Challenges to Body Fluid Homeostasis Recruit Mesolimbic Dopamine Signaling

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THESIS

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AUTHORSHIP STATEMENT

The following dissertation contains data that have not yet been published.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

LIST OF FIGURES

LIST OF FIGURES (continued)

PAGE

LIST OF TABLES

LIST OF ABBREVIATIONS

HSD2	11-beta-hydroxysteriod dehydrogenase type 2
ANOVA	analysis of variance
AP	anterior-posterior
AQP5	Aquaporin-5
BLA	basolateral amygdala
DOCA	deoxycorticosterone acetate
DAT	dopamine reuptake transporter
DV	dorsal-ventral
FoxP2	forkhead box protein 2
FSCV	fast-scan cyclic voltammetry
g	grams
Hz	Hertz
hr	hour
PBel-inner	inner subdivision of the external lateral parabrachial nucleus
IP	Intraperitoneal
kg	kilograms
L	liters
LiCl	lithium chloride
LC	locus coerelus
ML	medial-lateral
MSNs	medium spiny neurons
MeV	mesencephalic trigeminal nucleus
μA	microamps
μM	micrometers
mg	milligrams
mL	milliliter
mm	millimeter
mmol	millimol
mV	millivolts
MR	mineralocorticoid receptor
M	molar
nM	nanomols
NIH	National Institute of Health
NAc	nucleus accumbens
NTS	nucleus of the solitary tract
Phox2b	paired-like homeobox 2b

PBN	parabrachial nucleus
PSTN	parasubthalamic nucleus
KCl	potassium chloride
pH	potential of hydrogen
pre-LC	pre-locus coeruleus
RAAS	renin-angiotensin-aldosterone system
s	seconds
Sgk	serum and glucocorticoid-regulated kinase
Ag/Agcl	silver/silver chloride
NaCl	sodium chloride
SEM	standard error of the mean
SC	subcutaneous
VTA	ventral tegmental area
VGLUT2	vesicular glutamate transporter 2
V	volts

SUMMARY

The internal environment of a living organism must remain stable in order to ensure optimal performance and ultimately survival. Maintaining equilibrium, or homeostasis, of the body requires regulation of a variety of tightly controlled physiological processes and adaptive behaviors. Motivated behaviors, for example, arise during physiological need to defend homeostasis. The neural systems which generate motivated behaviors must, therefore, be in tune to physiological state. A proposed neurobiological substrate for the generation of motivated behaviors is mesolimbic dopamine signaling. However, the modulation of dopamine signaling by physiological state remains underexplored. This thesis aims to shed light on the neural circuitry underlying motivated behavior by exploring the impact of physiological state on mesolimbic dopamine signaling.

I have elected to manipulate physiological state through two challenges of body fluid homeostasis, sodium deprivation and water restriction. The robust motivated behaviors that these homeostatic perturbations induce, sodium appetite and thirst, represent two opportunities to study the influence of homeostasis on dopamine signaling. Sodium appetite is particularly well-suited for my investigation because sodium depletion drastically transforms the behavior directed at hypertonic sodium chloride (NaCl), a stimulus that is avoided by rats under replete conditions but avidly consumed when the physiological need for it arises. Furthermore, sodium appetite is an incredibly stimulus-specific behavior. The gustatory system permits the discrimination of NaCl from other salts like KCl, which differ by a single ion. The taste-specificity of sodium appetite allows for the selective consumption of NaCl, the physiologically required stimulus, by sodium deplete rats. Manipulations of body fluid

homeostasis powerfully illustrate how physiological state and taste modulate the value of a stimulus and behaviors directed towards it. It is likely that, similar to the motivated behaviors consequent of sodium and water deprivation, dopamine signaling evoked by sodium or water stimuli will also be state and taste-dependent. However, how circuits that drive motivated behavior are recruited by sodium appetite and thirst is not well understood.

My dissertation aims to analyze real-time dopamine responses evoked by sodium or water stimuli and the modulation of these responses by physiological state (Chapter II) and taste (Chapter III). I go on to use immunohistochemistry and tracing techniques to identify sodium deprivation-responsive inputs to the VTA (Chapters IV and V) that may modulate the excitability of mesolimbic dopamine neurons and consequent dopamine release. The two projections that I identify represent components of a circuit that may drive state-specific dopamine signaling (as demonstrated in Chapter II) and the motivated behaviors observed during sodium appetite. Using sodium appetite as a platform to expand our understanding of the neurobiological basis of motivated behaviors is promising for the development of therapeutics to treat diseases rooted in maladaptive motivated behaviors such as obesity and drug addiction.

Chapter I

Introduction

A. The motivation behind studying the neural underpinnings of motivated behavior

The internal environment of a living organism must remain stable in order to ensure optimal performance and ultimately survival. Maintaining equilibrium, or homeostasis, of the body requires active regulation of a variety of tightly controlled physiological processes (i.e. energy balance, body fluid composition). In order to regulate these processes, an organism must be able to identify, approach and consume specific stimuli that restore disruptions of homeostasis. Innate drives exist within living organisms to ensure that the necessary behavioral output defends homeostasis. For example, in a state of negative energy balance, there exists an innate drive, or motivation, to approach and consume calories. In this way, motivation is an adaptive and protective biopsychological process that serves to ensure survival.

Evolution has ensured that ingesta necessary for maintaining homeostasis are hedonically rewarding (Stellar, 1980). While rewarding in nature, consumed stimuli are not necessarily beneficial for the health and well-being of the organism. Rewarding stimuli include fats, sugars, salt and drugs of abuse which, when overconsumed, lead to the development of diseases and disorders like obesity and drug addiction. Without question, these stimuli vary in nature (natural and artificial) and the etiology of the public health issues that they potentiate is diverse. As such, specific and tailored investigations are necessary. However, motivating stimuli all come to acquire control through behavioral reinforcement- perhaps through similar mechanisms and overlapping neural substrates and circuits. Expanding our general understanding of the neurobiological basis of motivated behavior represents a gap in our

current knowledge of the brain and a potential avenue to interfere with the progression of seemingly unrelated diseases that share an aberrant motivation component.

B. Perturbations of body fluid homeostasis give rise to sodium appetite and thirst, ideal entry points to studying motivated behavior

The generation of motivation arises from perturbations of homeostasis which require correction through goal-directed behaviors. It is likely that the generation of all motivated behaviors share neural substrates and circuits. Investigation of the intricacies of motivation's neural underpinnings requires an ideal entry point- a homeostatic perturbation from which the nuances of motivated behavior and underlying circuitry can be studied. I believe that the neurobiology of motivation is best explored through disruption of body fluid homeostasis for reasons described throughout this thesis.

Body fluid regulation is a tightly controlled homeostatic process that is necessary for survival. The internal environment of the body is primarily fluid (blood plasma, interstitial fluid, intracellular fluid) that is regulated in its composition, temperature, pressure, and pH. Body fluids are distributed between two defined compartments, the intracellular and extracellular (comprised of interstitial and intravascular) compartments. Continuous exchange of fluids between these two compartments is necessary to keep body fluid concentration within a narrow physiological range and volume at an appropriate ratio (for humans, ~23:19 L for intracellular vs extracellular compartments) (Bianchetti et al., 2009). As water freely moves across plasma membranes and between compartments, osmotic and volume changes in intra and extracellular compartments is primarily regulated by changes in the concentration of body

fluid solutes. The dominant solute in extracellular fluid is sodium. Sodium concentration must be maintained within a narrow range for optimal body fluid osmolality (for humans, within the range of 135-146 mmol/L (Hall and Guyton, 2011; Bianchetti et al., 2009). An abundance or insufficiency of extracellular sodium causes disruptions on a cellular level, with cells shrinking or swelling, respectively. On a larger scale, body fluid homeostasis ensures the optimal functioning of a variety of physiological processes including cardiac output, regulation of blood pressure, capillary exchange, renal excretion of waste and ion conductance across membranes [see (Daniels and Fluharty, 2004) for review]. Regulation of internal sodium levels is, therefore, critical for survival.

Maintenance of osmolality and body fluid volume requires regulated intake and excretion of both sodium and water. With the exception of marine life, which possesses the ability to passively intake sodium and chloride through their skin, animals must obtain sodium and water through ingestion (Denton, 1965). The process of ingestion is complex, as salt and water need to be identified and actively consumed. Importantly, drives to seek and consume required stimuli (salt or water) exist to direct behavior in situations of need. These drive states are termed sodium appetite and thirst. Both thirst and sodium appetite produce robust, innate and specific motivated behaviors that can be modelled in a laboratory setting with a variety of experimental approaches that utilize well-investigated hormone systems. It is known that the drive states of sodium appetite and thirst are under hormonal control and are guided by sensory experience (Richter, 1947; Schulkin, 1992; Leib et al., 2016). However, how circuits related to motivated behavior are recruited by sodium appetite and thirst are not well understood and is the focus of my dissertation.

1. The generation of sodium appetite and thirst are under hormonal control

Decades of research have contributed to our understanding of the systems that stimulate intake of sodium and water in the service of defending body fluid homeostasis. An imbalance of water and sodium levels results in deviations from ideal body fluid osmolality and volume. The body detects and corrects for body fluid perturbations through various peripheral and central mechanism. Although the biological responses to sodium and water deficit involve multiple organ systems of the body working in concert (brain, liver, adrenals, lung), the responses are well characterized.

With respect to osmolality, increases in plasma osmolality levels are more common than decreases, and result from a variety of physiological disturbances including dehydration, excessive sweating, or extreme diarrhea or vomiting. Increases in osmolality are also observed following excessive intake of sodium, which is a common occurrence in for humans in modern day society. The concept of specialized cells (osmosensors) monitoring extracellular fluid osmolality was first proposed by Verney in 1947 (Verney, 1947). Osmoreceptors have since been identified in the central nervous system, primarily in the supraoptic nucleus of the hypothalamus (Bourque et al., 2002) as well as areas of the brain devoid of the blood-brain barrier including the subfornical organ, median preoptic nucleus, organum vasculosum of the lamina terminalis and the area postrema (Anderson, 1977; Andersson and McCann, 1955). Osmoreceptors have also been identified in the periphery on afferent nerve endings around the hepatic vessels, kidneys and bowels (Arsenijevic and Baertschi, 1985; Choi-Kwon and Baertschi, 1991). Activation of either central or peripheral osmoreceptors triggers a variety of

downstream hormonal responses including the release of vasopressin, angiotensin-II (Ang-II), oxytocin and atrial natriuretic peptide (Antunes-Rodrigues et al., 2004; Bourque et al., 2002; Verney, 1947). All of these hormones act in unique ways (that ultimately lead to the conservation or excretion of sodium or water) in the service of restoring body fluid homeostasis.

Compensatory physiological responses are also generated when circulating body fluid volume levels are disrupted (i.e. cases of blood loss). To ensure proper tissue perfusion, blood pressure must be maintained through balance of extracellular sodium and water. The volume of circulating fluids is detected by cells called baroreceptors, found primarily around the heart. In multiple investigated species, these cells respond to changes in blood volume that are between 10-20% (Share, 1988). Like osmoreceptors, baroreceptors trigger a release or inhibition of central hormones (Jhamandas and Renaud, 1986; Johnson et al., 1970; Shade and Share, 1975; Kimura et al., 1994), which regulate body fluid homeostasis through the conservation or excretion of salt or water. The individual mechanisms of action of hormones which regulate homeostasis in this way falls outside of the scope of this dissertation. However, yet another component of body fluid regulation beyond the internal mechanism described above is the generation of intake behaviors. The neurobiological basis for the generation of sodium and water- directed behavior in a state of need, termed sodium and appetite and thirst, is a focus of my work.

The renin-angiotensin- aldosterone system (RAAS) that is activated downstream of osmoreceptor and baroreceptor activation is one hormone system implicated in driving motivated behavior in conditions of both sodium and water deprivation (Fitzsimons, 1998).

Renin, produced in the kidney, is an acid protease which facilitates the production of angiotensin I from angiotensinogen. Angiotensin I is then converted to Angiotensin II (Ang-II) by the enzyme angiotensin-converting enzyme. Ang-II is a powerful stimulator of both thirst and sodium appetite across many species of animals (Fitzsimons, 1998). The majority of the work surrounding Ang-II-induced water or salt intake has been conducted in the rat. Central injections of Ang-II cause water replete rats to immediately (usually within 1 min of injection) drink significant amounts of water (Epstein et al., 1970; Simpson et al., 1978). Rats will also lever press more than 60 times for a single 0.1 mL reward of water (Rolls et al., 1972). Central brain sites mediating the water-intake stimulatory effects of Ang-II have been studied extensively and include the circumventricular organs like the subfornical organ and the organ vasculosum of the lateral terminalis [see (Daniels and Fluharty, 2004) for review].

At higher doses, central Ang-II elicits sodium intake (Buggy and Fisher, 1974). This occurs both directly (Avrith, DB, Wiselka, MJ, Fitzsimons, 1980) and secondary to stimulating release of the mineralocorticoid aldosterone from the adrenal cortex (Fitzsimons, 1998). Aldosterone release following RAAS system activation works to conserve sodium in the service of restoring body fluid homeostasis. It does so by increasing uptake of sodium in the tubular lumen of kidney through increased expression of sodium channels (Fitzsimons, 1998). In addition to "activational" effects on body fluid homeostasis, aldosterone has "organizational" effects (Epstein, 1982). Aldosterone stimulates sodium intake through mineralocorticoid receptors (MR), first identified in the hippocampus, amygdala and septum (Birmingham et al., 1979; McEwen et al., 1986). It was later found that aldosterone stimulates long and short term body fluid homeostasis through two central mechanisms. First, aldosterone acts as a transcription

factor to increase expression of the protein Sgk (serum and glucocorticoid- regulated kinase). Increased Sgk supports increased phosphorylation of the ubiquitin ligase Nedd4-2, ultimately preventing internalization and disposal of sodium receptors at the cell surface (Izzo et al., 2008). Aldosterone also causes nongenomic and rapid changes in ligand gated ion channels at the surface of neurons, similarly to in kidney cells, which mediate neuronal firing (Fluharty, SJ and Sakai, 1996; Sakai et al., 2000). Much of the work investigating sodium appetite has revealed that while angiotensin and aldosterone both play a role, it is the synergistic action of the two hormones which is biologically relevant for the stimulation of sodium intake [see (Daniels and Fluharty, 2004; Stellar, 1993) for review]. The mechanism by which aldosterone induces the motivated behavior of sodium appetite is unknown and explored in this thesis.

In an experimental setting, stimulation of the RAAS system, and therefore aldosterone secretion, can be achieved through multiple approaches. Dietary sodium depletion over the course of days to weeks is certainly the most naturalistic method of chronically activating the RAAS system (Geerling and Loewy, 2006b; Wagman, 1963; Huang and Yan, 2008; Stricker et al., 1991). More invasive chronic manipulations include adrenalectomy, ligation of the vena cava, and hemorrhage (Grossman, 1990). In order to avoid the neural changes that accompany long term sodium deprivation (Roitman et al., 2002; Lucas et al., 2000), acute manipulations of sodium balance are utilized. Pharmacological agents that induce a sodium appetite take advantage of the 70 years of knowledge that we have accumulated to directly target hormonal systems underlying body fluid homeostasis. They include, but are not limited to, administration of exogenous angiotensin (Fitzsimons, 1998), hypertonic polyethylene glycol which sequesters plasma fluid into interstitial space (Stricker, 1981), aldosterone or its synthetic precursor

deoxycorticosterone acetate (DOCA) (Morris et al., 2006). Another acute method that has been validated as a potent driver of sodium appetite is use of furosemide. Furosemide is a loop diuretic which acts in the Loop of Henle within nephrons of the kidney to prevent reabsorption of sodium, leading to hypovolemia and stimulation of the classical RAAS pathway (Fitzsimons, 1998) – a hormonal cascade which includes the release of aldosterone (Vonder and Carlson; Ames et al., 2016; Rowland and Morian, 1999). The reliable and rapid induction of a sodium appetite by furosemide along with its aldosterone-releasing effects have contributed to its selection as the method of sodium depletion used throughout my dissertation studies. A circuit that I propose through my work here begins with central aldosterone action and ends with forebrain dopamine release.

