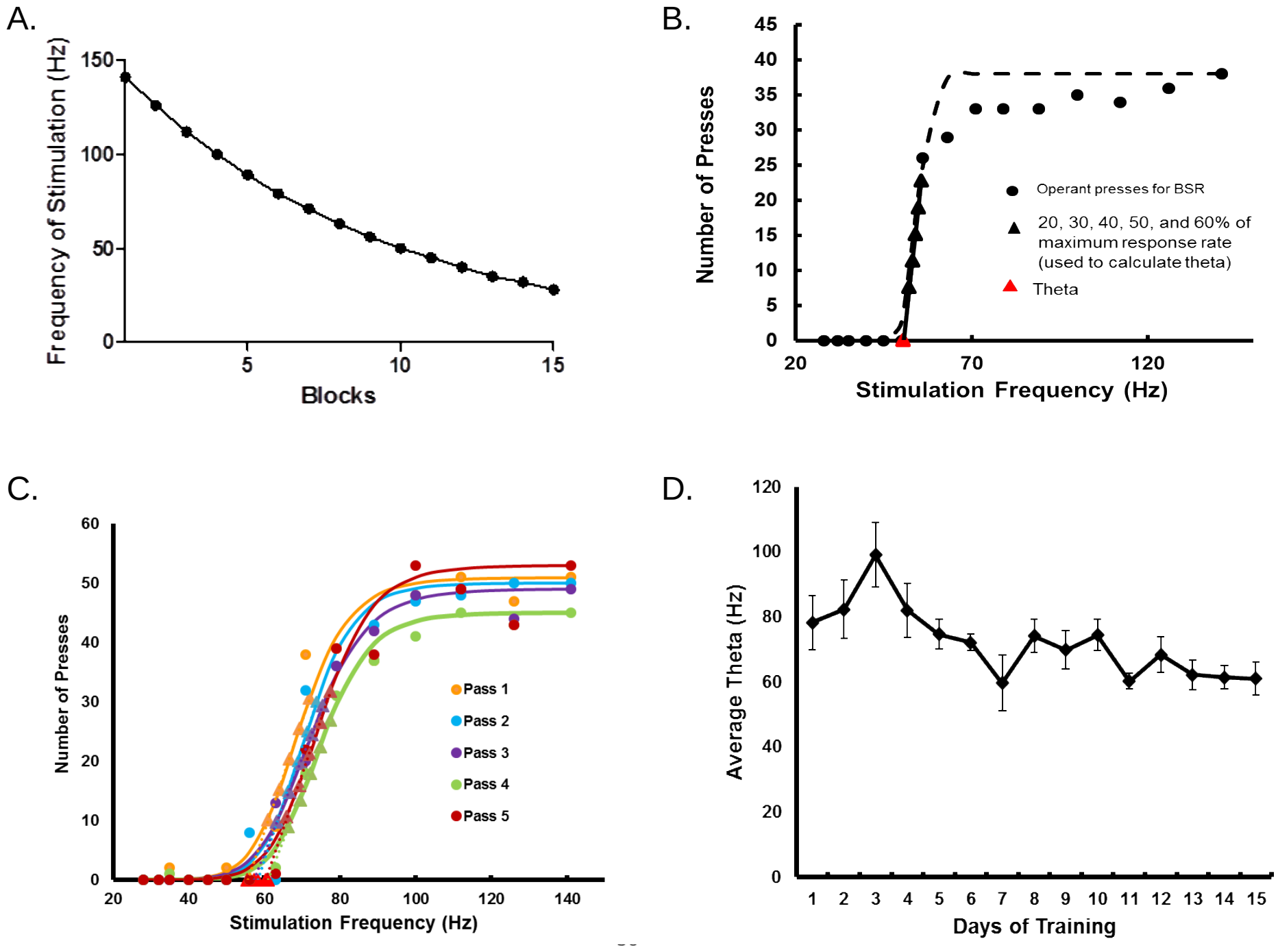
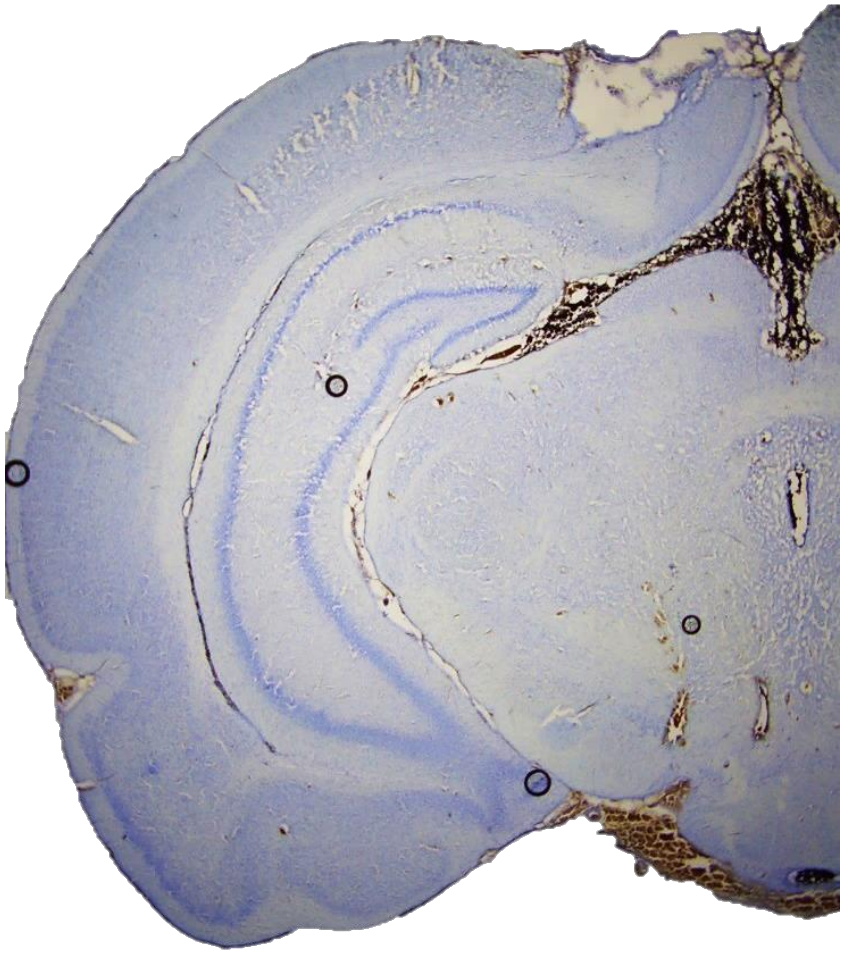

A.
Frequency of Stimulation (Hz)
150
100
50
0
5
10
15
Blocks
B.
Number of Presses
45
40
35
30
25
20
15
10
5
0
20
70
120
Stimulation Frequency (Hz)
Operant presses for BSR
20, 30, 40, 50, and 60% of maximum response rate (used to calculate theta)
Theta
C.
Number of Presses
60
50
40
30
20
10
0
20
40
60
80
100
120
140
Stimulation Frequency (Hz)
Pass 1
Pass 2
Pass 3
Pass 4
Pass 5
D.
Average Theta (Hz)
120
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Days of Training

Figure 2.

A.



B.

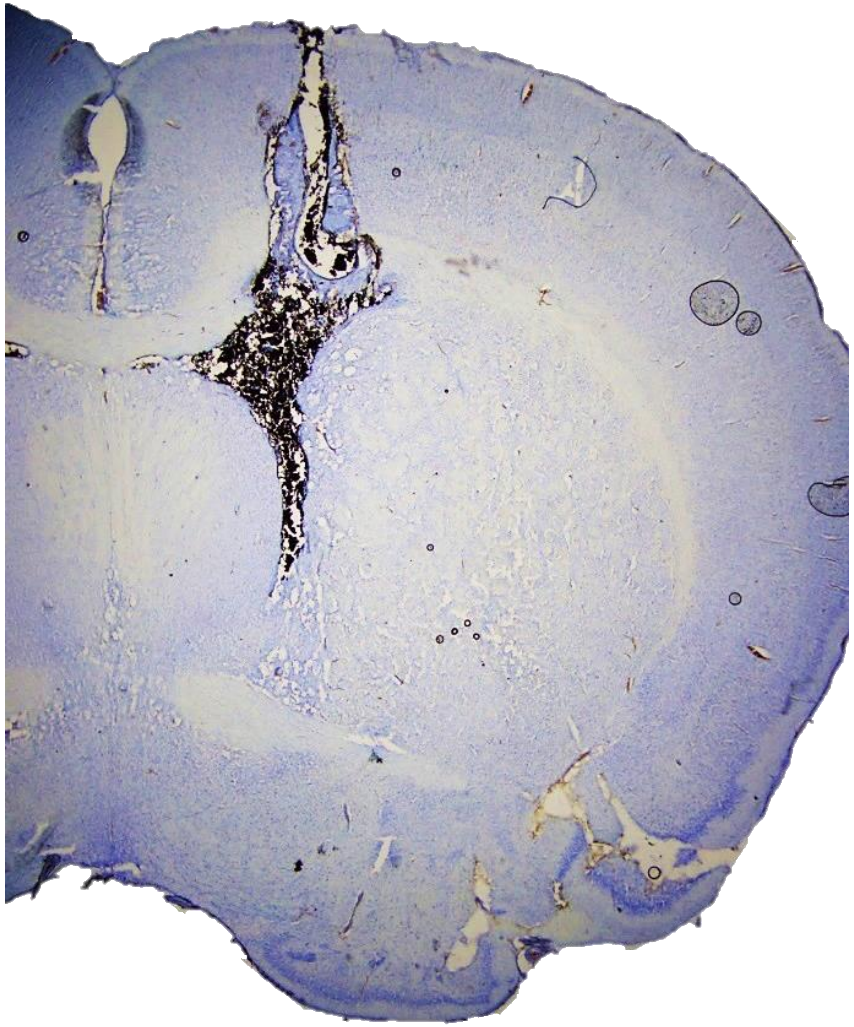


Figure 3.

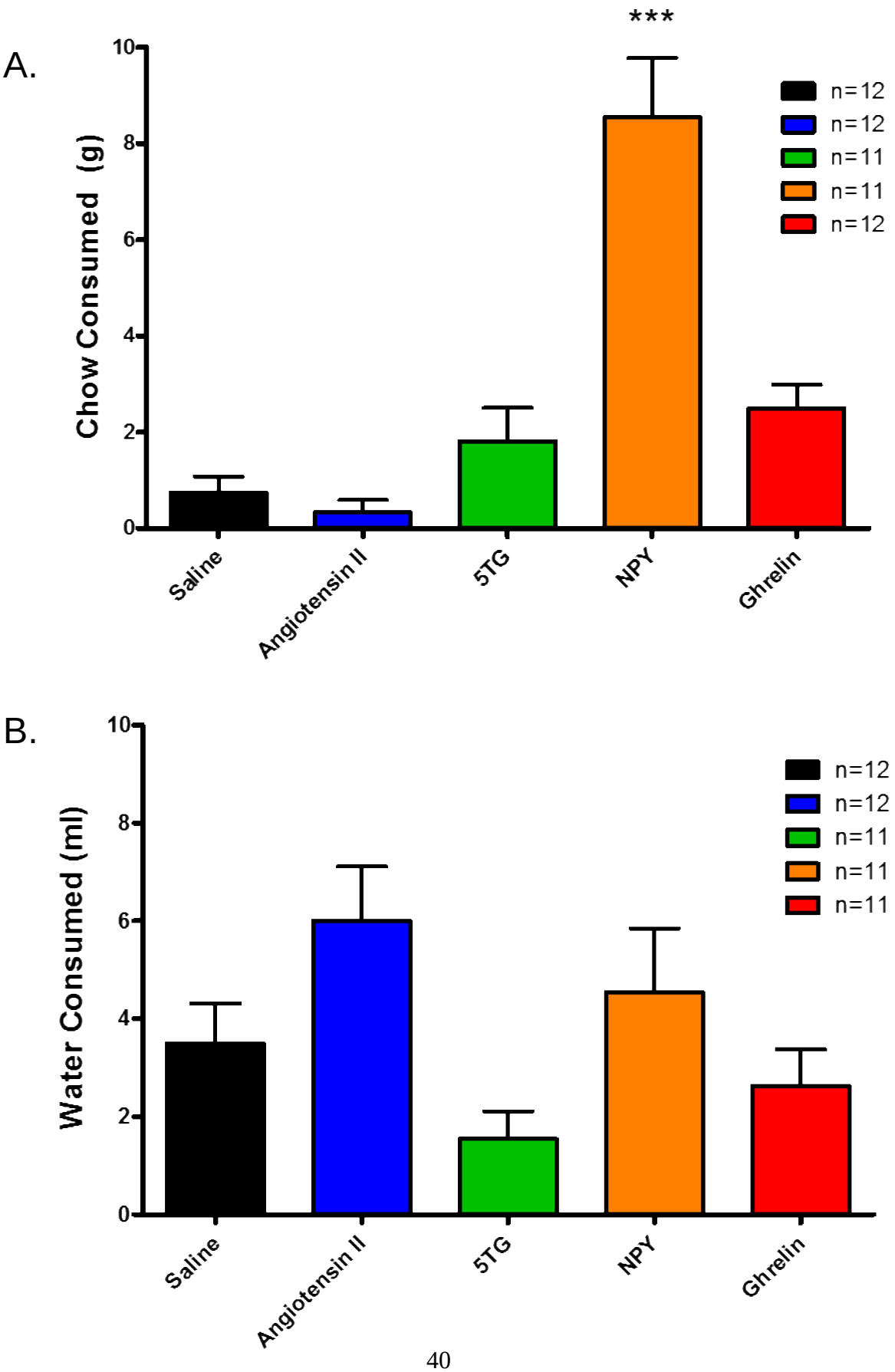

A.
***
Chow Consumed (g)
10
8
6
4
2
0
n=12
n=12
n=11
n=11
n=12
Saline
Angiotensin II
5TG
NPY
Ghrelin
B.
Water Consumed (ml)
10
8
6
4
2
0
n=12
n=12
n=11
n=11
n=11
Saline
Angiotensin II
5TG
NPY
Ghrelin

Figure 4.