2. Sodium appetite and thirst produce state dependent motivated behaviors

Physiological state dynamically modulates the value of stimuli such that behaviors directed towards solutions which restore body fluid homeostasis of the animal are drastically transformed in instances of need. This is powerfully illustrated by the value of sodium following the induction of a sodium appetite. Sodium appetite is a concept that is perhaps hard for humans to conceptualize, given the abundance of salt in our diets. However, powerful sodium appetites have been reported in humans with the either adrenal insufficiency, which causes the inability to retain sodium, or those who voluntarily restrict sodium consumption [see (Daniels and Fluharty, 2004) for review]. Examples of sodium appetite are more commonly observed among animals in the wild, where sodium is harder to obtain. Sodium is a valuable resource that is sought out by species ranging from elephants to crickets by various extreme means

including pilgrimages to salt mine caves and cannibalism (Bowell et al., 1996; Simpson et al., 2006).

In a laboratory setting, powerful motivated behavior can be observed in lab rats following experimental induction of a sodium appetite (Richter, 1936). Homeostatically balanced, sodium replete, rats will avoid concentrated sodium solutions. Following sodium depletion, identical concentrations of sodium solutions are avidly consumed (Handal, 1965; Nachman, 1962). Prior experience with sodium solutions or deprivation is not needed for animals to approach and consume solutions of sodium that are normally avoided, indicating that the appetite for sodium is innate (Handal, 1965; Epstein and Stellar, 1955). The transformation of behavior by sodium depletion is accompanied by a shift in sodium's palatability. Sodium deplete rats find hypertonic NaCl more palatable than replete animals (Berridge et al., 1984). Other drive states used to study motivation lack this unique property of sodium appetite. For example, neither hungry nor sated rats show avoidance or aversive responses to food unless some negative experience [i.e. pairing with visceral illness via lithium chloride (LiCl)] is associated with intake. In the case of sodium appetite, the hedonic value of concentrated sodium is changed merely by altering the physiological state of the animal. Sodium appetite provides a powerful example of how changes in physiological state can alter the value of a stimulus and the behavior directed towards it.

Sodium intake and the behavior directed towards sodium is intentional and motivated, not a consequence of a reflexive motor response. Sodium deplete rats will work harder for sodium by increasing the rate of lever pressing for sodium solutions (Quartermain et al., 1967; Krieckhaus and Wolf, 1968; Clark and Bernstein, 2006). The performance rate of sodium

deplete animals scales to the amount of deprivation, with rats receiving higher doses of sodium-depleting formalin or those maintained on a sodium-free diet for longer periods of time working harder (Quartermain et al., 1967; Wagman, 1963). Another example of rats working to the extent that they satisfy their deficit can be observed in rats that are lever pressing under a progressive ratio task. In this instance, the amount of effort that an animal is willing to invest in obtaining a fixed amount of saline increases with sodium deprivation until the point (breakpoint) at which they have consumed the amount of sodium necessary to restore their deficit (Starr and Rowland, 2006). Perhaps a more naturalistic experimental measure of reward directed behavior than lever pressing is the speed at which animals will run towards a reward. Indeed, sodium depletion increases running speed towards a sodium solution (Zhang et al., 1984; Schulkin et al., 1985). Sodium seeking in a deplete state is intentional, as cues associated with sodium availability also come to elicit approach behaviors (Robinson and Berridge, 2013; Cone et al., 2016). Sodium depletion clearly provides a powerful example of the influence of physiological state on the generation of motivated behavior.

While less dramatic than in the case of sodium appetite, thirst also changes behaviors directed towards water rewards. In the rat, the level of water deprivation is reflected in both the amount of water it will voluntarily intake and the rate of water consumption (Stellar and Hill). If adequately water deprived (21 hours), rats deprived of water can be trained to lever press for a single drop of water. Once lever-pressing is acquired, the rate of pressing scales to the level of water deprivation (Hughes et al., 1994). Similarly, rats will increase lever pressing under a progressive ratio reinforcement schedule for a single 0.1 mL water reward when water deprived (Rolls et al., 1972). It has recently been shown that thirsty animals will optogenetically

self-stimulate water responsive taste cells, which causes licking behavior in the absence of water. Licking behavior driven by receptor stimulation was not induced in food- or sodium-deplete rats, indicating that the drive for water is state dependent (Zocchi et al., 2017).

Hormonal manipulation of thirst, via angiotensin II injection, recapitulates motivation-inducing effects of water restriction (Rolls et al., 1972). Non-human primate work has extended these findings, demonstrating that blood osmolality level is proportional to intake (Wood et al., 1982) as well as how long and hard monkeys will work for a water reward (Yamada et al., 2010). In addition to operant work, increased motivation for water in a thirsty state has also been explored by testing maze running speed or animal's tolerance for negative experiences to receive water rewards. Water deprived rats will run faster to receive water rewards (Khavari and Russell, 1966). They will also endure higher levels of for shock (Warden, 1931) and will tolerate water adulterated with the bitter taste of quinine (Rolls et al., 1972). Humans report changes in the hedonic value of a water stimulus when thirsty; reporting that water tastes better, that they want it more and will work harder for it (Rolls and Rolls, 1982). Ingestive motivated behavior directed at both sodium and water during sodium appetite and thirst clearly state dependent. The central mechanism which drives the consumption of sodium and water during challenges of body fluid homoeostasis likely involve the forebrain, as chronic decerebrate rats with severed hindbrain-forebrain connections fail to properly increase their intake of sodium or water in a sodium deplete or dehydrated state, respectively (Grill et al., 1986; Grill and Miselis, 1981). While forebrain signaling is implicated in driving sodium appetite and thirst, communication of central sodium appetite and thirst-responsive sites with circuitry

that drives motivation and therefore the consumption of sodium or water remains unknown and will be explored in this dissertation.

3. Sodium appetite and thirst produce motivated behaviors that are stimulus specific

In order to restore homeostasis, physiologically required stimuli must first be detected. In the case of sodium appetite, sodium chloride (NaCl) must be identified before it can be consumed. However, salts often differ by a single ion (i.e. NaCl, LiCl, KCl). A highly refined sensory system must therefore exist for the detection of NaCl, specifically, during sodium appetite. Taste transduction mechanisms for NaCl have been extensively studied (Geran and Spector, 2004; Breslin et al., 1993; Spector and Grill, 1992; Spector et al., 1996), in part because of the incredible ability of animals to discriminate various salts by taste. Sodium deplete animals will selectively consume NaCl over other non-sodium salts like potassium chloride (KCl) (Nachman, 1962), suggesting that the taste system is refined enough to detect chemical differences on a single ion-level. Importantly for purposes of rapid restoration of body fluid homeostasis, prior experience with NaCl and learning is not necessary for this to occur (Handal, 1965; Epstein and Stellar, 1955).

The taste of sodium is detected by two distinct mechanisms- a transcellular and paracellular pathway. The transcellular pathway consists of epithelial sodium channels which are blocked by oral application of the drug amiloride and are thus referred to as amiloride-sensitive ion channels (Doolin and Gilbertson, 1996). The paracellular pathway consists of submucosal receptor sites which are unaffected by oral application of amiloride but are blocked by the large anion salt sodium gluconate (Elliott and Simon, 1990). Electrophysiological and behavioral

studies utilizing lingual application of amiloride and sodium gluconate have provided evidence that the transcellular pathway is the only transduction pathway that is both necessary and sufficient for the normal detection of sodium (Geran and Spector, 2000b, 2000a). Importantly, blockade of amiloride-sensitive ion channels attenuates the expression of a sodium appetite, indicating that this cation-selective mechanism is important for sodium-directed behaviors under conditions of physiological need (Bernstein and Hennessy, 1987; Roitman and Bernstein, 1999; Spector et al., 1996). In addition to supporting the consumption of NaCl during sodium appetite, amiloride sensitive ion channels are necessary for the discrimination of salts by rats. Amiloride-sensitive ion channels are selective for the cations Na^+ and Li^+, as both cations (Na^+ and Li^+) are small enough to permeate the ion channel to activate the receptor (Heck et al., 1984; Avenet and Lindemann, 1988; Geran and Spector, 2004). Consequently, the taste of NaCl and LiCl salts are transduced through this pathway, while the taste of KCl is not (Kellenberger et al., 1999; Heck et al., 1984). Amiloride prevents the selective consumption of NaCl over non-sodium salts like KCl that is reliably observed in sodium-deplete animals, indicating that amiloride-sensitive ion channels of the tongue are necessary for the discrimination and selective consumption of sodium-containing salts (Spector et al., 1996).

Three cranial nerves (vagus, glossopharyngeal and facial nerve) that innervate the tongue carry gustatory information to the central gustatory neuraxis. While the majority of taste receptors are innervated by the glossopharyngeal nerve (Miller, 1977), taste coding of salt is dependent on a branch of the facial nerve called the chorda tympani nerve (Breslin et al., 1993, 1995; St John et al., 1997; Spector and Grill, 1992). Electrophysiological recordings of the chorda tympani nerve demonstrate finely tuned responses to NaCl (Pfaffmann, 1955; Beidler,

1953; Frank et al., 1983) and surgical transection of the chorda tympani nerve interferes with the specificity of a sodium appetite in sodium-deplete animals (Breslin et al., 1993, 1995; St John et al., 1997; Spector and Grill, 1992). The chorda tympani nerve transmits sodium taste coding from sodium taste receptors of the anterior portion of the tongue to the rostral NTS for central processing (Hamilton and Norgren, 1984).

From the NTS, taste information is communicated to the medial and waist subdivisions of the parabrachial nucleus (PBN) (Norgren, 1978). From the PBN, gustatory signals are sent to forebrain sites through divergent dorsal and a ventral pathways, proposed in 1977 by Pfaffmann and colleagues [(Pfaffmann et al., 1977) but also see (Schulkin, 1991) for review]. The necessity of forebrain signaling in sodium appetite was later demonstrated by work with chronic supracollicular decerebrate rats. These animals have severed connections between the rostral NTS, where taste information is first processed centrally, and forebrain nuclei. While these animals maintain some intact responses to sodium deprivation, they do not display a sodium appetite or display oral-facial patterns indicative of palatability shifts following sodium depletion (Grill et al., 1986). It is likely that the forebrain nuclei implicated in the gustatory processing of NaCl reside within the ventral taste pathway, proposed by Pfaffmann to direct taste-guided motivation and affect, as lesions of nuclei of ventral gustatory nuclei (i.e. lateral hypothalamus and amygdala), impair or abolish NaCl intake in sodium deplete rats (Wolf and Quartermain, 1967; Cox et al., 1978; Pfaffmann et al., 1977). Conversely, lesions of the gustatory thalamus or insular cortex (nuclei of the dorsal taste pathway nuclei) do not completely impair rat's ability to identify and consume NaCl (Wolf et al., 1970; Flynn et al., 1991).

Gustation also provides a highly refined system for the detection water which may guide water-directed behavior during thirst (Zocchi et al., 2017). Despite the misconception that water is intrinsically tasteless, water stimuli are recognized at every level of the gustatory neuraxis. Water is a gustatory stimulus, and arguably a “basic taste modality” (Rosen et al., 2010) in that it: 1. has a dedicated peripheral transduction mechanism; 2. generates activity in gustatory afferents of the oral cavity; and 3. evokes central gustatory neuron activity. The mechanism by which gustatory afferents are activated by water stimuli has been largely overlooked, in part due to the focus placed on understanding responses to classic taste stimuli (salty, sour, sweet, bitter and umami). However, a few peripheral transduction mechanisms (criteria 1 for basic taste modalities) have been proposed. Gilbertson and colleagues have hypothesized that taste receptor cells act as osmotic sensors. The transduction pathway is believed to begin with the exposure to water causing swelling of taste receptor cells via influx through water channels called aquaporins (Watson et al.), specifically Aquaporin-5 (AQP5) on the apical membrane. Consequent swelling leads to activation of volume regulated anion channels and downstream depolarization of the taste receptor cell and neurotransmitter release (Gilbertson et al., 2006; Gilbertson, 2002). Very recently, an alternative peripheral transduction mechanism has been revealed in acid-sensing taste receptors previously thought to transduce sour tastes. Acid-sensing taste receptors were found to be necessary for water evoked responses in taste nerves and sufficient for eliciting water-specific drinking responses (Zocchi et al., 2017). With respect to generating activity in gustatory afferents (criteria 2), electrophysiological recordings of the chorda tympani, glossopharyngeal and laryngeal nerves have effectively demonstrated a peripheral “water response” across vertebrate models (Cohen

et al., 1955; Zotterman, 1956; Shingai, 1980; Sakaguchi et al., 1989). Similarly to other taste stimuli, orally delivered water stimuli evoke neural responses at multiple levels of the gustatory neuraxis (criteria 3) including the NTS (Nakamura and Norgren, 1991; Rosen et al., 2010), PBN (Nishijo and Norgren, 1990; Rosen et al., 2010), thalamus (Verhagen et al., 2003) and gustatory cortex (de Araujo et al., 2003). Interestingly, "water specialist" cells within the PBN were found to exclusively respond to water and not taste stimuli of equivalent tactile and thermal sensation, providing support for the idea that the central representation for water is unique and that water is a basic taste modality.

Thirst elicits water-directed behaviors that are guided by the orosensory experience of water ingestion. Animals will work for single drops or 0.1 mL volumes of a water reward, amounts which are detected in the oral cavity but do not reach the gut, therefore eliminating the opportunity for postingestive feedback (Hughes et al., 1994; Rolls et al., 1972). Similarly, the orosensory experience of water ingestion supports the reinforcing value of water across multiple species of thirty sham-drinking animals passively consuming (Rolls and Rolls, 1982) or lever pressing for water rewards (Mook and Wagner, 1988). Human reports of water tasting pleasant in a thirsty state but not in a satiated state have supported the idea that the affective value of water's taste drives the motivation to consume water when thirsty (Rolls et al., 1980; Rolls, 1999).

Unlike with sodium appetite, the high-specificity of an appetite for water in thirsty animals is less clear. Some studies have found that water deprivation leads to preferences for sucrose over water, which would not support a water-specific drive of thirsty rats (Johnson and Fisher, 1973; Rolls and Rolls, 1973). Conversely, other work has observed clear preferences for

water over sucrose or other solutions like NaCl (Falk and Young, 1956; Trowill JA, Panksepp J, 1969). Likewise, thirsty rats will increase runway running speed for water, but not for NaCl (Schulkin et al., 1985). Experimental differences in the method of water deprivation may be responsible for these conflicting results. More studies are required to experimentally demonstrate the stimulus-selectivity of water by thirst rats. Nevertheless, both sodium appetite and thirst produce drive states that require animals to seek out and consume stimuli that satisfy homeostatic deficit in a targeted and selective manner. The high specificity of the gustatory system permits the immediate identification and consumption of the goal object, NaCl or water during sodium appetite and thirst.