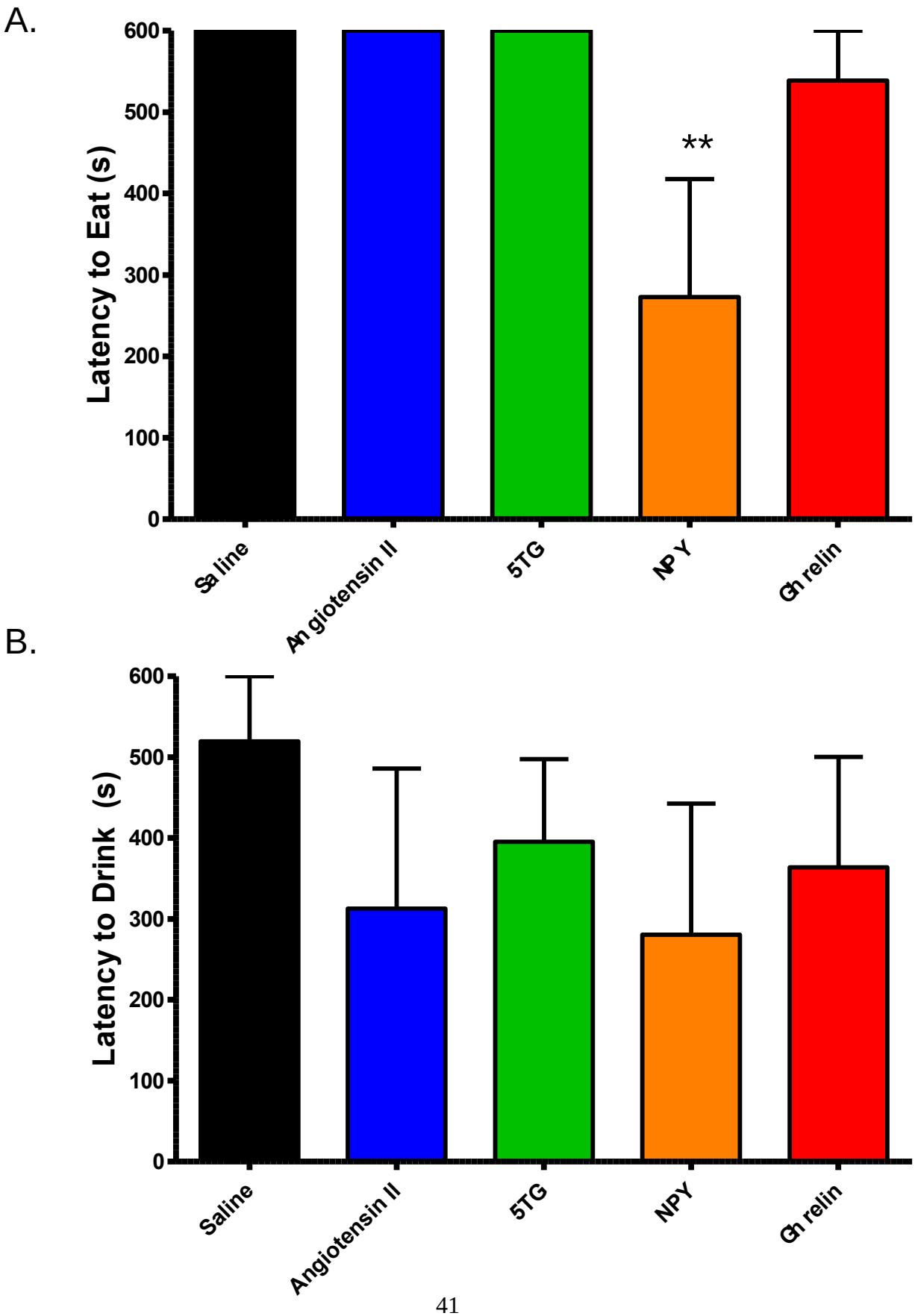

A.
Latency to Eat (s)
600
500
400
300
200
100
0
**
Saline
Angiotensin II
5TG
NPY
Ghrelin
B.
Latency to Drink (s)
600
500
400
300
200
100
0
Saline
Angiotensin II
5TG
NPY
Ghrelin

Figure 5.

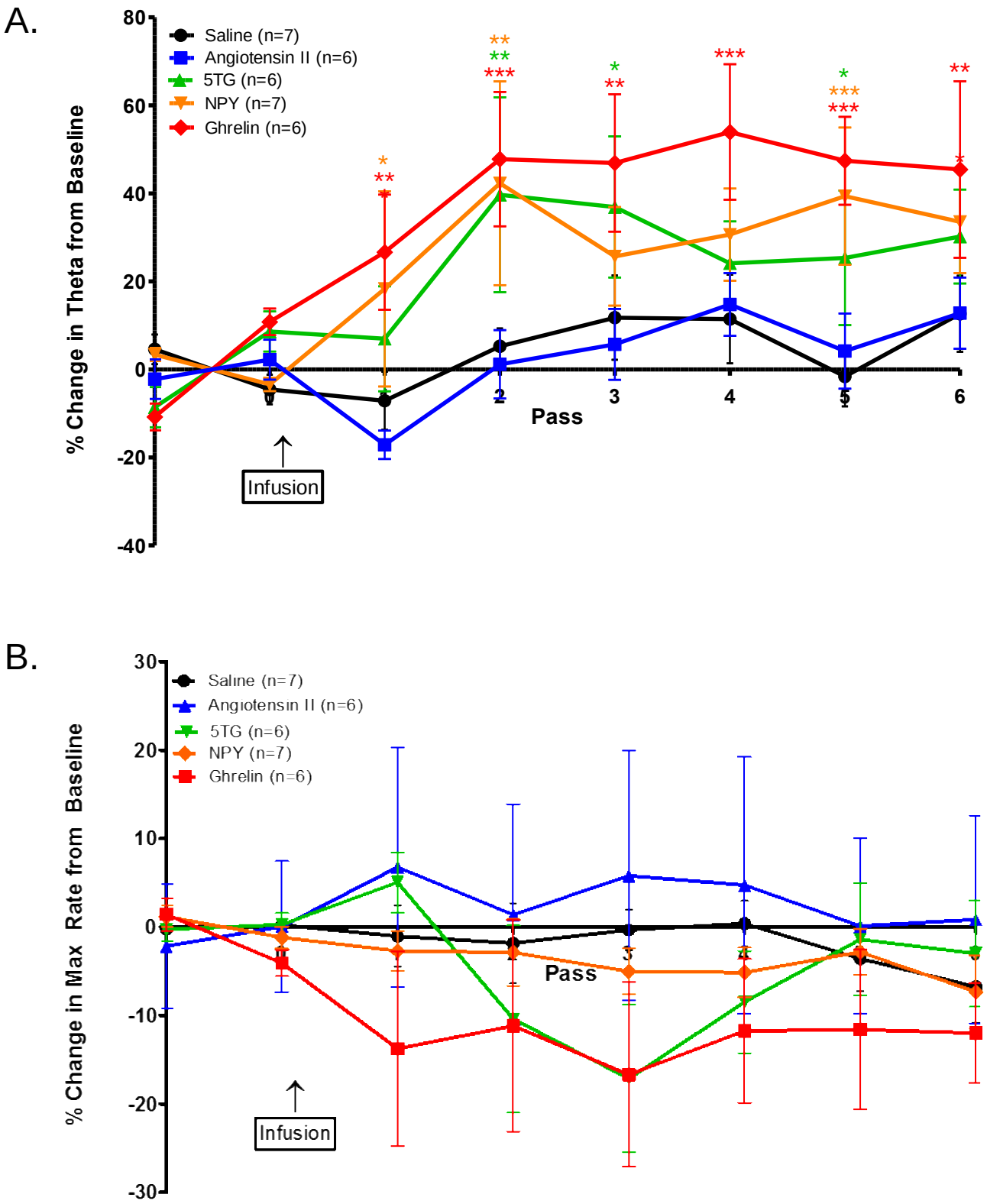

A.
Saline (n=7)
Angiotensin II (n=6)
5TG (n=6)
NPY (n=7)
Ghrelin (n=6)
% Change in Theta from Baseline
Pass
Infusion
B.
Saline (n=7)
Angiotensin II (n=6)
5TG (n=6)
NPY (n=7)
Ghrelin (n=6)
% Change in Max Rate from Baseline
Pass
Infusion

Figure 6.

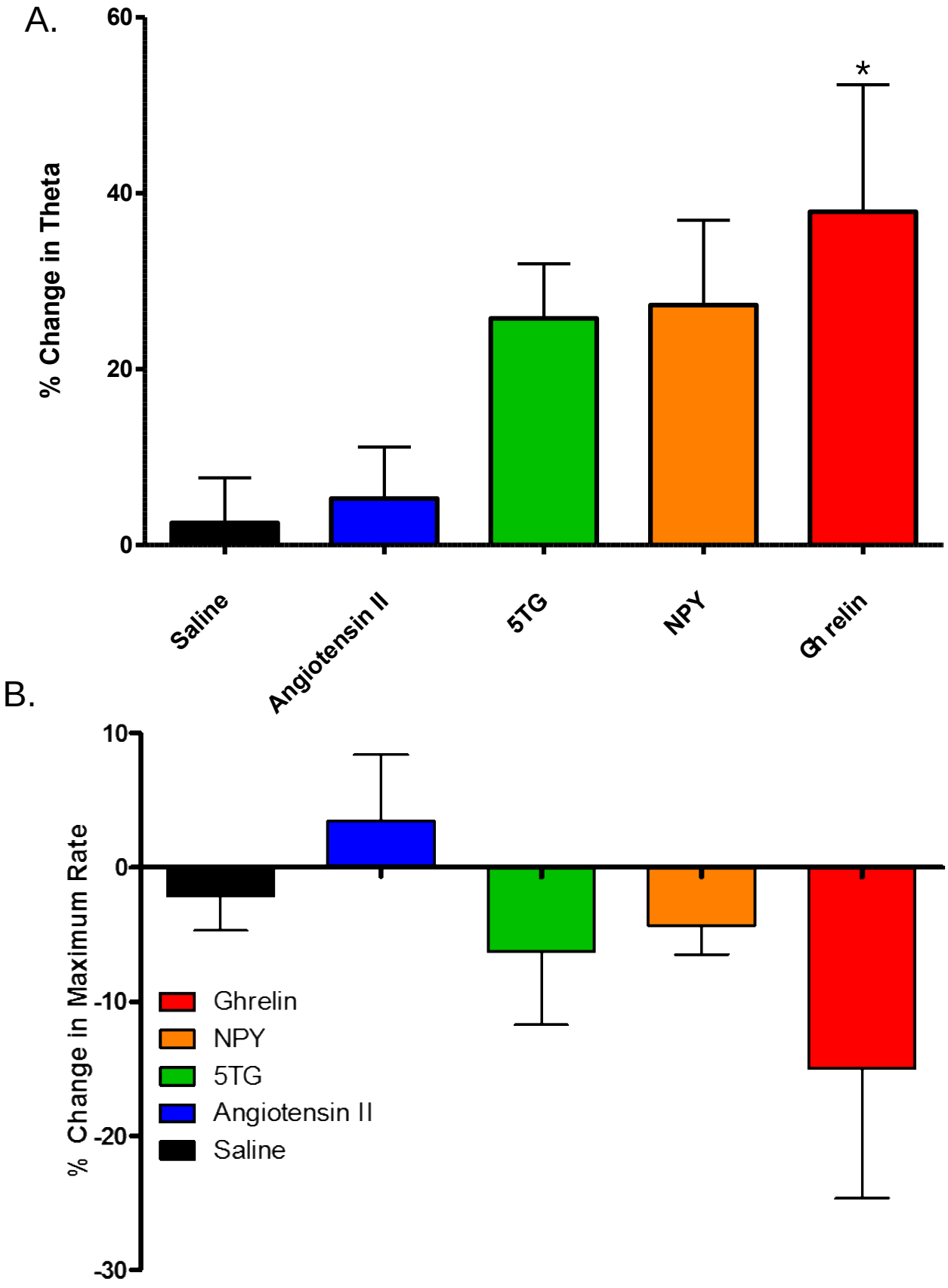

A.
60
40
20
0
% Change in Theta
*
Saline
Angiotensin II
5TG
NPY
Ghrelin
B.
10
0
-10
-20
-30
% Change in Maximum Rate
Ghrelin
NPY
5TG
Angiotensin II
Saline

Figure 7.