C. Mesolimbic dopamine signaling drives motivated behavior

While the central circuits that identify need states are well characterized, how that information gets translated into motor plans to seek and consume stimuli in a targeted manner remain poorly understood. The neurotransmitter dopamine, however, is a likely candidate. Release of the neurotransmitter dopamine in forebrain targets (e.g. NAc and dorsal striatum) was first proposed by Roy Wise and colleagues to play an important role in reward (Wise, 2004; Wise et al., 1978). Decades later, a precise role for dopamine signaling is continuously evolving in depth and subtlety. Proposed roles for dopamine include, but are not limited to, promoting behavioral reinforcement (Tsai et al., 2009; Witten et al., 2011; Steinberg et al., 2014) and motivation (Ilango et al., 2014), enhancing the salience of reward-predictive cues (Berridge and Robinson, 1998a) and promoting effortful responses to obtain rewards (Salamone et al., 2016; Hamid et al., 2016). While there are aspects of these psychological constructs that are

incongruent, a prevailing hypothesis is that dopamine serves as a "teaching signal". During associative learning, brief, high concentration (phasic) bursts of dopamine are released in response to rewarding stimuli as well as salient environmental stimuli which predict reward receipt (Schultz et al., 1997; Steinberg et al., 2013). Phasic release of dopamine during the associative learning process helps to maximize the procurement of rewards from the external environment by directing behavior towards relevant stimuli (Hamid et al., 2016). Sodium appetite and thirst are powerful motivated behaviors directed towards physiologically relevant stimuli. For this reason, the two drive states become ideal models for exploring the neural underpinnings of value assignment, goal directed behavior and reinforcement. This dissertation aims to shed light on the neural circuitry underlying motivated behavior by exploring the impact of physiological state on mesolimbic dopamine signaling.

1. Dopamine serves as an interface between physiological state and motivation

Often times stimuli that evoke dopamine responses are those which satisfy a physiological need. In this way, dopamine facilitates associative learning necessary for survival by serving as an interface between physiological state and motivation. Under conditions of physiological need, behaviors directed towards need-satisfying stimuli are invigorated. Hungry rats will increase their food intake and work harder for receipt of food rewards. With these heightened goal directed behaviors, we observe increased responsivity of the dopamine system. Burst firing of VTA dopamine neurons is increased in food restricted animals (Marinelli et al., 2006; Branch et al., 2013). In response to food, food restricted animals have potentiated striatal dopamine release (Cone et al., 2014; Abizaid et al., 2006). Similar observations have been made with

administration of hormones that serve as proxies for hunger (Cone et al., 2014; Abizaid et al., 2006; Jerlhag et al., 2006; Cone et al., 2015). Physiological state modulates goal directed behavior and dopamine signaling in a bi-directional fashion. Inducing a state of satiety with food or hormonal manipulation decreases reward directed behavior (Hayes and Schmidt, 2016), VTA neuron firing rate (Hommel et al., 2006; Labouèbe et al., 2013) and NAc dopamine release (Krügel et al., 2003; Egecioglu et al., 2013; Stouffer et al., 2015). The studies above illustrate the state-dependency of dopamine responses using hunger as a manipulation of physiological state. Unfortunately, there are limited investigations of dopamine release patterns during sodium appetite and thirst, despite the powerful motivated behaviors that are evoked by manipulations of body fluid homeostasis.

The existing literature exploring changes in dopamine signaling during sodium appetite are limited and indirect. Sodium deplete rats that are ingesting NaCl have increased dopamine levels, assessed through microdialysis, and dopamine metabolism in the NAc (Frankmann et al., 1994; Hoebel et al., 1989). The rate of dopamine metabolism is an indirect and crude way of assessing dopamine release which requires many assumptions to be made about dopamine kinetics including a constant rate of reuptake by the dopamine reuptake transporter (DAT). Increased dopamine levels are potentially a consequence of altered function of the dopamine reuptake transporter (DAT). Measured dopamine concentration is a function of both the amount of dopamine released and the rate of reuptake by the DAT. There is evidence to suggest that DAT changes contribute to altered dopamine signaling observed during sodium depletion, with sodium depletion decreasing the rate of reuptake (Roitman et al., 1999b; Figlewicz et al., 1999). Aldosterone administration to the NAc is sufficient to recapitulate this

effect in sodium replete rats (Roitman et al., 1999b). Furthermore, DOCA treatment reduces DAT binding in sodium replete animals (Lucas et al., 2000). The expected result from either a reduction in DAT number or function is a net increase in dopamine release and diffusion (Cragg and Rice, 2004; Sulzer et al., 2016). Prolonged dopamine signaling, through DAT changes, remains a potential mechanism by which sodium depletion generates motivation and increases the vigor directed towards sodium. Additional mechanisms include alterations in mesolimbic dopamine signaling by increasing dopamine production. This mechanism is supported by a study in which manipulations consequent of sodium deprivation (DOCA and Ang-II treatment) increased phosphorylated tyrosine hydroxylase (Grafe and Flanagan-Cato, 2016), thereby augmenting the activity of the rate-limiting enzyme in dopamine production (Dunkley et al., 2004). Currently, there are limited studies which examine the effect of sodium depletion on sodium-evoked dopamine release (Cone et al., 2016). My thesis aims to analyze real-time dopamine responses during sodium exposure in sodium deplete and replete rats.

2. Using FSCV to monitor concentration changes in dopamine release

My work aims to investigate dopamine release evoked by brief exposure to taste stimuli. Correlating dopamine release patterns with coincident events (e.g. the sensory experience of a taste exposure) or behavioral responses requires sampling dopamine changes in real-time in awake and behaving animals. Quantification of phasic dopamine release, known to play a role in motivated behavior (Phillips et al., 2003; Roitman et al., 2004), requires the electrochemical technique of fast-scan cyclic voltammetry (FSCV).

Measuring real-time dopamine concentration fluctuations requires FSCV which permits the opportunity to observe changes in dopamine with subsecond temporal resolution, micrometer spatial resolution and a chemical sensitivity which captures nanomolar concentration changes. These characteristics make FSCV unparalleled by other *in vivo* techniques that are used to measure dopamine neurotransmission (Watson et al., 2006; Sossi and Ruth, 2005). Here, I use FSCV to investigate fluctuations in NAc dopamine signaling during taste exposure to salt or water solutions. My studies require that rats consume the taste stimuli in order to analyze dopamine release resulting from the sensory experience of ingestion. Unfortunately, sodium replete rats will not voluntarily ingest the hypertonic concentration of NaCl used in my investigation (Handal, 1965). In order to compare dopamine responses in sodium replete and deplete rats to a stimulus that is avoided by replete animals, I utilized intraoral catheters. Intraoral catheters allowed for the direct infusion of discrete fluid boluses into the oral cavity of rats (Grill and Norgren, 1978). The ability to temporally control the administration of the intraoral infusions also allowed for analysis of dopamine concentration changes, captured with concurrent FSCV, surrounding the taste.

D. Potential circuits by which perturbations of sodium balance influence dopamine signaling

The central targets of hormones which regulate sodium balance have been extensively studied. Relevant nuclei include sites which monitor the physiological need for salt, as well as those which respond to deficit by internal compensatory mechanisms. The generation of motivated behavior directed at sodium is also a compensatory mechanism for the correction of

sodium deficit. However, how motivated behavior is generated following sodium depletion is unknown.

The intention of this dissertation is to determine the influence of physiological state on mesolimbic circuitry. Here, I look to identify a potential circuit by which a perturbation of body fluid homeostasis is sensed and relayed to mesolimbic circuitry to drive dopamine signaling and potentially the intake behavior aimed to restore physiological deficit. There are surely many central nodes to probe in exploration of a circuit. I elected to restrict my investigation to a candidate circuit involved exclusively with maintaining sodium balance (Geerling and Loewy, 2006d). I have chosen to focus on a sodium-appetite specific pathway by investigating a circuit which begins with HSD2 neurons, the only known viscerosensory neurons that are activated specifically by sodium appetite (Geerling and Loewy, 2006d). The neural systems that drive dopamine responses to water in the instance of thirst are important but will not be explored here.

1. <u>HSD2 neurons are activated by sodium deprivation</u>

Sodium appetite is, in part, driven by activation of HSD2 neurons (Geerling et al., 2006a; Jarvie and Palmiter, 2016). HSD2 neurons do not appear to be activated generally by body fluid homeostasis disruption. They are not responsive to dehydration and play no role in the generation of thirst (Geerling and Loewy, 2006d; Jarvie and Palmiter, 2016). HSD2 neurons are, instead, sensitive to increases in circulating aldosterone induced by sodium deprivation (Geerling et al., 2006a; Davis et al., 1963). Aldosterone activates these neurons through MRs. Under normal conditions, the MR has equal affinity for mineralocorticoids (like aldosterone) and glucocorticoids (i.e. cortisol or corticosterone) (Funder et al., 1988; Sheppard and Funder,

1987). However, in HSD2 neurons, hormone binding is altered in HSD2 neurons by the enzyme for which they were named, 11-beta-hydroxysteriod dehydrogenase type 2 (Geerling et al., 2006a). HSD2 metabolizes cortisol, which circulates in high concentrations, to inert cortisone (Amelung et al., 1953). This process allows for increased access of aldosterone to MRs on HSD2 neurons (Geerling et al., 2006b). While MR expression is found throughout the brain (Arriza et al., 1988), and HSD2-immunoreactive neurons are found in a few brain regions including the NTS, ventrolateral division of the ventromedial hypothalamus, medial vestibular nucleus and ependymal cells of the subcommissural organ, co-expression of MR and HSD2 represents an extremely rare population of neurons (Geerling et al., 2006b). Aldosterone-sensitive HSD2 neurons of the NTS therefore represent a unique population of neurons that are responsive to sodium appetite.

The location of HSD2 neurons that are highly selective to aldosterone within the brain was established by Geerling and colleagues in 2006 through immunoreactivity studies for MR and HSD2 (Geerling et al., 2006b). The neurons are found in the caudal NTS where they extend from just rostral to the area postrema though the caudal end of the NTS. The most dense population of HSD2 cells lies along the medial NTS border with the caudal fourth ventricle, beneath the area postrema (Geerling et al., 2006a, 2006b). This blood-brain barrier deficient area of the brain (Broadwell and Sofroniew, 1993; Gross et al., 1990) allows for circulating aldosterone to access HSD2 neurons. Various manipulations of sodium balance which cause elevations in aldosterone (dietary sodium deprivation, diuresis with furosemide, high doses of DOCA) all result in HSD2 c-Fos activation (Geerling et al., 2006b). The timecourse of c-Fos activation in HSD2 neurons nicely parallels the behavioral expression of a sodium appetite. Furthermore, the

ingestion of sodium after deprivation results in rapid inactivation of c-Fos (Geerling et al., 2006b).

In addition to aldosterone activation, HSD2 neurons receive sensory information from the periphery via direct projections from the vagus nerve (Shin et al., 2009). It is possible that sodium balance is communicated to HSD2 neurons from peripheral sites. Tracing studies from peripheral sites to HSD2 neurons have shown that afferent input arises mainly from the stomach via nodose-ganglion neurons (Shin and Loewy, 2009). Additional organ sources and the functional subtypes of vagal afferents remains unclear (Shin et al., 2009). Beyond the vagus, neural inputs to HSD2 cells include the area postrema, medial subdivision of the central nucleus of the amygdala and local NTS cells which receive baroafferent input (Sequeira et al., 2006; Geerling and Loewy, 2006b). The contribution of these sources to induction of a sodium appetite via HSD2 neuron activation will not be explored here.

HSD2 neurons are a unique subpopulation of NTS neurons. Their activity pattern, molecular phenotype and output connections are all dissimilar from that of surrounding NTS cells. While HSD2 neurons express c-Fos during sodium depletion and are silenced after sodium ingestion, surrounding cells exhibit the opposite pattern of responses (Geerling and Loewy, 2006c, 2007). Molecular markers for neuropeptides, calcium-binding proteins and enzymes that are characteristic of NTS neurons fail to co-localize with NTS HSD2 neurons (Geerling et al., 2006b). Furthermore, HSD2 neurons possess a unique rostral projection pattern. The autonomic regions of the brainstem and hypothalamus that NTS neurons commonly innervate are not targets of HSD2 neurons (Geerling and Loewy, 2006a, 2006b). Instead, HSD2 neurons project to more rostral sites including the BNST and forebrain-projecting relay nuclei in the

pons (Geerling and Loewy, 2006a, 2006b). Two of these relay nuclei, the pre-locus coeruleus (pre-LC) and inner subdivision of the external lateral parabrachial nucleus (PBel-inner) will be discussed in more detail below.

Overwhelming evidence suggests that HSD2 neurons are glutamatergic. HSD2 neurons ubiquitously express the transcription factor paired-like homeobox 2b (Phox2b) (Stornetta et al., 2006; Kang et al., 2007; Geerling et al., 2008). Beyond being critical for the development of chemosensory integration neurons in various brainstem regions including the NTS (Stornetta et al., 2006), Phox2b is co-localized with mRNA for the vesicular glutamate transporter (VGLUT2) (Kang et al., 2007). Phox2b is not expressed in GABAergic cells, identified by the enzyme GAD67 (Kang et al., 2007). In addition to molecular evidence, many of the projection target nuclei of HSD2 neurons also show c-Fos expression upon sodium depletion (Geerling and Loewy, 2007). While these results strongly suggest that HSD2 neurons are excitatory, electrophysiological and pharmacological studies have yet to be conducted. HSD2 neurons represent a central node of hormonal action (aldosterone) and the projections of HSD2 neurons are interesting candidates for the regulation of sodium appetite.

2. FoxP2 neurons in the pre-LC and PBel-inner receive HSD2 projections and are activated by sodium deprivation

Two of the sites most heavily innervated by HSD2 neurons of the NTS are the pre-LC and the PBel-inner. Both pre-LC and PBel-inner neurons can be identified by the constitutive expression of the transcription factor Forkhead box protein 2 (FoxP2) (Geerling et al., 2011). The shared expression of this stable molecular marker suggests not only a common developmental

phenotype of the pre-LC and PBel-inner, but also functional similarities- although the phenotype of these neurons is unknown. Indeed, almost all neurons that become c-Fos activated upon sodium depletion in both the pre-LC and PBel are FoxP2 expressing (Geerling et al., 2011). The sodium- depletion induced c-Fos expression in these two areas of the brain parallels that of the HSD2 NTS neurons. However, while HSD2 c-Fos activation has been shown across multiple experimental means of inducing a sodium appetite (Geerling et al., 2006b), FoxP2 c-Fos activation has only been explored following prolonged dietary sodium depletion (Geerling et al., 2011). Whether or not manipulations that more rapidly induce a sodium appetite, like those that have been used here, also cause c-Fos activation in the pre-LC and PBel-inner is unknown and will be explored here.