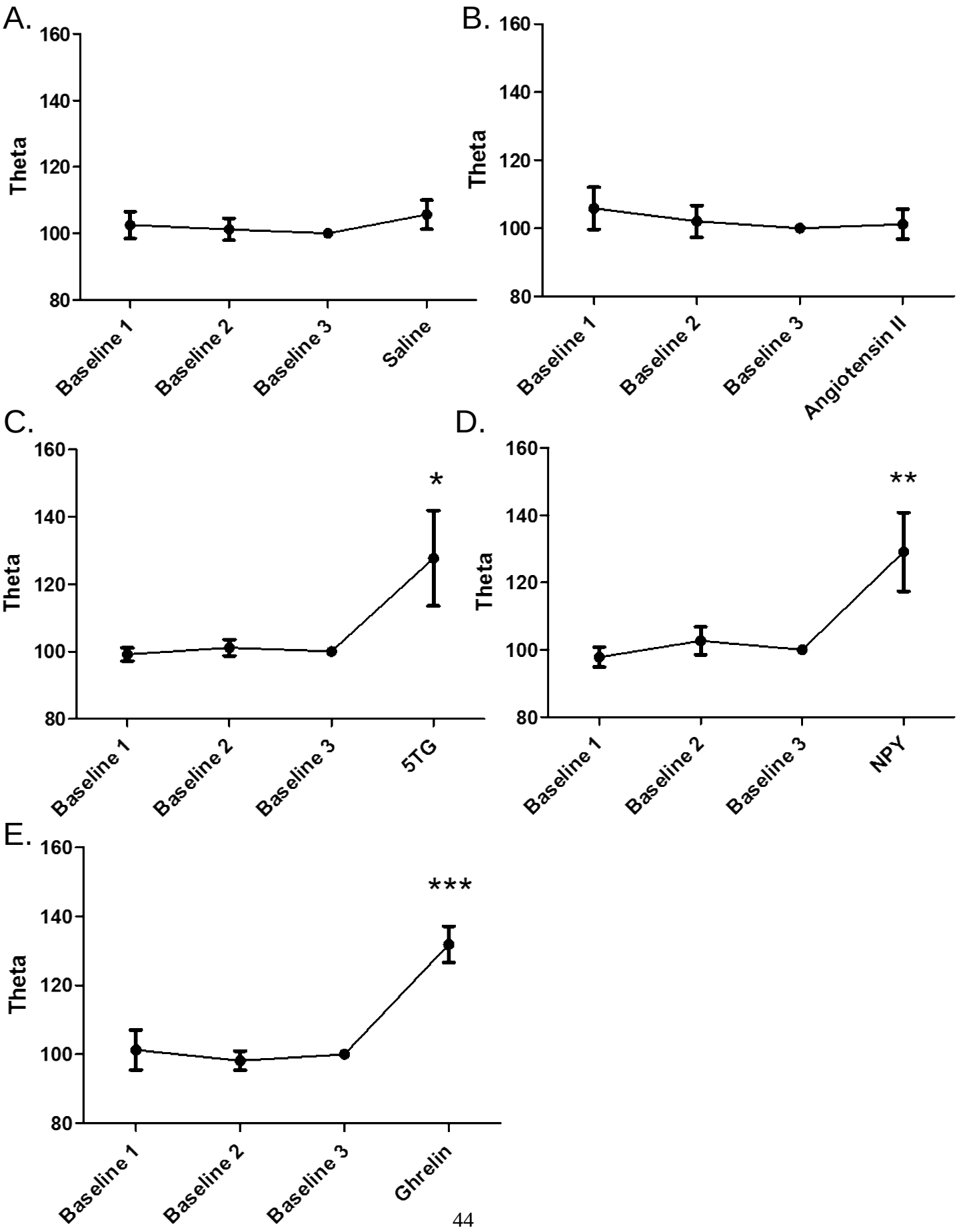

A.
Theta
160
140
120
100
80
Baseline 1
Baseline 2
Baseline 3
Saline
B.
Theta
160
140
120
100
80
Baseline 1
Baseline 2
Baseline 3
Angiotensin II
C.
Theta
160
140
120
100
80
*
Baseline 1
Baseline 2
Baseline 3
5TG
D.
Theta
160
140
120
100
80
**
Baseline 1
Baseline 2
Baseline 3
NPY
E.
Theta
160
140
120
100
80
***
Baseline 1
Baseline 2
Baseline 3
Ghrelin

Figure 8.

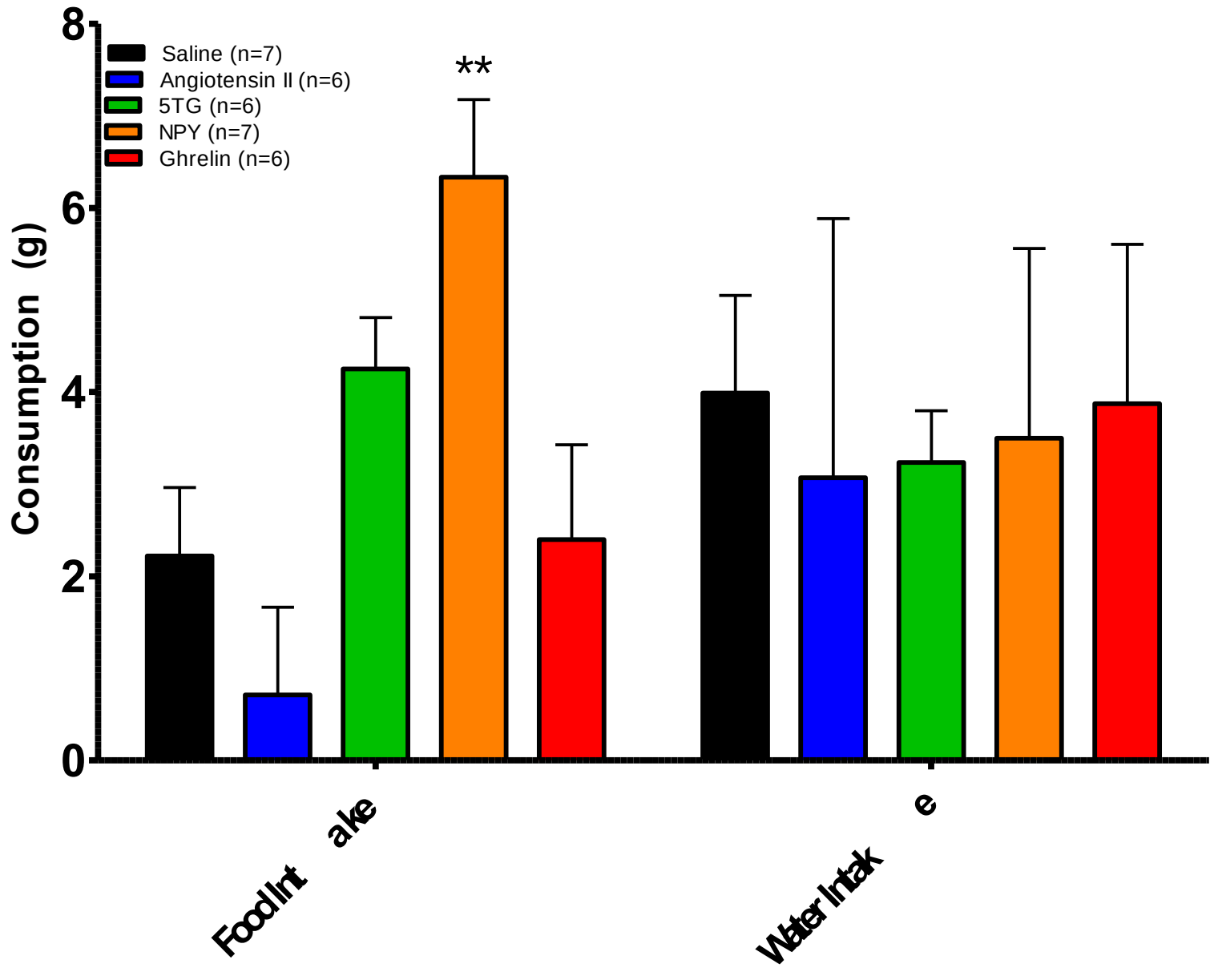

Saline (n=7)
Angiotensin II (n=6)
5TG (n=6)
NPY (n=7)
Ghrelin (n=6)
**
Consumption (g)
0
2
4
6
8
Food Intake
Water Intake

Figure 9.

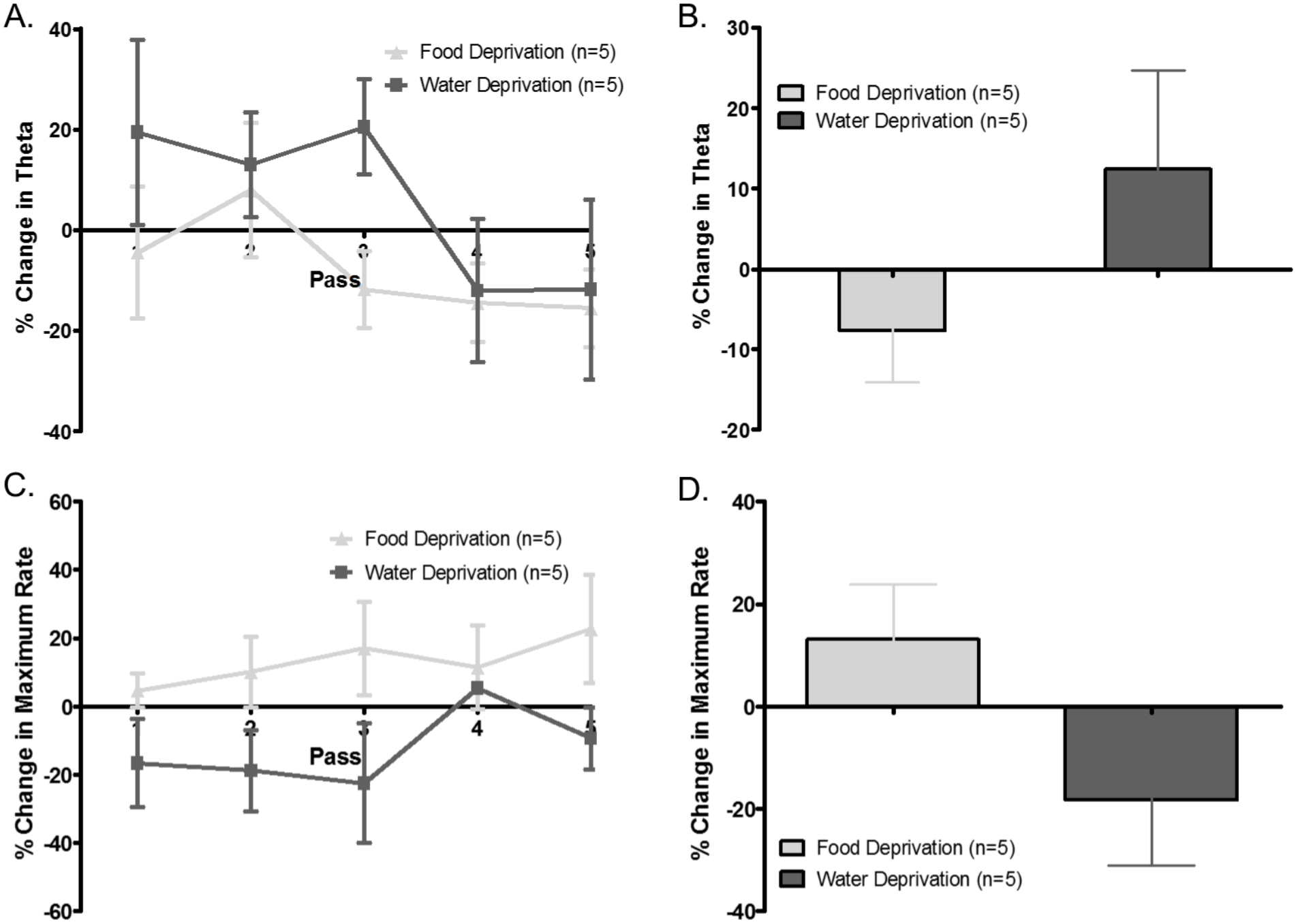

A.
Food Deprivation (n=5)
Water Deprivation (n=5)
% Change in Theta
Pass
B.
Food Deprivation (n=5)
Water Deprivation (n=5)
% Change in Theta
C.
Food Deprivation (n=5)
Water Deprivation (n=5)
% Change in Maximum Rate
Pass
D.
% Change in Maximum Rate
Food Deprivation (n=5)
Water Deprivation (n=5)

Figure 10.

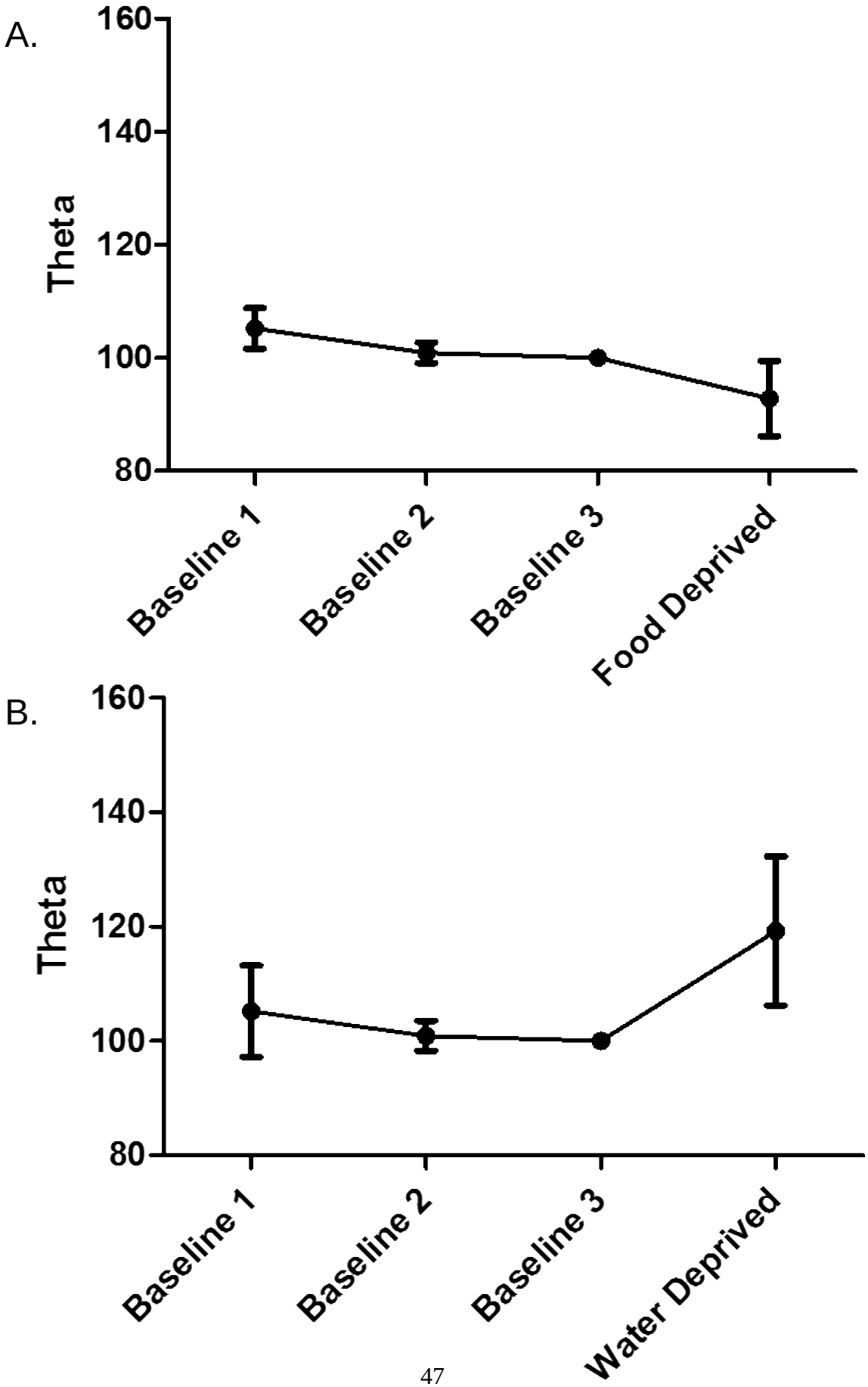

A.
160
140
120
100
80
Theta
Baseline 1
Baseline 2
Baseline 3
Food Deprived
B.
160
140
120
100
80
Theta
Baseline 1
Baseline 2
Baseline 3
Water Deprived

Table 1.

Drug Dosages

Drug	Dosage
Ghrelin	1 µg/µl
Neuropeptide Y	5 µg/µl
5-thio-d-glucose (5TG)	100 µg/µl
Angiotensin II	100 ng/µl

Table 2.