The pre-LC consists of a small cluster of neurons in the periventricular gray matter of the dorsolateral pons. The nucleus is immediately medial to the mesencephalic trigeminal nucleus (MeV). Some pre-LC neurons can be found within this nucleus (Stein and Loewy, 2010). The pre-LC is rostral to and molecularly distinct from the locus coerelus (LC). Unlike the LC, neurons of the pre-LC do not express tyrosine hydroxylase (Shin et al., 2011). Little is known about the pre-LC, in part because studies have failed to differentiate the nucleus from the LC (Shin et al., 2011; Krout and Loewy, 2000). In addition to being densely innervated by the NTS (Geerling and Loewy, 2006a), the pre-LC receives significant projections from the paraventricular nucleus and area postrema, neither of which project to the LC (Stein and Loewy, 2010; Geerling et al., 2010). Retrograde tracing techniques paired with immunohistochemical identification of FoxP2 neurons, have been used to define the pre-LC projection targets within the brain (Shin et al., 2011). Many of these hold the potential to be involved with regulation of sodium appetite. Pre-

LC FoxP2 neurons project to sites include those implicated in general homeostatic regulation (paraventricular hypothalamus and dorsomedial hypothalamus among other hypothalamic nuclei), sodium appetite regulation (bed nucleus of the stria terminalis) (Geerling and Loewy, 2008; Zardetto-Smith et al., 1994; Reilly et al., 1994), and reward circuitry (VTA) that potentially drives behavior towards sodium (Shin et al., 2011). The contribution of these output nuclei to the generation of a sodium appetite and sodium seeking behavior is currently unknown.

The PBN is a complex nucleus with many cytochemically unique subnuclei with diverse inputs and outputs (Fulwiler and Saper, 1984; Krout and Loewy, 2000) . The major subdivisions of the PBN include the medial and lateral divisions, both of which are involved with the control of sodium and water intake (Johnson and Thunhorst, 1997). FoxP2 immunoreactivity in the PBN is found in a limited subset of PBN nuclei, including the dorsal lateral and central lateral subnuclei. While the outer portion of the external lateral region of the PBN is devoid of FoxP2 neurons, a thin band of cells along the ventrolateral aspect of the PBel (PBel-inner) highly expresses FoxP2 (Shin et al., 2011). Sodium deprivation activates c-Fos in FoxP2 neurons of the PBel-inner (Geerling et al., 2011). It has been suggested, that like pre-LC, FoxP2 neurons of the PBel-inner receive information regarding sodium balance from hindbrain HSD2 neurons. Indeed, axons from sodium responsive HSD2 neurons of the NTS terminate within this thin band of PBel-inner cells (Geerling and Loewy, 2007). Consistent with the hypothesis that the pre-LC and PBel-inner share similar roles in sodium balance maintenance, the output targets of the Pbel-inner greatly overlap with those of the pre-LC (Shin et al., 2011). Importantly for the studies conducted here, the VTA receives a dense projection from FoxP2 neurons of the PBel-inner (Shin et al., 2011). Projections to the VTA from FoxP2 neurons could represent a circuit

that communicates sodium balance to mesolimbic circuitry to alter dopaminergic responses to sodium stimuli. Whether or not these known pre-LC and PBel-inner to VTA projections are responsive to sodium appetite is not known and will be investigated here. I will use immunohistochemistry to identify pre-LC or PBel-inner to VTA projections that are activated by sodium deprivation. These projections represent an unknown component of a potential circuit by which sodium deprivation drives the motivated behavior observed during sodium appetite. Currently, the mechanism by which sodium appetite generates state-dependent behavioral responses is unknown.

Chapter II

Dopamine responses to sodium and water are state-dependent

A. Introduction

Restoration of body fluid homeostasis requires that animals seek and consume stimuli which satisfy their need state when the need arises. Sodium appetite and thirst drive state-dependent behaviors such that sodium and water come to elicit appetitive and consummatory behaviors during times of homeostatic deficit. These powerful innate motivated states, are termed sodium appetite and thirst. While thirst, the drive to seek and consume water in defense of dehydration, is a motivated state that humans frequently experience, sodium appetite is challenging for humans to conceptualize. Nevertheless, sodium appetite is a biologically conserved motivated state that perhaps best illustrates state-dependent behavior and the powerful influence of physiological state on the assignment of value to a stimulus. For homeostatically balanced, sodium replete rats, the taste of hypertonic NaCl is avoided (Nachman, 1962) and, when experienced, appears to be unpalatable (Nachman, 1962; Berridge et al., 1984). However, in a state of sodium deprivation, the same concentration of NaCl supports voluntary consumption and animals will work to obtain access to it (Nachman, 1962; Quartermain et al., 1967). In addition, there is a shift in the palatability of hypertonic sodium solutions following sodium depletion (Berridge et al., 1984).

How drives towards sodium and water are translated into goal-directed action and how this behavior is reinforced are not currently known. However, mesolimbic dopamine and the NAc are thought to play a key role. Brief, high concentration (phasic) dopamine release in the NAc has been associated with driving motivation and reinforcing appetitive behaviors (Tsai et

al., 2009; Witten et al., 2011; Steinberg et al., 2014; Ilango et al., 2014). The following experiments seek to determine if phasic dopamine signaling is recruited by the taste of sodium and water stimuli after the induction of a sodium appetite or thirst.

I hypothesize that like the motivated behavior directed towards fluid stimuli, the dopamine responses to the stimuli will be drastically transformed. I use FSCV to measure phasic dopamine release patterns within the NAc shell of sodium replete/deplete and *ad libitum* watered/water restricted rats during intraoral exposure to sodium or water stimuli. Administering discrete boluses of stimuli allows for forced exposure of sodium replete or *ad libitum* watered rats to stimuli which they won't voluntarily ingest, as the need for them doesn't exist. Intraoral infusion also allows for precise control of the sensory experience of ingestion and temporally accurate investigation of dopamine responses at the onset and offset of intraoral infusion. As detailed below, the results provide support for a role for dopamine in the generation of sodium appetite and thirst and reinforcing the consumption of stimuli that meet generated needs.

B. Experimental Methods

1. Subjects

Adult, male Sprague Dawley rats (*n*=20) weighing ~350 g at the time of testing were used. Rats were individually housed in plastic cages with lights on from 7 AM to 7 PM. All FSCV testing took place in a standard operant chamber during the light phase. Animals were given *ad libitum* access to standard laboratory chow (2010 Teklad global 18% protein diet) and tap water, unless noted otherwise. Details for housing as well as food and water access during the experiment

can be found under “Deprivation Protocol”. All animals tested were naïve to sodium deprivation or water restriction before the experiments were conducted. They were also naïve to intraoral infusions of NaCl or water. Animal care and use was in accordance with the National Institute of Health (NIH) *Guide for the Care and Use of Laboratory Animals* and was approved by the Institutional Animal Care and Use Committee at the University of Illinois at Chicago.

2. Surgery

Animals were deeply anesthetized with intraperitoneal (IP) ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (20 mg/kg). An in-depth description of the surgical preparation of animals for FSCV is described elsewhere (Fortin et al., 2015). Briefly, a FSCV cannula (Bioanalytical Systems) was implanted above the NAc shell using coordinates [+1.7 mm anterior-posterior (AP), 0.9 mm medial-lateral (ML), and -2.5 mm dorsal-ventral (DV)] obtained from the rat brain atlas of Paxinos and Watson (Paxinos and Watson, 2007). A chlorinated silver (Ag/AgCl) reference electrode was placed in the contralateral cortex. A bipolar stimulating electrode (0.20-mm diameter; Plastics One) was implanted into the VTA (-5.2 mm AP, +0.8 mm ML, -8.4 mm DV from the brain surface). All implants were secured with stainless steel surgical screws and dental cement.

All animals were also implanted with an intraoral catheter as described elsewhere (Grill and Norgren, 1978). Briefly, a catheter, constructed from ~6 cm of PE6 (Scientific Commodities, Inc.) tubing with a Teflon washer-flanged end, was inserted through the mouth and exteriorized out of a small incision (~ 5 mm) in the scalp. The catheter was implanted anterolateral to the first maxillary molar and pushed through until the washer lay flush against the molar. The

catheter was secured in place with the addition of a second Teflon washer fitted over the exteriorized portion of the catheter and secured in place using expoxy.

Following surgery, rats were injected with subcutaneous (SC) meloxicam (1 mg/Kg) . Rats were given 5-7 days of postoperative recovery time. All animals returned to pre-surgical body weight during this time. In order to maintain patency, intraoral catheters were flushed daily with distilled water throughout the post-operative period.

3. Sodium Depletion Protocol

Animals (*n*=5) were made sodium deplete by two, equal volume injections of the diuretic furosemide (20 mg/kg, 0.5 mL/kg, SC, Sigma Aldrich). Animals tested in a replete state (*n*=5) were injected with vehicle (100% DMSO) in place of furosemide. The two injections were spaced one hour apart. FSCV recordings began 24 hours after the first injection. During the 24-hr depletion period, all animals were housed in hanging wire-bottomed cages to prevent the ingestion of urine.

Prior to injections, food and water were removed and the rats were weighed. Diuresis was evaluated by weighing the animal 2 hours following the first injection. Animals were deemed 'sodium deplete' if they lost at least 15 grams of body weight during the two-hour period following furosemide injection. At this time, sodium deplete animals were provided *ad libitum* distilled water and sodium deficient diet (Teklad Sodium Deficient Diet). Sodium replete animals were provided with *ad libitum* water and standard laboratory chow.

Sodium appetite was confirmed following the FSCV recording session. During the 24 hours following FSCV testing, all animals were housed in hanging wire bottom cages with access

to standard laboratory chow, 0.45 M NaCl and water *ad libitum*. Intake of chow and both solutions was recorded and compared between sodium deplete and replete rats.

4. Water Restriction Protocol

Both water restricted (*n*=5) and *ad libitum* watered (*n*=5) animals were housed in hanging wire bottomed cages for the 24 hours preceding their FSCV recording session. During this time water restricted animals were provided with only *ad libitum* standard rodent chow. *Ad libitum* watered animals were provided with *ad libitum* standard rodent chow and water. Water restriction was confirmed by weighing the animals following the restriction period. Animals were included in analysis if they lost at least 15 grams of body weight during this 24-hr period.

Thirst was confirmed following FSCV testing. During the 24 hours following voltammetric testing, all animals were housed in hanging wire bottom cages with *ad libitum* access to standard laboratory chow, 0.45 M NaCl and water. Intake of chow and both solutions was recorded to confirm increased voluntary intake of water in water restricted rats during this repletion period.

5. Intraoral Infusion Session Protocol

Prior to FSCV testing, intraoral cannula were flushed with water to ensure patency. The intraoral cannula of the animal was then connected to an infusion line that ran through a commutator (Christ Instruments) and a solenoid valve. The infusion line was connected, via a solenoid valve, to a reservoir containing either 0.45 M NaCl or distilled water. The reservoir

was outside of a sound attenuating chamber and passed through a fluid swivel to ensure that movement on the part of the animal would not tangle the infusion line.

FSCV recording in awake and behaving animals is described in detail elsewhere (Fortin et al., 2015). Briefly, rats were connected to a FSCV head-mounted voltammetric amplifier in a sound-attenuated standard operant chamber by both their FSCV recording electrode and Ag/AgCl reference electrode. The FSCV carbon-fiber recording electrode was lowered into NAc shell dopamine terminals by means of a micromanipulator. A triangular voltage waveform was applied to the carbon-fiber (from −0.4 to 1.3 to −0.4 V relative to the Ag/AgCl reference electrode, 400 V/s, 60 Hz) for 30 minutes to allow for electrode equilibration. The rate was then changed to 10 Hz for 15 minutes which was then followed by data acquisition. Application of each waveform resulted in a background current, which was subtracted from the current resulting from the oxidation and reduction of dopamine evoked during recording. Current changes resulting from the oxidation and reduction of dopamine were converted into concentration changes using a calibration factor obtained from exposing the electrode to a known concentration of dopamine after data collection concluded (Sinkala et al., 2012). Application of the waveform as well as current measurements were computer controlled (National Instruments, Austin, TX and Tarheel CV, UNC Electronics Facility).

While continuously sampling dopamine release patterns in the NAc shell, remotely controlled (Med Associates, Inc) intraoral infusions occurred. The solenoid valve was opened (flow rate= 50 μl/s) for 4 seconds to allow for discrete infusions (200 μl) of either 0.45M NaCl or water to the mouth of the rat. Each experimental session consisted of 10 discrete intraoral infusions with a variable intertrial interval (range: 30-90 s, mean: 60 ± 8.2 s) to

prevent anticipation of delivery. Following the intraoral infusion session, the VTA was electrically stimulated at 120 μA with a range of frequencies (30-60 Hz) and pulse numbers (5,8,10, 20, 24). Resulting current traces and cyclic voltammograms (current by voltage plots) were later used to extract dopamine concentration changes throughout the session (see FSCV Data Analysis). FSCV hardware and intraoral cannula were disconnected and rats were returned to hanging wire-bottom cages for 24-hr post-recording measurements of food intake and body weight.

6. <u>Behavioral Data Analysis</u>

Diuresis in furosemide treated rats was confirmed with a two-tailed Student's t-test which compared body weight loss in the two hours following vehicle (Sodium Replete rats) or furosemide (Sodium Deplete rats) injection. Sodium appetite was confirmed with a two-tailed Student's t-test which compared voluntary intake of 0.45M NaCl in the 24-hrs following the recording session in Sodium Replete and Sodium Deplete rats. Intake of distilled water during this 24-hr time period was similarly compared between Sodium Replete and Sodium Deplete rats.

Water deprivation in water restricted rats was confirmed with a two-tailed Student's t-test which compared body weight loss in the 24 hours preceding testing when rats had either *ad libitum* (*Ad Libitum* rats) or no access (Water Restricted) to water. Thirst was confirmed with a two-tailed Student's t-test which compared voluntary intake of distilled water in the 24-hrs following the recording session in *Ad Libitum* and Water Restricted rats. Intake of 0.45M NaCl during this 24-hr time period was similarly compared between *Ad Libitum* and Water Restricted

rats. Statistical analyses for body weight changes and intake were performed with GraphPad 5.0 (Prism, Inc.).

7. FSCV Data Analysis

Individual infusion trials (5 s preceding and 10 s following the onset of the intraoral infusion) were isolated from the entirety of the experimental session. Trials were background-subtracted and dopamine concentration changes were extracted using template cyclic voltammograms obtained from VTA stimulation, a current to concentration conversion factor obtained through a post-calibration step (Sinkala et al., 2012) and principal component analysis (Heien et al., 2004). The average dopamine concentration during the 5 seconds prior to infusion of 0.45M NaCl distilled water (baseline, -5 to 0 s) was compared to the average dopamine concentration during the 4 second infusion period (infusion, 0 to 4 s). Statistical comparisons of dopamine concentration during the baseline ("B") and infusion ("I") periods were conducted for Sodium Replete, Sodium Deplete, *Ad Libitum* and Water Restricted rats using two-tailed Student's t-tests. Statistical analyses were performed with GraphPad 5.0 (Prism, Inc.).

8. Verification of Recording Sites

Following experimentation, rats were deeply anesthetized with sodium pentobarbital (100 mg/kg; Sigma-Aldrich). A polyamide-insulated stainless steel electrode (A-M Systems, Inc.) was lowered through the guide cannula to the same depth as the recording electrode and current was passed to create an electrolytic lesion. Next, brains were extracted and stored in

formalin for 24 hours before transferring them to 30% sucrose in 0.1 M phosphate buffer. The brain was then sectioned (35uM) on a cryostat. Coronal sections were mounted on slides. Light microscopy, together with the rat atlas of Paxinos and Watson (Paxinos and Watson, 2007) were used to determine the location of the electrolytic lesion. Only those recordings sites verified to have been in the NAc shell were analyzed.