Saline vs Angiotensin II

Pass	Saline	Angiotensin II	Difference	95% CI of diff.
1.000	-7.091	-17.13	-10.04	-39.58 to 19.50
2.000	5.274	1.169	-4.105	-33.65 to 25.44
3.000	11.78	5.694	-6.086	-35.63 to 23.46
4.000	11.44	14.77	3.321	-26.22 to 32.86
5.000	-1.589	4.188	5.777	-23.77 to 35.32
6.000	12.65	12.81	0.1602	-29.38 to 29.70

Pass	Difference	t	P value	Summary
1.000	-10.04	1.201	P > 0.05	ns
2.000	-4.105	0.4911	P > 0.05	ns
3.000	-6.086	0.7281	P > 0.05	ns
4.000	3.321	0.3973	P > 0.05	ns
5.000	5.777	0.6911	P > 0.05	ns
6.000	0.1602	0.01916	P > 0.05	ns

Saline vs 5TG

Pass	Saline	5TG	Difference	95% CI of diff.
1.000	-7.091	6.961	14.05	-15.49 to 43.59
2.000	5.274	39.69	34.41	4.872 to 63.96
3.000	11.78	36.91	25.13	-4.414 to 54.67
4.000	11.44	24.11	12.67	-16.87 to 42.21
5.000	-1.589	25.34	26.93	-2.611 to 56.47
6.000	12.65	30.20	17.55	-11.99 to 47.09

Pass	Difference	t	P value	Summary
1.000	14.05	1.681	P > 0.05	ns
2.000	34.41	4.117	P<0.01	**
3.000	25.13	3.006	P < 0.05	*
4.000	12.67	1.516	P > 0.05	ns
5.000	26.93	3.222	P < 0.05	*
6.000	17.55	2.100	P > 0.05	ns

Saline vs NPY

Pass	Saline	NPY	Difference	95% CI of diff.
1.000	-7.091	18.29	25.38	-4.159 to 54.93
2.000	5.274	42.28	37.01	7.468 to 66.55
3.000	11.78	25.69	13.91	-15.64 to 43.45
4.000	11.44	30.63	19.18	-10.36 to 48.73
5.000	-1.589	39.37	40.96	11.42 to 70.50
6.000	12.65	33.56	20.91	-8.628 to 50.46

Pass	Difference	t	P value	Summary
1.000	25.38	3.037	P < 0.05	*
2.000	37.01	4.428	P<0.01	**
3.000	13.91	1.664	P > 0.05	ns
4.000	19.18	2.295	P > 0.05	ns
5.000	40.96	4.900	P<0.001	***
6.000	20.91	2.502	P > 0.05	ns

Saline vs Ghrelin

Pass	Saline	Ghrelin	Difference	95% CI of diff.
1.000	-7.091	26.64	33.73	4.190 to 63.28
2.000	5.274	47.78	42.51	12.97 to 72.05
3.000	11.78	46.93	35.15	5.602 to 64.69
4.000	11.44	53.94	42.50	12.96 to 72.04
5.000	-1.589	47.41	49.00	19.46 to 78.54
6.000	12.65	45.43	32.78	3.239 to 62.32

Pass	Difference	t	P value	Summary
1.000	33.73	4.036	P<0.01	**
2.000	42.51	5.085	P<0.001	***
3.000	35.15	4.204	P<0.01	**
4.000	42.50	5.084	P<0.001	***
5.000	49.00	5.862	P<0.001	***
6.000	32.78	3.922	P<0.01	**

Table 3.

Authors, Year	Stimulation Site	Food Restriction Paradigm	Effect on ICSS
Blundell & Herberg, 1968	LH	2 schedules, chronic and acute	Facilitation, far greater with chronic food restriction (FR)
Brady, Boren, Conrad, & Sidman, 1957	Caudate nucleus	1,4, 24, 48 hrs	Facilitation, varies with time of FR
Cabeza de Vaca & Carr, 1998	MFB	Chronic restriction until -20% body weight	Facilitation in all but nicotine condition
Carr & Wolinsky, 1993	LH—perifornical and non-perifornical	Chronic	Only perifornical LH sites showed facilitation
Frutiger, 1986	LH	0, 24, 48 hrs	Facilitation
Hodos & Valenstein, 1960	Septal nucleus	48 hr fast	Facilitation
Hoebel & Teitelbaum, 1962	Various hypothalamic sites	Stimulation/ablation of hypothalamic sites	Facilitation
Katz, Baldrighi, & Roth, 1978	Substantia nigra	48 hrs	Facilitation
Lin, Bruijnzeel, Schmidt, & Markou, 2002	MFB	>24 hrs, chronic mild stress procedure	No effect
Margules & Olds, 1962	LH	24 hrs	Facilitation, only in feeding sensitive sites
Moreau et al., 1992	VTA	> 24 hrs, chronic mild stress	Attenuation
Olds, 1958	Septal nucleus, LH, MH, Subcallosal cortex	Overnight fast	LH and medial hypothalamus: no effect Septal nuc and subcallosal cortex: facilitation
Reynolds, 1958	Ventromedial hypothalamus (VMH)	24 hrs and below	No effect

Figure 1. Rate frequency analysis of ICSS **A.** Graphical representation of the descending logarithmic scale of frequencies that are presented to an animal throughout a pass. **B.** Lever presses for stimulation made at each frequency by a representative animal. Theta is calculated by creating a vertical line through 20, 30, 40, 50, and 60% of maximum response rate in a pass and calculating the point at which that line crosses the x-axis. **C.** Lever presses for stimulation made at each frequency by a representative animal across five passes. Red triangles represent theta on each pass. **D.** Average theta value for a representative animal across days of rate frequency training. Error bars represent SEM.

Figure 2. Histological verification of placements. **A.** Representative image of coronal slice stained with cresyl violet showing placement of stimulating electrode in the VTA. **B.** Representative image of a coronal slice stained with cresyl violet showing cannula placement and ink in the lateral ventricle.

Figure 3. Food and water intake following ICV infusions. **A.** Total consumption of chow for one hour and thirty minutes following administration of drugs. There was a significant main effect of infusion on food intake ($F(4,57)= 24.22$, $p<0.001$). Animals that received NPY had significantly increased food intake versus saline (95% CI [-10.18, -5.413]). **B.** Total consumption of water for one hour and thirty minutes following administration of drugs. There was a significant main effect of infusion type on water intake ($F(4,56)= 3.313$, $p=0.0172$). Error bars represent SEM.

Figure 4. Latency to begin eating and drinking following ICV infusions. Latency to initiate (**A**) eating and (**B**) drinking were measured in a subset of animals (n=3) following ICV infusions. Time began as soon as animals were placed back into their home cage. If animals had not initiated eating or drinking after a period of 600 seconds, the timer was stopped. Error bars represent SEM.

Figure 5. Change in theta and maximum rate following infusions by pass. **A.** Percent change in theta from pre-treatment theta by pass. Graph shows pre-treatment (drug-free) passes as -1 and 0. Infusions were made immediately following pass 0. Color of asterisk corresponds to infusion that was significantly different from saline at each pass * $p<0.05$, ** $p<0.01$, *** $p<0.001$ **B.** Percent change in maximum rate of lever pressing from pre-treatment theta by pass. Graph shows pre-treatment (drug-free) passes as -1 and 0. Infusions were made immediately following pass 0. Error bars represent SEM.

Figure 6. Change in theta and maximum rate following infusions across passes. **A.** Percent change in theta from pre-treatment theta averaged across post-infusion passes. * represents $p<0.05$ **B.** Percent change in maximum rate of lever pressing from pre-treatment theta by averaged across post-infusion passes. Error bars represent SEM.