C. Results

1. Verification of diuresis and sodium appetite in Sodium Deplete rats

Furosemide-treated (Sodium Deplete; *n*=5) rats lost a significant ($p<0.001$) amount of body weight in the two hours following drug treatment compared to vehicle-treated (Sodium Replete; *n*=5) control rats (-23.2 ± 1.74 vs 0.8 ± 1.46 g; Figure 2.1 A). During sodium appetite assessment, Sodium Deplete rats consumed significantly more 0.45M NaCl than Sodium Replete rats (15.2 ± 2.13 vs 4.7 ± 1.66 mL; $p<0.01$; Fig. 2.1 B).

2. Intraoral NaCl evokes phasic dopamine release in the NAc shell of only sodium deplete rats

Intraoral infusion of 0.45M NaCl in Sodium Replete rats (*n*=5) caused a modest decrease in dopamine concentration relative to baseline (Fig. 2.2 A). The average dopamine concentration during the infusion period ("I") was not, however, significantly different from the average baseline ("B") dopamine concentration (6.7 ± 3.69 vs 15.9 ± 6.85 nM; $p=0.07$; Fig. 2.2 B). Interestingly, in Sodium Deplete rats (*n*=5), intraoral infusion of the same hypertonic sodium

solution (0.45M NaCl) caused a markedly different response: a sharp and sustained increase in dopamine concentration relative to baseline (Fig. 2.2 C). The dopamine concentration during the infusion period observed in these animals was significantly different relative to baseline dopamine concentration (37.7 ± 7.47 vs 10.8 ± 2.43 nM; $p<0.05$; Fig. 2.2 D). Histology confirmed that all recordings from Sodium Replete and Sodium Deplete rats were made in the NAc shell (Fig. 2.5 A).

3. Verification of water deprivation and thirst in Water Restricted rats

Water Restricted animals (*n*=5) lost a significant ($p<0.01$) amount of body weight in the 24 hours following water deprivation compared to rats with *ad libitum* access to water (*Ad Libitum*; *n*=5; -26.0 ± 5.82 vs -0.6 ± 1.36 g; Figure 2.3 A). During the assessment of thirst, Water Restricted rats consumed significantly more distilled water than *Ad Libitum* watered rats (62.8 ± 9.72 mL vs 41.2 ± 3.64 mL; $p<0.05$). Intake of 0.45M NaCl during the assessment of thirst was not significantly different between Water Restricted and *Ad Libitum* watered animals (6.25 ± 0.85 vs 6.2 ± 1.06 mL; Fig. 2.3 B).

4. Intraoral water evokes phasic dopamine release in the NAc shell of only water restricted rats

Intraoral infusion of distilled water in *Ad Libitum* watered rats (*n*=5) caused a modest decrease in dopamine concentration relative to baseline (Fig. 2.4 A). The average dopamine concentration during the infusion period ("I") was not, however, significantly different from the

average baseline ("B") dopamine concentration (10.9 $\pm$ 3.60 vs 15.84 $\pm$ 3.40 nM; Fig. 2.4 B). Interestingly, in Water Restricted rats (*n*=5), intraoral infusion of distilled water caused a markedly different response: a sharp and sustained increase in dopamine concentration relative to baseline (Fig. 2.4 C). The dopamine concentration during the infusion period observed in these animals was significantly different relative to baseline dopamine concentration (28.5 $\pm$ 4.25 vs 8.2 $\pm$ 2.41 nM; $p<0.05$; Fig. 2.4 D). Histology confirmed that all recordings from *Ad Libitum* and Water Restricted rats were made in the NAc shell (Fig. 2.5 B).

D. Discussion

The studies here used sodium appetite and thirst as a platform to examine the influence of physiological state on phasic dopamine signaling. I found that phasic dopamine release in the NAc shell is state-dependent. Drastic changes in dopamine release patterns are observed in response to an identical stimulus merely by changing the physiological state of the animal. Phasic dopamine signaling is postulated to underlie reinforcement, motivation and approach behaviors (Tsai et al., 2009; Steinberg et al., 2014; Witten et al., 2011; Ilango et al., 2014).The ability to modulate phasic dopamine signaling with changes in homeostasis therefore represents a potential means to alter the expression of motivated behaviors.

Perturbations of body fluid homeostasis, by either the induction of sodium appetite or thirst, are inducible laboratory setting. To induce a sodium appetite, I used 24-hour sodium deprivation by means of the diuretic furosemide. Furosemide treatment is a well- established method of sodium depletion that causes rapid diuresis and the expression of a sodium appetite (Fitzsimons, 1998). Only animals that demonstrated evidence of diuresis, established as a loss

of >15 g of body weight in the two hours following injection, were used in subsequent FSCV data analysis. Rats treated with furosemide (Sodium Deplete) lost significantly more body weight than vehicle treated (Sodium Replete) rats (Fig. 2.1 A). In addition, Sodium Deplete rats displayed evidence of a sodium appetite in the 24 hours following FSCV testing, with significantly enhanced consumption of 0.45 M NaCl (Fig. 2.1 B). I also chose to examine the effects of water restriction on the dopamine system by inducing a state of thirst. Rats with 24-hour water restriction (Water Restricted) lost a significant amount of body weight compared to *Ad Libitum* watered rats (Fig. 2.3 A). During the 24-hour repletion period following FSCV testing, Water Restricted animals consumed significantly more water than *Ad Libitum* watered rats, suggesting successful induction of thirst in the animals tested (Fig. 2.3 B). Inducing states of sodium appetite and thirst allowed for examination of two opposing states of body fluid homeostasis and the state-dependency of phasic dopamine signaling under each condition.

Sodium deprivation is an ideal manipulation of homeostasis because sodium deprivation drastically switches the hedonic valence of a NaCl stimulus. Hypertonic NaCl is avoided by sodium replete rats but avidly consumed by rats in negative sodium balance (Handal, 1965). In this way, the valence of the same stimulus is transformed by changing the physiological state of the animal. Other manipulations of physiological state do not provide such a scenario. For example, a sucrose pellets are avidly consumed by both hungry and satiated rats alike. In this case, it is not possible to flip the hedonic valence of the sucrose pellet from negative to positive with metabolic state alone. As such, dopamine responses to sucrose pellets merely change in magnitude, with proxies of hunger augmenting the response (Cone et al., 2014). I hypothesized that sodium depletion would cause drastic changes in dopamine signaling evoked by sodium, as

the valence of NaCl is drastically transformed during a sodium appetite. A role for dopamine signaling in driving sodium appetite is inconclusive. Neither systemic nor intra-NAc dopamine antagonists have been shown to alter the expression of a sodium appetite (Roitman et al., 1997; Lucas et al., 2007). However, some indirect measures of dopamine release (dopamine reuptake activity and binding) are affected by induction of a sodium appetite (Roitman et al., 1999a; Lucas et al., 2000). My studies directly measure dopamine release directly using FSCV.

Intraoral catheters were necessary to examine dopamine release dynamics before, during and after exposure to NaCl, as sodium replete rats won't voluntarily consume 0.45 M NaCl. In addition, intraoral catheters allow for precise control of the subject's sensory experience (Grill and Norgren, 1978) to then align with concurrent dopamine concentration changes. The design used here permitted observation of dopamine concentration changes in response to taste stimuli in real-time. Using intraoral delivery of 0.45 M NaCl during real-time phasic dopamine sampling with FSCV, I found that in a state of body fluid homeostasis, hypertonic NaCl fails to evoke a change in phasic dopamine concentration from baseline (Fig. 2.2 A-B). However, after sodium depletion, a rapid and robust increase from baseline aligned to the infusion onset is observed (Fig. 2.2 C-D). I observed similar state-dependent dopamine responses with the induction of thirst. Intraoral water infusion only increased dopamine concentration from baseline in Water Restricted rats (Fig. 2.4).

The temporal dynamics of the observed dopamine responses to sodium and water in the need states of sodium appetite and thirst are typical of NAc shell (Fig. 2.5) responses following palatable intraoral infusions (Roitman et al., 2008). Basal frequency of spontaneous dopamine release events is higher in the NAc shell than core (Aragona et al., 2008).

Spontaneous transients contribute to the baseline dopamine concentration observed before infusion onset (Fig. 2.2 and 2.4). Intraoral infusion reliably evokes dopamine release in all animals tested, which is seen as a spike in dopamine concentration aligned to the infusion onset. While dopamine transient frequency stays elevated throughout and after termination of the infusion, the transients are temporally offset between animals. When averaging dopamine responses (Fig. 2.2 and 2.4), dopamine concentration in the NAc shell appears to remain elevated for a prolonged amount of time relative to observations made in the NAc core (Cone et al., 2016).

The increase from baseline observed in Sodium Deplete and Water Restricted rats is suggestive of NaCl and water adopting rewarding profiles, like that of sucrose, which elicits similar increases in NAc shell dopamine (Roitman et al., 2008). Indeed, both NaCl and water generate motivated behavior in a state of homeostatic deficit (Quartermain et al., 1967; Krieckhaus and Wolf, 1968; Clark and Bernstein, 2006; Hughes et al., 1994; Rolls et al., 1972). While my experiments did not use taste reactivity measures, the well-established method of assessing the hedonic value of a stimulus (Grill and Norgren, 1978) has previously been used to examine changes in palatability of NaCl and water following sodium depletion and water restriction, respectively. Both NaCl and water generate an increased number of positive ingestive responses, those typical of palatable solutions like sucrose, under conditions of homeostatic deficit (sodium depletion or water restriction) relative to a state of homeostatic balance (Grill and Norgren, 1978; Berridge et al., 1984; Eckel and Ossenkopp, 1995). These studies suggest that body fluid homeostasis shifts can alter perceived palatability of tastes. A shift in palatability is supported by human reports (Takamata et al., 1994) as well as rodent

studies in which sodium depletion increases ingestive taste reactivity responses (Berridge et al., 1984) and causes rats to prefer sodium over highly palatable glucose solutions (Smith et al., 1968).

The dopamine increase that I observed under conditions of sodium depletion or water restriction are typical of stimuli which generate appetitive and consummatory behaviors. It is hypothesized that these increases drive motivated behavior. While less clear, the literature addressing dopamine responses to aversive stimuli suggests that aversive stimuli generate pauses in phasic dopamine signaling [see (McCutcheon et al., 2012) for review]. I did not observe a significant pause in dopamine signaling from baseline in Sodium Replete rats infused with hypertonic NaCl or *Ad Libitum* watered rats infused with water (Fig. 2.2 and 2.4). Despite the fact that sodium replete rats won't voluntarily ingest hypertonic NaCl, failure to observe a pause in dopamine signaling is perhaps not surprising given that hypertonic NaCl is not entirely aversive to sodium replete rats. Taste reactivity tests show that sodium deplete rats display mixed ingestive and aversive responses (Berridge et al., 1984; Grill and Norgren, 1978). This result highlights the important distinction between avoided and aversive stimuli. My work contributes to the characterization of dopamine signaling patterns for avoided and aversive stimuli, however this subject requires further investigation.

The studies described here illustrate state-dependent dopamine signaling. Information regarding body fluid homeostasis is somehow conveyed to mesolimbic circuitry to generate increases in phasic dopamine signaling only in need states. The circuits by which body fluid homeostasis is relayed to dopamine neurons are currently unknown and will be the focus of future studies. It is likely that key nuclei for the expression of sodium and water seeking

behaviors reside in the forebrain, as the forebrain as the forebrain is necessary for at least some aspects of the control of sodium and water balance (Grill et al., 1986; Grill and Miselis, 1981). I plan to examine direct projections from sodium appetite responsive neurons of the parabrachial nucleus and pre-locus coeruleus (Geerling et al., 2011) to the VTA. Both site represent potential modulators dopamine neuron excitability and dopamine release in the NAc, a forebrain structure, under conditions of sodium depletion. It is well established that the SFO is a thirst control center of the brain [see (Daniels and Fluharty, 2004) for review]. Future work should examine the potential of the SFO to modulate motivated behavior through communication with the VTA. Once circuits have been identified, optogenetic, chemogenetic and pharmacological methods can be utilized in an attempt to modulate dopamine signaling and possibly, as a consequence, motivated behaviors. The ability to interfere with motivated behaviors may hold promise for the therapeutic intervention of diseases progression for diseases (obesity, drug addiction) rooted in maladaptive motivated behavior.

Figure 2.1

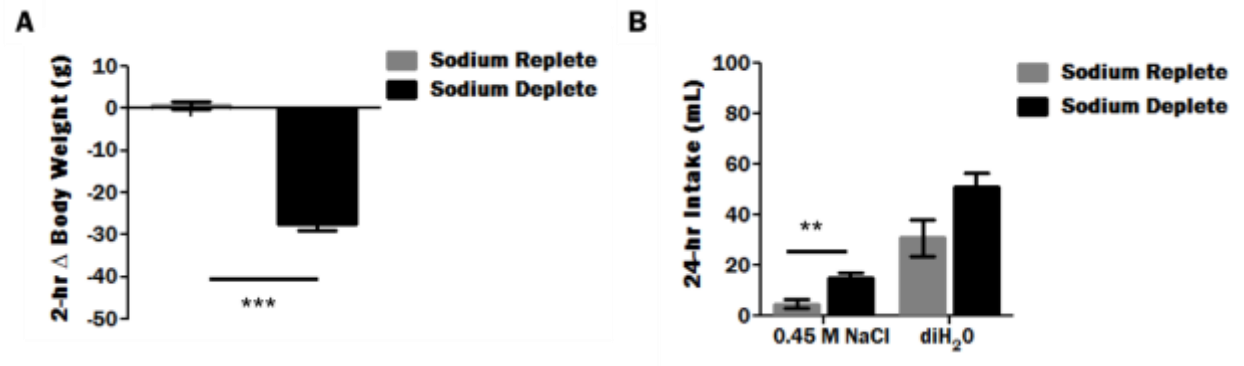

Figure 2.1: Confirmation of diuresis and sodium appetite in furosemide-treated rats. ***A,*** Rats injected with the diuretic furosemide [Sodium Deplete (*n*=5); 20 mg/kg, 0.5 mL/kg, SC, two injections spaced one hour apart] lose significantly more body weight in the two hours following the first injection relative to vehicle-treated [Sodium Replete (*n*=5); DMSO, 0.5 mL/kg, SC] controls. ***B,*** During the 24-h4 repletion period following testing, rats treated with furosemide prior to testing [Sodium Deplete (*n*=5)] consume significantly more 0.45 M NaCl and diH20 than vehicle treated [Sodium Replete (*n*=5)] controls. Bar data represent mean ± SEM; **p<0.01, *** p<0.001.

Figure 2.2

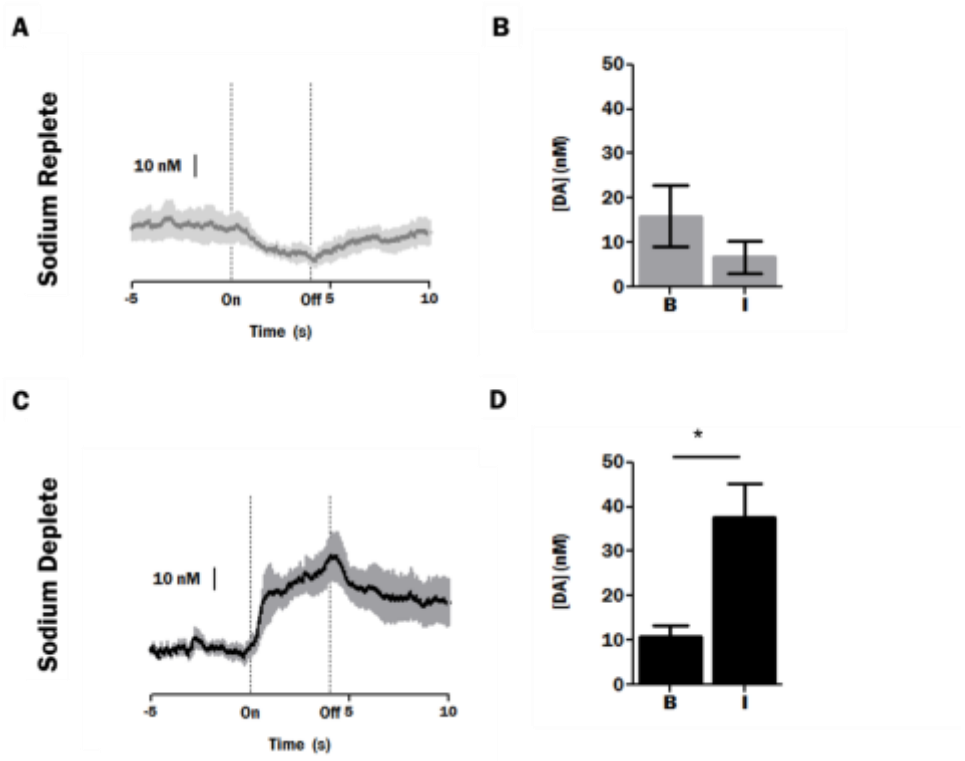

Figure 2.2: Phasic dopamine signaling evoked by hypertonic NaCl is state-dependent. ***A,*** Average dopamine concentration response in Sodium Replete (*n*=5) rats during the 15 seconds (x-axis) surrounding the onset (On) and offset (Off) a 4-second intraoral infusion of 0.45 M NaCl. ***B,*** Average dopamine concentration during the 5-second baseline period (B; -5 to On) vs the 4-second infusion period (I; On-Off) of 0.45 M NaCl in Sodium Replete rats. ***C,*** Same as *A* in Sodium Deplete (*n*=5) rats. ***D,*** Same as *B* in Sodium Deplete rats. For *A*-*D*, average dopamine concentration (mean ± SEM) is representative of 10 intraoral infusion trials. There were no baseline differences between Sodium Replete and Sodium Deplete rats. *p<0.05.

Figure 2.3

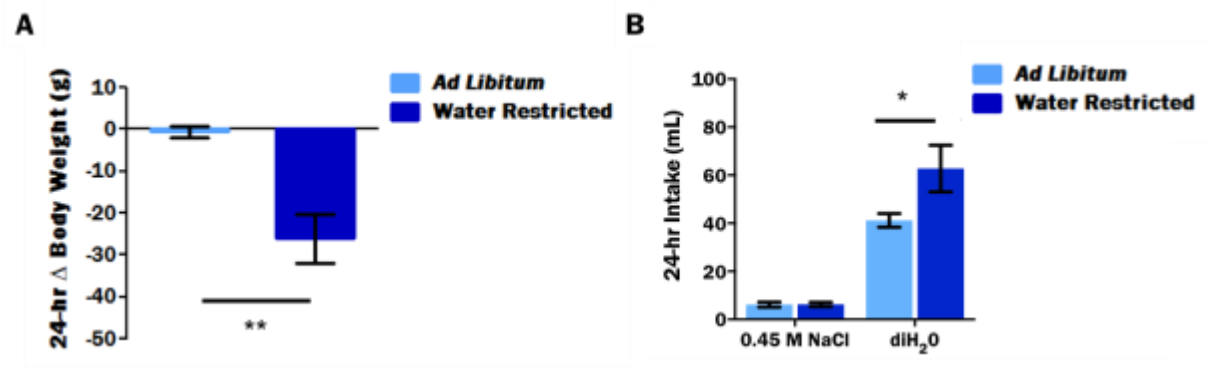

Figure 2.3: Confirmation of water restriction and thirst in water-restricted rats. **A,** Rats maintained under water restriction [Water Restricted (*n*=5)] lose significantly more body weight in the 24 hours following deprivation relative to rats with *ad libitum* water access [*Ad Libitum* (*n*=5)]. **B,** During the 24-hr repletion period following testing, previously water restricted rats [Water Restricted (*n*=5)] consume significantly more diH20 than rats with previous *ad libitum* water access [*Ad libitum* (*n*=5)]. Bar data represent mean ± SEM; *p<0.05, **p<0.01

Figure 2.4

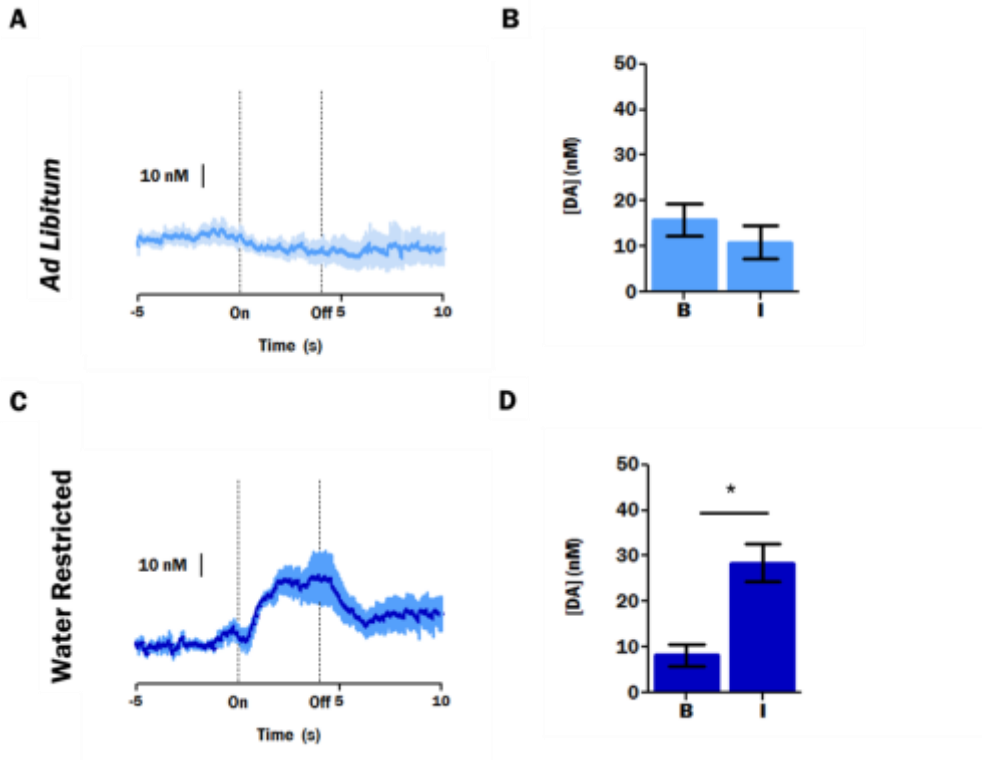

Figure 2.4: Phasic dopamine signaling evoked by water is state-dependent. ***A,*** Average dopamine concentration response in *Ad Libitum* (*n*=5) watered rats during the 15 seconds (x-axis) surrounding the onset (On) and offset (Off) a 4-second intraoral infusion of distilled water. ***B,*** Average dopamine concentration during the 5-second baseline period (B; -5 to On) vs the 4-second infusion period (I; On-Off) of distilled water in *Ad Libitum* watered rats. ***C,*** Same as *A* in Water Restricted (*n*=5) rats. ***D,*** Same as *B* in Water Restricted rats. For *A-D*, average dopamine concentration (mean ± SEM) is representative of 10 intraoral infusion trials. There were no baseline differences between *Ad Libitum* and Water Restricted rats. *p<0.05.

Figure 2.5

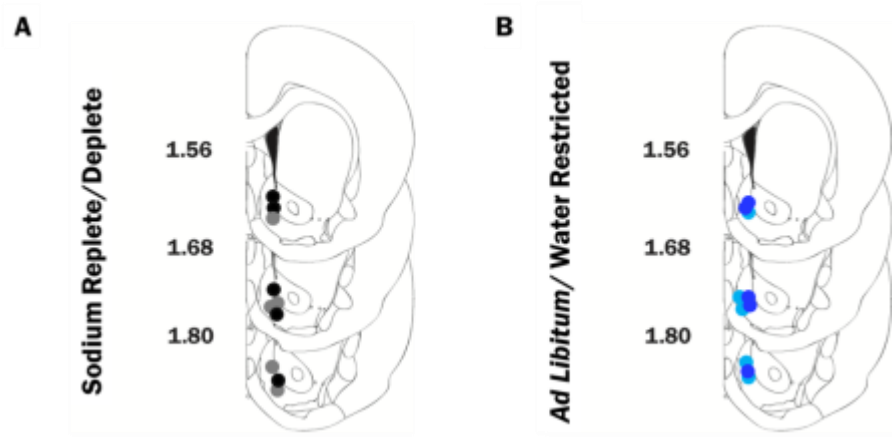

Figure 2.5: Summary of Nac shell recording sites. Approximate sites of FSCV recording are depicted as circles on coronal sections modified from Paxinos and Watson (2007). Numbers to the left indicate approximate distance from bregma. ***A,*** Recording locations from Sodium Replete [grey circles (*n*=5)] and Sodium Deplete [black circles (*n*=5)] rats infused with 0.45M NaCl. ***B,*** Recording locations from *Ad Libitum* [light blue circles (*n*=5)] watered or Water Restricted [royal blue circles (*n*=5)] rats infused with distilled water.

Chapter III

Dopamine responses to sodium and water are taste-specific

A. Introduction

The motivated behaviors of sodium appetite and thirst require that an animal identify the specific stimulus needed to restore their body fluid homeostasis. The taste system has evolved for highly selective processing which supports sensory identification and discrimination of stimuli. While opposing views on how taste qualities are encoded exist, the consensus is that taste receptor cells are tuned to respond to a single taste. Taste receptor cells are innervated by non-overlapping fibers (e.g. salt-best fibers) which transmit the taste quality to the central nervous system along "labeled lines" (Chandrashekar et al., 2006). With respect to sodium taste, amiloride-sensitive primary taste receptors are necessary and sufficient for the detection of NaCl (Geran and Spector, 2000b, 2000a). Sodium deplete rats use amiloride-sensitive taste receptors to selectively consume NaCl over non-sodium salts like KCl (Spector et al., 1996). The selective permeability of lingual amiloride-sensitive ion channels allows only cations of the appropriate size, like Na^+, to pass through the ion channel to activate sodium taste receptors (Kellenberger et al., 1999; Heck et al., 1984). Blockade of these lingual amiloride-sensitive channels abolishes the behavioral expression of a sodium appetite (Brot et al.; Bernstein and Hennessy, 1987; Roitman and Bernstein, 1999). It is through amiloride-sensitive receptors that rats are able to process the taste of NaCl, an important physiological process for identifying and consuming NaCl in the service of maintaining body fluid homeostasis.

The potassium ion of KCl does not pass through the pore of amiloride-sensitive ion channels (Kellenberger et al., 1999). As a result, the taste system, and presumably downstream

signaling which reinforces the drive to consume the salt solution, is not activated and sodium deplete rats will not consume KCl. By having a sensory system that allows for the discrimination of salts on a single ion level, rats are able to identify and consume the only salt that is physiologically relevant. There is one exception to the precision of the finely tuned taste system in regards to it supporting taste-specific behaviors directed at salts. The lithium ion of LiCl passes through epithelial sodium-ion channels and activates sodium taste receptors similarly to NaCl (Kellenberger et al., 1999). As a result, sodium deplete rats are not able to use taste to distinguish NaCl from LiCl. This phenomenon has only been explored in rats made chronically sodium deplete by adrenalectomy (Nachman, 1962). Here, I aim to determine cation selectivity of sodium appetite that is aroused acutely via diuretic (furosemide) injection using a two-bottle intake test.

Replenishing need states of sodium appetite and thirst requires reinforcement of appetitive and consummatory intake behaviors. The reinforcement of intake behaviors must be directed at physiologically required stimuli (sodium or water), which are identified by sensory properties including taste. It is likely that during consumption, the taste of the stimulus is what is being reinforced and thereby supports intake which replenishes the physiological need. The neurobiological substrates of reinforcement must, therefore, be in tune to taste qualities of required ingesta. Here, I examine the taste-specificity of the dopamine system. I examined this phenomenon in both sodium deplete and replete rats as well as in *ad libitum* watered and water restricted rats. Both sodium and water stimuli support taste-specific behaviors in a state of homeostatic deficit (Nachman, 1962; Quartermain et al., 1967; Trowill JA, Panksepp J, 1969; Mook and Wagner, 1988). I hypothesized that like the taste-guided behavior observed during

sodium appetite and thirst, the dopamine responses to intraorally infused stimuli would be taste specific such that only those taste stimuli which satisfy the need state of the animal would evoke dopamine responses.

B. Experimental Methods

1. Subjects

Adult, male Sprague Dawley rats weighing ~350 g at the time of testing were used. Rats were individually housed in plastic cages with lights on from 7 AM to 7 PM. All FSCV testing took place in a standard operant chamber during the light phase. Animals were given *ad libitum* access to standard laboratory chow (2010 Teklad global 18% protein diet) and tap water, unless noted otherwise. Details for housing as well as food and water access during the experiment can be found under "Deprivation Protocol". All animals tested were naïve to sodium deprivation or water restriction before the experiments were conducted. They were also naïve to intraoral infusions of NaCl or water. Animal care and use was in accordance with the National Institute of Health (NIH) *Guide for the Care and Use of Laboratory Animals* and was approved by the Institutional Animal Care and Use Committee at the University of Illinois at Chicago.

2. Surgery

Animals were deeply anesthetized with intraperitoneal (IP) ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (20 mg/kg). An in-depth description of the surgical preparation of animals for FSCV is described elsewhere (Fortin et al., 2015). Briefly, a FSCV

cannula (Bioanalytical Systems) was implanted above the NAc shell using coordinates [+1.7 mm anterior-posterior (AP), 0.9 mm medial-lateral (ML), and -2.5 mm dorsal-ventral (DV)] obtained from the rat brain atlas of Paxinos and Watson (Paxinos and Watson, 2007). A chlorinated silver (Ag/AgCl) reference electrode was placed in the contralateral cortex. A bipolar stimulating electrode (0.20-mm diameter; Plastics One) was implanted into the VTA (-5.2 mm AP, +0.8 mm ML, -8.4 mm DV from the brain surface). All implants were secured with stainless steel surgical screws and dental cement.

All animals were also implanted with an intraoral catheter as described elsewhere (Grill and Norgren, 1978). Briefly, a catheter, constructed from ~6 cm of PE6 (Scientific Commodities, Inc.) tubing with a Teflon washer-flanged end, was inserted through the mouth and exteriorized out of a small incision (~ 5 mm) in the scalp. The catheter was implanted anterolateral to the first maxillary molar and pushed through until the washer lay flush against the molar. The catheter was secured in place with the addition of a second Teflon washer fitted over the exteriorized portion of the catheter and secured in place using expoxy.

Following surgery, rats were injected with subcutaneous (SC) meloxicam (1 mg/Kg) . Rats were given 5-7 days of postoperative recovery time. All animals returned to pre-surgical body weight during this time. In order to maintain patency, intraoral catheters were flushed daily with distilled water throughout the post-operative period.