Figure 7. Normalized theta for baseline (untreated) days and post ICV infusions. Each animal's theta value was normalized to the baseline day before testing (Baseline 3) in order to account for individual variability of theta values across animals. **A.** Normalized theta values for baseline and post saline infusion (n=7). **B.** Normalized theta values for baseline and post angiotensin II

infusion (n=6). **C.** Normalized theta values for baseline and post 5TG infusion (n=7). Normalized theta after 5TG was significantly larger than baseline 3 (95% CI [-51.9, -3.506]). * represents $p<0.05$ **D.** Normalized theta values for baseline and post NPY infusion (n=6). Normalized theta after NPY was significantly larger than baseline 3 (95% CI [-50.55, -7.764]).** represents $p<0.01$ **E.** Normalized theta values for baseline and post ghrelin infusion (n=6). Normalized theta after ghrelin was significantly larger than baseline 3 (95% CI [-47.47, -16.33]). *** represents $p<0.001$ Error bars represent SEM.

Figure 8. Food and water intake following ICSS testing. A. Total consumption of chow and water for one hour following ICSS testing on the day of peptide infusions. Animals that received NPY had significantly increased food intake versus saline (95% CI [-7.028, -2.201]). ** represents $p<0.01$. Error bars represent SEM.

Figure 9. Change in theta and maximum rate following food and water deprivation. **A.** Percent change in theta from untreated days theta (calculated as the average theta for the preceding three days) by pass following 24-hour food or water deprivation. **B.** Percent change in maximum rate of lever pressing from untreated maximum rate by pass following 24-hour food or water deprivation. **C.** Percent change in theta from untreated days averaged across all six passes following 24-hour food or water deprivation. **D.** Percent change in maximum rate of lever pressing from untreated days averaged across all six passes following food or water deprivation. Error bars represent SEM.

Figure 10. Normalized theta during baseline (untreated) and post food and water deprivation. Each animal's theta value was normalized to the baseline day before testing (Baseline 3) in order

to account for individual variability of theta values across animals. **A.** Normalized theta values for baseline and ICSS following food deprivation (n=5). **B.** Normalized theta values for baseline and ICSS following water deprivation (n=5). Error bars represent SEM.

VIII. VITA

Education

University of Illinois at Chicago. Chicago, IL — 2014-present
PhD Program, Behavioral Neuroscience, Psychology Department

University of Michigan. Ann Arbor, MI — 2009-2013
B.S. in Biopsychology, Cognition and Neuroscience
Senior Thesis: *In-vivo* Identification of Dopamine Transmission During Reward Omission

Experience

Teaching Assistant, University of Illinois at Chicago — 2014-present
Classes Taught: Laboratory in Behavioral Neuroscience, Behavioral Neuroscience, Learning and Conditioning, Laboratory in Cognitive Neuroscience, Introduction to Research in Psychology

Research Assistant, University of Illinois at Chicago — 2014-present
Graduate Advisor: Dr. Mitch Roitman

Laboratory Manager and Technician, University of Michigan — 2012-2014
Advisor: Dr. Brandon Aragona & Dr. Terry Robinson

Summer Laboratory Technician, University of Illinois at Chicago — 2012
Advisor: Dr. Mitch Roitman

Undergraduate Research Assistant — 2010-2013
Advisor: Dr. Brandon Aragona

Publications

Singer, B. F., Guptaroy, B., Austin, C. J., Wohl, I., Lovic, V., **Seiler, J. L.,** Vaughan, R. A., Gnegy, M. E., Robinson, T. E., Aragona, B. J. (2016), Individual variation in incentive salience attribution and accumbens dopamine transporter expression and function. European Journal of Neuroscience, 43: 662–670. doi: 10.1111/ejn.13134

Porter-Stransky KA, **Seiler J.L.**, Mabrouk OS, Hamid A, Berke JD, Kennedy RT, & Aragona BJ. Tonic and phasic dopamine transmission in the nucleus accumbens during unexpected reward omission. (*in preparation*)

Porter-Stranksy, K.A., **Seiler, J.L.,** Day, J.J., and Aragona, B.J. (2013), Development of behavioral preferences for the optimal choice following unexpected reward omission is mediated by a reduction of D2-like receptor tone in the nucleus accumbens. European Journal of Neuroscience, 38: 2572-2588. doj: 10.1111/ejn.12253

Presentations

Seiler, J.L., Conway, S.M., and Roitman, M.F., Society for Neuroscience Annual Meeting, 2016, San Diego, CA. "Effects of pharmacologically induced physiological need states on reward processing through intracranial self-stimulation."

Singer, B. F., Guptaroy, B., Austin, C. J., Wohl, I., Lovic, V., **Seiler, J. L.,** Vaughan, R. A., Gnegy, M. E., Robinson, T. E., Aragona, B. J., Society for Neuroscience Annual Meeting, 2015. Chicago, IL. "Individual variation in incentive salience attribution and accumbens dopamine transporter expression and function."

Singer, B.F, Austin, C.J., Wohl, I., Guptaroy, B., Lovic, V., **Seiler, J.L.,** Bryan, M.A., Gnegy, M.E., Aragona, B.J. and Robinson, T.E., Society for Neuroscience Annual Meeting, 2014, Washington, DC. "Differential dopamine signaling in sign-tracker and goal-tracker rats following amphetamine."

Porter-Stransky, K.A., **Seiler, J.L.,** Mabrouk, O.S., Kennedy, R.T., and Aragona, B.J., Society for Neuroscience Annual Meeting, 2013, San Diego, CA. "Phasic and tonic dopamine transmission dynamics in the nucleus accumbens during unexpected reward omission."

Porter-Stransky, K.A., **Seiler, J.L,** Mabrouk, O.S., Jenkins, B., Kennedy, R.T., & Aragona, B.J. Catecholamines Gordon Research Conference, 2013, West Dover, VT. "Differential tonic and phasic dopamine transmission in the nucleus accumbens during unexpected reward omission."

Porter-Stransky, K.A., **Seiler, J.L.,** Mabrouk O.S., Kennedy, R.T., and Aragona, B.J., Dopamine Conference, 2013, Alghero, Sassari, Italy. "Dopamine transmission dynamics in the nucleus accumbens during unexpected reward omission."

Seiler, J.L. Department of Psychology Honors Research Symposium, 2013, University of Michigan, Ann Arbor, MI. "*In-vivo* identification of dopamine transmission during reward omission."

Porter-Stransky, K.A., **Seiler, J.L.,** Klinger, M.C., and Aragona, B.J., Society for Neuroscience Annual Meeting, 2012, New Orleans, LA. "Disrupting D2, but not D1, tone within the nucleus accumbens core impacts the rapid establishment of behavioral preferences resulting from unexpected reward omission."

Training/Skills

Training and mentorship of undergraduate research assistants
Experimental skills:

- Optical manipulation of neuronal circuits (optogenetics)
- Detection of *in vivo* dopamine release using *fast-scan cyclic voltammetry* (FSCV)
- High efficiency detection of extracellular molecules using *in vivo* microdialysis
- Stereotaxic surgeries
- Immunohistochemistry
- Operant behavioral conditioning
- Vaginal lavaging and identification of estrous stage in female rats to monitor cycle

Software proficiency: SPSS, GraphPad, Microsoft Excel, Tarheel CV, MedPC, BehaviorTracker, MiniAnalysis, Arduino, basic MATLAB
Languages: English, conversational Spanish

Awards

LAS Travel Award	2014 & 2016
University Honors	2010

Affiliations/Memberships

Society for Neuroscience	2012-present
Expanding Your Horizons (EYH)	2016-present
Delta Phi Epsilon Sorority	2009-2013
Females Excelling More in Math, Engineering, and Sciences	2012-2013
Order of Omega	2011-2013