3. Sodium Depletion Protocol

Animals (*n*=22) were made sodium deplete by two, equal volume injections of the diuretic furosemide (20 mg/kg, 0.5 mL/kg, SC, Sigma Aldrich). Animals tested in a replete state

(*n*=23) were injected with vehicle (100% DMSO) in place of furosemide. The two injections were spaced one hour apart. FSCV recordings began 24 hours after the first injection. During the 24-hr depletion period, all animals were housed in hanging wire-bottomed cages to prevent the ingestion of urine.

Prior to injections, food and water were removed and the rats were weighed. Diuresis was evaluated by weighing the animal 2 hours following the first injection. Animals were deemed 'sodium deplete' if they lost at least 15 grams of body weight during the two-hour period following furosemide injection. At this time, sodium deplete animals were provided *ad libitum* distilled water and sodium deficient diet (Teklad Sodium Deficient Diet). Sodium replete animals were provided with *ad libitum* water and standard laboratory chow.

Sodium appetite was confirmed following the FSCV recording session. During the 24 hours following FSCV testing, all animals were housed in hanging wire bottom cages with access to standard laboratory chow, 0.45 M NaCl and water *ad libitum*. Intake of chow and both solutions was recorded and compared between sodium deplete and replete rats.

4. <u>Water Restriction Protocol</u>

Both water restricted (*n*=10) and *ad libitum* watered (*n*=5) animals were housed in hanging wire bottomed cages for the 24 hours preceding their FSCV recording session. During this time water restricted animals were provided with only *ad libitum* standard rodent chow. *Ad libitum* watered animals were provided with *ad libitum* standard rodent chow and water. Water restriction was confirmed by weighing the animals following the restriction period. Animals were included in analysis if they lost at least 15 grams of body weight during this 24-hr period.

Thirst was confirmed following FSCV testing. During the 24 hours following voltammetric testing, all animals were housed in hanging wire bottom cages with *ad libitum* access to standard laboratory chow, 0.45 M NaCl and water. Intake of chow and both solutions was recorded to confirm increased voluntary intake of water in water restricted rats during this repletion period.

5. Two Bottle Test Protocol

All rats (*n*=29) were made 24-hr sodium deplete. Diuresis was confirmed in all animals (see Sodium Depletion Protocol above). A two bottle test was conducted in hanging wire bottom cages. Rats were presented with two graduated cylinder sipper tubes containing either 0.15 M KCl and 0.15M NaCl (*n*=14) or 0.15 M LiCl and 0.15 M NaCl (*n*=15). Placement of the NaCl-containing sipper tube on the right or left-hand side of the cage was pseudorandomly counterbalanced. Rats were allowed 10 minutes to voluntarily consume either solution.

6. Intraoral Infusion Session Protocol

Prior to testing, intraoral cannula were flushed with water to ensure patency. The intraoral cannula of the animal was then connected to an infusion line that ran through a commutator (Christ Instruments) and a solenoid valve. The infusion line terminated on the outside of the sound-attenuated chamber in a suspended syringe containing either 0.15 M NaCl, KCl, LiCl or distilled water (for Sodium Replete/Deplete rats) or 0.45 M NaCl or distilled water (for Ad Libitum watered/ Water Restricted Rats).

FSCV recording in awake and behaving animals is described in detail elsewhere (Fortin et al., 2015). Briefly, rats were connected to a FSCV head-mounted voltammetric amplifier in a sound-attenuated standard operant chamber by both their FSCV recording electrode and Ag/AgCl reference electrode. The FSCV carbon-fiber recording electrode was lowered into NAc shell dopamine terminals by means of a micromanipulator. A triangular voltage waveform was applied to the carbon-fiber (from −0.4 to 1.3 to −0.4 V relative to the Ag/AgCl reference electrode, 400 V/s, 60 Hz) for 30 minutes to allow for electrode equilibration. The rate was then changed to 10 Hz for 15 minutes following data acquisition. Application of each waveform resulted in a background current, which was subtracted from the current resulting from the oxidation and reduction of dopamine evoked during recording. Current changes resulting from the oxidation and reduction of dopamine were converted into concentration changes after data collection (Sinkala et al., 2012). Application of the waveform as well as current measurements were computer controlled (National Instruments, Austin, TX and Tarheel CV, UNC Electronics Facility).

While continuously sampling dopamine release patterns in the NAc shell, remotely controlled (Med Associates, Inc) intraoral infusions occurred. The solenoid valve was opened (flow rate= 50 μl/s) for 4 seconds to allow for discrete infusions (200 μl) of a salt or water solution to the mouth of the rat. Each experimental session consisted of 10 discrete intraoral infusions with a variable intertrial interval (range: 30-90 s, mean: 60 ± 8.2 s) to prevent anticipation of delivery. Following the intraoral infusion session, the VTA was electrically stimulated at 120 μA with a range of frequencies (30-60 Hz) and pulse numbers (5,8,10, 20, 24). Resulting current traces and cyclic voltammograms (current by voltage plots) were later

used to extract dopamine concentration changes throughout the session (see FSCV Data Analysis. FSCV hardware and intraoral cannula were disconnected and rats were returned to hanging wire-bottom cages for 24-hr post-recording measurements of food intake and body weight.

7. Behavioral Data Analysis

Intake of each presented solution during the 10-minute test was measured. Cumulative intake was calculated by adding intake volumes of the two solutions presented. Preference scores for the non-sodium salt were calculated for each animal (intake volume of non-sodium salt/ total intake volume). Cumulative intake and preference scores of rats presented with LiCl and NaCl were compared to rats presented with KCl and NaCl using a two-tailed Student's t-tests.

8. FSCV Data Analysis

Individual infusion trials (5 s preceding and 10 s following the onset of the intraoral infusion) were isolated from the entirety of the experimental session. Trials were background-subtracted and dopamine concentration changes were extracted using template cyclic voltammograms obtained from VTA stimulation, a current to concentration conversion factor obtained through a post-calibration step (Sinkala et al., 2012) and principal component analysis (Heien et al., 2004). The average dopamine concentration during the 5 seconds prior to infusion of 0.45M NaCl distilled water (baseline, -5 to 0 s) was compared to the average dopamine

concentration during the 4 second infusion period (infusion, 0 to 4 s). Statistical comparisons of dopamine concentration during the baseline ("B") and infusion ("I") periods were conducted for Sodium Replete, Sodium Deplete, *Ad Libitum* and Water Restricted rats using two-tailed Student's t-tests. Statistical analyses were performed with GraphPad 5.0 (Prism, Inc.).

9. Verification of Recording Sites

Following experimentation, rats were deeply anesthetized with sodium pentobarbital (100 mg/kg; Sigma-Aldrich). A polyamide-insulated stainless steel electrode (A-M Systems, Inc.) was lowered through the guide cannula to the same depth as the recording electrode and current was passed to create an electrolytic lesion. Next, brains were extracted and stored in formalin for 24 hours before transferring them to 30% sucrose in 0.1 M phosphate buffer. The brain was then sectioned (35uM) on a cryostat. Coronal sections were mounted on slides. Light microscopy, together with the rat atlas of Paxinos and Watson (Paxinos and Watson, 2007) were used to determine the location of the electrolytic lesion. Only those recordings sites verified to have been in the NAc shell were analyzed.

C. Results

1. Sodium Deplete rats are able to discriminate NaCl from KCl but not NaCl from LiCl

A two-bottle preference test in Sodium Deplete rats revealed no group differences in cumulative intake when comparing rats that received LiCl and NaCl (n=15) to those that received KCl and NaCl (n=14; 10.5 $\pm$ 1.50 vs 10.4 $\pm$ 1.84 mL; p>0.05; Fig. 3.1 A). Rats presented

with LiCl and NaCl had no preference for LiCl over NaCl (preference score for non-sodium salt = 0.6 ± 0.09). Conversely, rats presented with KCl and NaCl had a strong preference for NaCl (preference score for non-sodium 0.09). The preference scores for the two groups of rats tested were significantly different from each other ($p<0.001$). Taken together, these results suggest that rats fail to discriminate NaCl from LiCl but do discriminate between NaCl and KCL. From the two-bottle intake test, clear predictions can be made regarding the dopamine responses to intraoral salt solutions, with only NaCl and LiCl evoking phasic dopamine increases.

2. <u>Only intraoral NaCl and LiCl evoke phasic dopamine release in the NAc shell of sodium deplete rats</u>

In Sodium Replete rats, intraoral infusion of 0.15M NaCl (*n*=6), KCl (*n*=6), LiCl (*n*=6) or water (*n*=5) did not evoke a change in dopamine concentration relative to baseline (Fig. 3.2 A). The average dopamine concentration during the infusion period ("I") was not significantly different from the average baseline ("B") dopamine concentration ($p>0.05$) for any of the tested solutions (16.0 ± 2.83 vs 13. 1 ± 1.89 nM for NaCl, 11.5 ± 1.73 vs 14.6 ± 2.71 nM for KCl, 13.4 ± 3.47 vs 13.4 ± 2.17 nM for LiCl, 13.6 ± 2.24 vs 18.6 ± 3.33 nM for water; Fig. 3.2 B).

In Sodium Deplete rats, intraoral infusion of 0.15M KCl (*n*=5) or water (*n*=6) did not evoke a change in dopamine concentration relative to baseline (Fig. 3.2 C). For KCl and water infused animals, the average dopamine concentration during the infusion period was not significantly different from the average baseline dopamine concentration (15.4 ± 2.91 vs 17.3 ± 5.42 nM and 15.6 ± 3.07 vs 14.6 ± 3.27 nM, respectively; $p>0.05$; Fig. 3.2 D). In stark contrast, in Sodium Deplete rats infused with 0.15M NaCl (*n*=6), there was a markedly different response: a

sharp and sustained increase in dopamine concentration relative to baseline (Fig. 3.2 C). The increase in dopamine concentration evoked by NaCl in Sodium Deplete rats replicates findings presented in Chapter 2. Critically, in Sodium Deplete animals, the dopamine response evoked by 0.15 M LiCl (*n*=5) looked similar to the response evoked by NaCl- both solutions evoked an increase in NAc dopamine concentration (Fig. 3.2 C). For NaCl and LiCl infused animals, the average dopamine concentration during the infusion period was significantly different from the average baseline dopamine concentration (40.4 ± 8.19 vs 13.6 ± 1.98 nM and 34.0 ± 6.59 vs 16.7 ± 3.90 nM, respectively; $p<0.05$; Fig. 3.2 D). Histology confirmed that all recordings from Sodium Replete and Sodium Deplete rats were made in the NAc shell (Fig. 3.4 A-B).

3. Only intraoral water evokes phasic dopamine release in the NAc shell of water restricted rats

Intraoral infusion of distilled water in *Ad Libitum* watered rats (*n*=5) caused a modest decrease in dopamine concentration relative to baseline (Fig. 3.3 A). The average dopamine concentration during the infusion period ("I") was not, however, significantly different ($p>0.05$) from the average baseline ("B") dopamine concentration (10.9 ± 3.60 vs 15.84 ± 3.40 nM; Fig. 3.3 B). Interestingly, in Water Restricted rats (*n*=5), intraoral infusion of distilled water caused an increase in dopamine concentration relative to baseline (Fig. 3.3 C). The dopamine concentration during the infusion period observed in these animals was significantly different relative to baseline dopamine concentration (28.5 ± 4.25 vs 8.2 ± 2.41 nM; $p<0.05$; Fig. 3.3 D). A modest increase in dopamine concentration relative to baseline (Fig. 3.3 A) was observed in Water Restricted rats infused with 0.45M NaCl (*n*=5), however this increase in dopamine

concentration from baseline was not significant (16. 9 $\pm$ 2.85 vs 6.8 $\pm$ 1.48 nM; $p>0.05$; Fig. 3.3 B). Histology confirmed that all recordings from Sodium Replete and Sodium Deplete rats were made in the NAc shell (Fig. 3.4 C-D).

D. Discussion

Behaviors directed at body fluid homeostasis-restoring stimuli arise during physiological need and are directed specifically at stimuli that are physiologically required. However, the state and taste specificity of dopamine signaling that likely plays a role in sodium appetite and thirst, is not clear. The results of the studies conducted here demonstrate that phasic dopamine signaling in response to body fluid homeostasis-restoring stimuli is both state-dependent and taste-specific. Only under the conditions of sodium depletion and water restriction do sodium and water stimuli, respectively and selectively, evoke increases in dopamine concentration from baseline (Fig. 3.2 and 3.4). Furthermore, the increases are stimulus-specific such that only those taste stimuli which satisfy the need state of the animal evoke dopamine increases.

Under sodium deplete conditions, intraoral infusion of NaCl, but not KCl or water, elicits an increase in dopamine concentration from baseline (Fig. 3.2 C-D) within the NAc shell (Fig. 3.4). The taste transduction mechanism for the detection of NaCl involves amiloride-sensitive sodium ion channels, which are also permeable to LiCl (cite). As a result, sodium deplete rats are unable to discriminate between LiCl and NaCl in a two-bottle test (Fig. 3.1) and intraoral LiCl infusion supports phasic dopamine responses that look similar to those evoked by NaCl (Fig. 3.2 C-D). Under conditions of thirst, water stimuli significantly augment phasic dopamine signaling (Fig. 3.3).

The taste system guides motivated behaviors towards physiologically relevant stimuli under conditions of homeostatic need (Spector and Grill, 1992; Spector et al., 1996). I have effectively demonstrated that here by replicating the two-bottle intake results of Nachman from 1962 and 1963 (Nachman, 1962, 1963b). Under conditions of furosemide-induced sodium depletion, rats presented with two sipper bottles of isomolar (0.15 M) salt solutions, one of NaCl and another of KCl, for 10 minutes almost exclusively consume the NaCl solution (Fig. 3.1, (Nachman, 1962)). While not quantified, I observed intake patterns of the rats during testing. The small amount of KCl intake that is reflected in the preference score for KCl of about 0.1 was due to rats sampling the KCl bottle before switching to the NaCl solution. Rats were very clearly able to identify and selectively consume the salt solution which restored their physiological deficit.

The taste transduction mechanism for the discrimination of NaCl and KCl involves the cation specificity of amiloride-sensitive sodium ion channels, which are permeable to NaCl but impermeable to KCl (Garty and Palmer, 1997). Amiloride-sensitive sodium ion channels are also permeable to LiCl, due to the size of the Li^+ ion and its ability to pass through the ion channel (Garty and Palmer, 1997). As a result, in Sodium Deplete rats, LiCl activates sodium receptors of the tongue similarly to NaCl. Rats presented with one bottle of NaCl and another of LiCl (both 0.15 M) consume a similar cumulative amount of fluid as those presented with NaCl and KCl (Fig. 3.1 A). However, they are unable to discriminate between LiCl and NaCl, and consume similar amounts of the two solutions (Nachman, 1963b), as indicated by the preference score for LiCl of 0.6 (Fig. 3.1). The identity of salts is processed at the level of primary taste receptors of the tongue and relayed to the brain via the chorda tympani nerve (Pfaffmann, 1955; Beidler,

1953; Frank et al., 1983). The chorda tympani nerve is necessary for the discrimination of salts, despite the fact that the glossopharyngeal nerve innervates the majority or rat taste buds (Miller, 1977). Transection of the chorda tympani but not the glossopharyngeal nerve impairs discrimination of NaCl and KCl salts (Breslin et al., 1993, 1995; St John et al., 1997; Spector and Grill, 1992; Markison et al., 1995). Taste coding and the discrimination of salts by peripheral mechanism allows for taste-specific intake that is necessary to replenish distinct (i.e. sodium) physiological deficits.

Here, sodium depletion was accomplished through administration of the diuretic furosemide and maintenance on a sodium free diet for 24 hours. While cation-selectivity for salts in deplete rats had been established in the 1960's (Nachman, 1962, 1963b), depletion was accomplished through adrenalectomy. It remained unclear if acute forms of sodium depletion would yield similar results. Results of the two-bottle test conducted here support furosemide-induced sodium depletion as a suitable model to examine taste-dependent dopamine signaling. I proceeded to examine the ability of the mesolimbic dopamine system to discriminate between taste stimuli. Like the intake behavior of the rats observed in the two-bottle test (Fig. 3.1), phasic dopamine responses within the NAc shell (Fig. 3.4 A-B) were selective for salt solutions that permeated amiloride-sensitive sodium ion channels. Intraoral infusion of both isomolar (0.15 M) NaCl and LiCl supported an increase in dopamine concentration from baseline in Sodium Deplete rats. The magnitude of the dopamine response in NaCl and LiCl infused animals was not compared. The technique of FSCV is best suited for within-subjects comparisons in which electrode placement and sensitivity remain constant (Fortin et al., 2015). It is clear, however, that both salt solutions supported NAc shell dopamine release.

Neither KCl (0.15 M) or water evoked changes in dopamine concentration from baseline in Sodium Deplete animals (Fig. 3.2 C-D). In addition to being taste-dependent, the dopamine responses were also state- dependent, as none of the four tested solutions evoked dopamine increases in Sodium Replete rats (Fig. 3.2 A-B). Under conditions of sodium appetite, taste system is discriminating between salt stimuli and relaying information to dopamergic circuitry. When rats are sodium deplete, the taste system selectively drives dopamine signaling in response to NaCl, a homoeostasis-restoring stimulus, or LiCl, which is the taste system perceives as NaCl. The dopamine signal generated by NaCl and LiCl in sodium deplete conditions is potentially providing the reinforcement signal that is driving continued consumption of the stimuli.

It is important to note that here both NaCl and LiCl are readily consumed by sodium deplete rats in a brief access (10-min) two-bottle test [(Nachman, 1963b), Fig. 3.1]. However, LiCl is toxic (Radomski et al., 1950) and while naïve rats will voluntarily consume LiCl, their intake does plateau during a 10-min intake session (Nachman, 1963a). Furthermore, 10-min of LiCl intake (~9 ml) is enough to cause avoidance upon subsequent exposure (Nachman, 1963a). The learned aversion is due to the association between the taste and the visceral effects of the substance, a phenomenon known as conditioned taste aversion (CTA) (Garcia et al., 1955; Parker, 1984). Generalization CTA tests demonstrate that rats previously given LiCl will avoid both LiCl and NaCl but will consume KCl in future tests (Nachman, 1963a), supporting the discrimination of salts by taste that my FSCV data reflect.

The FSCV test session that was conducted involved administration 2 ml of an intraoral solution (one of which was LiCl) to naïve animals over the course of a 10-min session. Work of

Nachman suggests that the post-ingestive effects of oral LiCl are not perceived until rats consume 8 ml of LiCl (Nachman, 1963a). It is therefore unlikely that the malaise-inducing effects of LiCl confounded the FSCV data collected. Future studies that administer larger volumes of LiCl over a longer period of time should be conducted in order to examine the post-ingestive effects of LiCl on dopamine signaling across a testing session. It is known that LiCl itself (Fortin et al., 2016) as well as tastes associated with systemically delivered LiCl-induced illness suppress NAc dopamine signaling, even if the taste was initially palatable and evoked an increase in dopamine signaling (McCutcheon et al., 2012). It is likely that in tests following oral LiCl-induced illness, both LiCl and the generalized taste of NaCl would also elicit dopamine pauses.

I also observed state and taste-specific dopamine signaling when manipulating thirst. An increase in dopamine concentration within the NAc shell (Fig. 3.4 C-D) was observed to water in Water Restricted but not *Ad Libitum* watered rats (Fig. 3.3). Intraoral infusion of 0.45 M NaCl did not evoke a significant increase in dopamine concentration from baseline in Water Restricted animals (Fig. 3.3), supporting the taste-specificity of dopamine signaling. While a significant increase in dopamine concentration did not occur, an increase in dopamine concentration was observed. The response is much smaller than that generated by water, although the magnitude of the two responses was not compared due to the preferred within-subjects nature of FSCV experiments. A few potential explanations for this increase exist. Firstly, the NaCl solution was water-based. The increase in dopamine concentration was potentially a response to the water, the physiologically required taste stimuli, and not the NaCl. Unfortunately, the design of this experiment makes dissociation of the two stimuli impossible.

Additionally, Weisinger and colleagues have shown that 24-hr water deprivation induces an appetite for hypertonic NaCl in rats (McKinley et al., 1983; Weisinger et al., 1985). Decreased food intake by water restricted rats was controlled for in these experiments and therefore does not account for the sodium appetite. One potential explanation is that rats maintained on an adequate sodium diet have increased sodium excretion when water deprived, perhaps to buffer increased plasma sodium and osmolality caused by water deprivation (McKinley et al., 1983). The non-significant increase in dopamine to NaCl in Water Restricted rats is potentially due to the physiologic need for NaCl. The salt appetite under water restricted conditions was specific to sodium chloride (Weisinger et al., 1985). While I did not test other salt solutions (i.e. KCl) for their ability to evoke dopamine responses in Water Restricted rats, the intake results of Weisinger suggest that no increase would be observed. As clearly observed using sodium appetite (Fig. 3.2), dopamine responses in states of physiologic need are taste-specific.

The circuit by which tastes are orally processed and the brain is fairly well understood (Rolls, 2015). However, the circuit by which taste stimuli, including the tastes of NaCl, are communicated to mesolimbic circuitry to drive dopamine responses and motivated behavior is unknown. Taste encoding projections to mesolimbic circuitry likely come from forebrain nuclei, as chronic decerebrate rats do not appropriately respond to the taste of NaCl in a sodium deplete state (Grill et al., 1986). Nuclei of the ventral taste pathway which are necessary for expression of a sodium appetite include the lateral hypothalamus and amygdala (Wolf and Quartermain, 1967; Cox et al., 1978; Pfaffmann et al., 1977). Future studies investigating these two candidate nuclei are necessary to reveal the mechanism by which the taste of NaCl drives dopamine signaling during sodium appetite. Once identified, optogenetic, chemogenetic and

pharmacological methods can be used to explore modulation of motivated behaviors by taste circuitry that modulates dopamine signaling. The ability to understand the neural circuitry that modulates motivated behaviors has important implications for the treatment of disorders that are potentiated by maladaptive motivated behaviors (obesity, drug addiction).

Figure 3.1

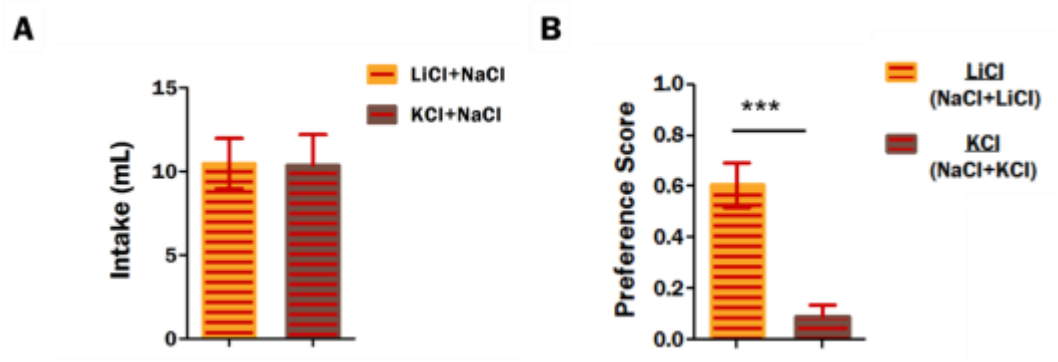

Figure 3.1: Sodium Deplete rats have no preference for LiCl over NaCl but prefer NaCl over KCl. **A,** During a 10- minute two-bottle test, Sodium Deplete rats presented with LiCl and NaCl [orange and red striped bars (*n*=15)] consume the same cumulative amount of liquid as rats presented with KCl and NaCl [brown and red striped bars (*n*=14)]. ***B,*** Sodium Deplete rats presented with LiCl and NaCl [orange and red striped bars (*n*=15)] consume similar amounts of the two solutions during a 10-minute two-bottle test. A preference score (for the non-sodium salt) of 0.5 is reflective of equal consumption of the two solutions. Sodium Deplete rats presented with KCl and NaCl [brown and red striped bars (*n*=14)] consume almost exclusively NaCl during a 10-minute two-bottle test. A preference score (for the non-sodium salt) of 0 is reflective of exclusive consumption of NaCl. The average preference score (mean ± SEM) for the group that received LiCl and NaCl was significantly different than the preference score for the group that received KCl and NaCl. *p<0.001.

Figure 3.2

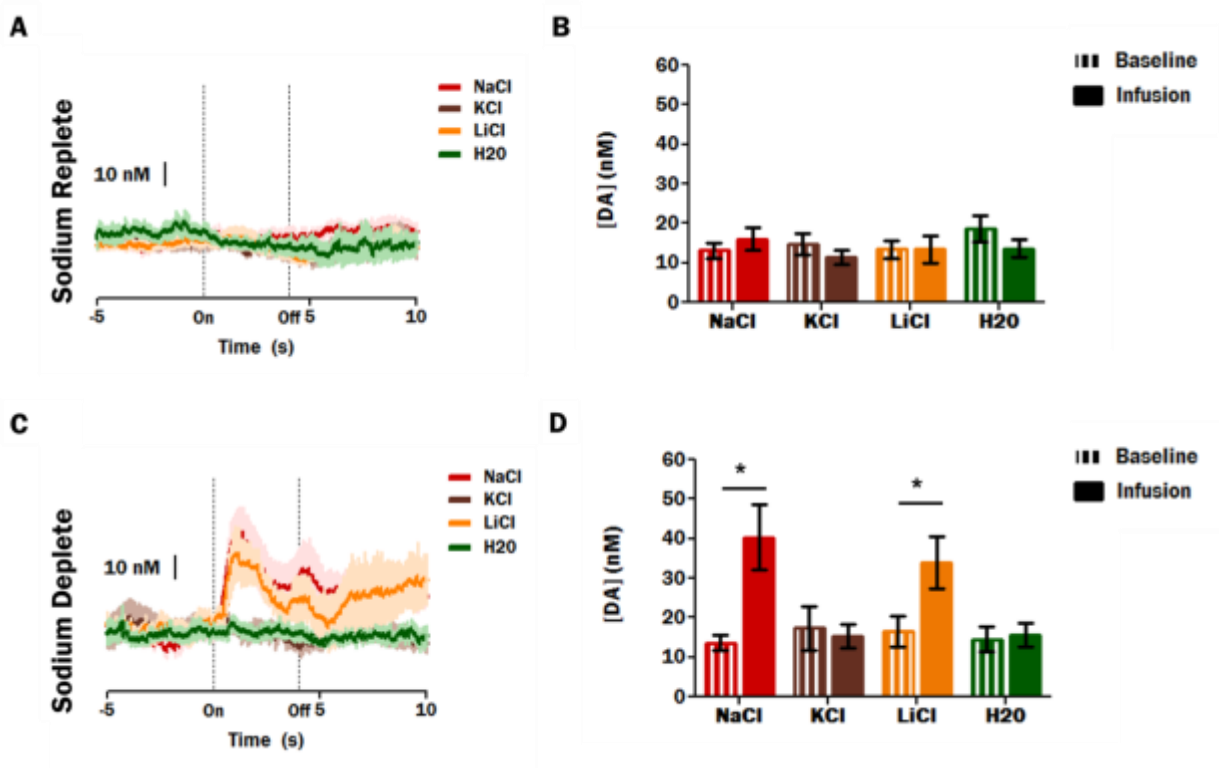

Figure 3.2: Phasic dopamine signaling evoked by salt solutions is taste-dependent. ***A,*** Average dopamine concentration response in Sodium Replete (*n*=23) rats during the 15 seconds (x-axis) surrounding the onset (On) and offset (Off) a 4-second intraoral infusion of a 0.15 M solution of NaCl [red trace (*n*= 6)], KCl [brown trace (*n*=6)], LiCl [orange trace (*n*=6)] or distilled water [green trace (*n*=5)]. ***B,*** Average dopamine concentration during the 5-second baseline period (Baseline, striped bars) vs the 4-second infusion period (Infusion, solid bars) of a 0.15 M salt solution (NaCl=red bars, KCl=brown bars, LiCl=orange bars) or distilled water (green bars) in Sodium Replete rats. ***C,*** Same as *A* in Sodium Deplete [*n*=22; NaCl (*n*=6), KCl (*n*=5), H2O (*n*=6), LiCl (*n*=5)] rats. ***D,*** Same as *B* in Sodium Deplete rats. For *A*-*D*, average dopamine concentration (mean ± SEM) is representative of 10 intraoral infusion trials. *p<0.05.

Figure 3.3

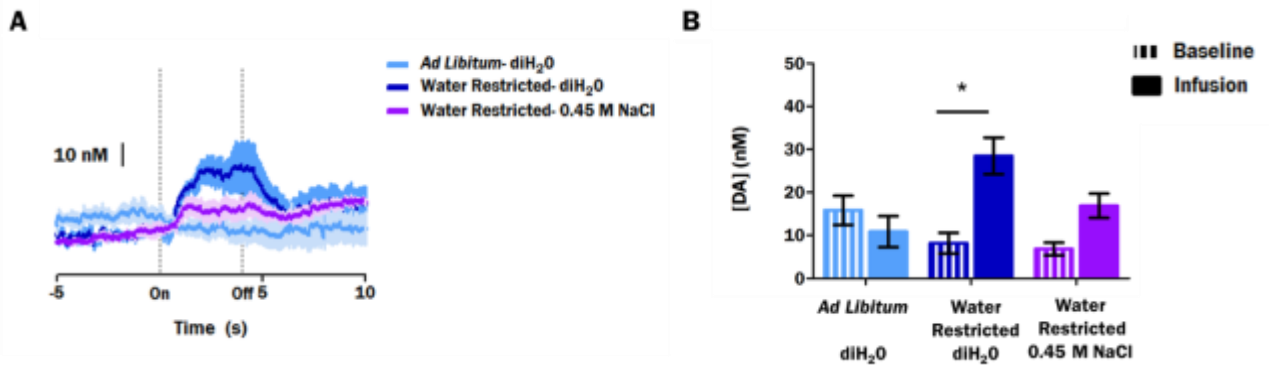

Figure 3.3: Phasic dopamine signaling evoked by water is state dependent and stimulus specific. ***A,*** Average dopamine concentration response surrounding the onset (On) and offset (Off) a 4-second intraoral infusion in *Ad Libitum* watered rats receiving intraoral water [light blue trace (*n*=5)], Water Restricted rats receiving intraoral water [royal blue trace (*n*=5)], and Water Restricted rats receiving intraoral 0.45M NaCl (purple trace (*n*=5)]. ***B,*** Average dopamine concentration during the 5-second baseline period (Baseline, striped bars) vs the 4-second infusion period (Infusion, solid bars) of a water solution (light and royal blue bars) or 0.45M NaCl (purple bars) solution in either *Ad Libitum* or Water Restricted rats. For *A*-*B*, average dopamine concentration (mean ± SEM) is representative of 10 intraoral infusion trials. There were no baseline differences between any of the three tested conditions. * $p<0.05$.

Figure 3.4

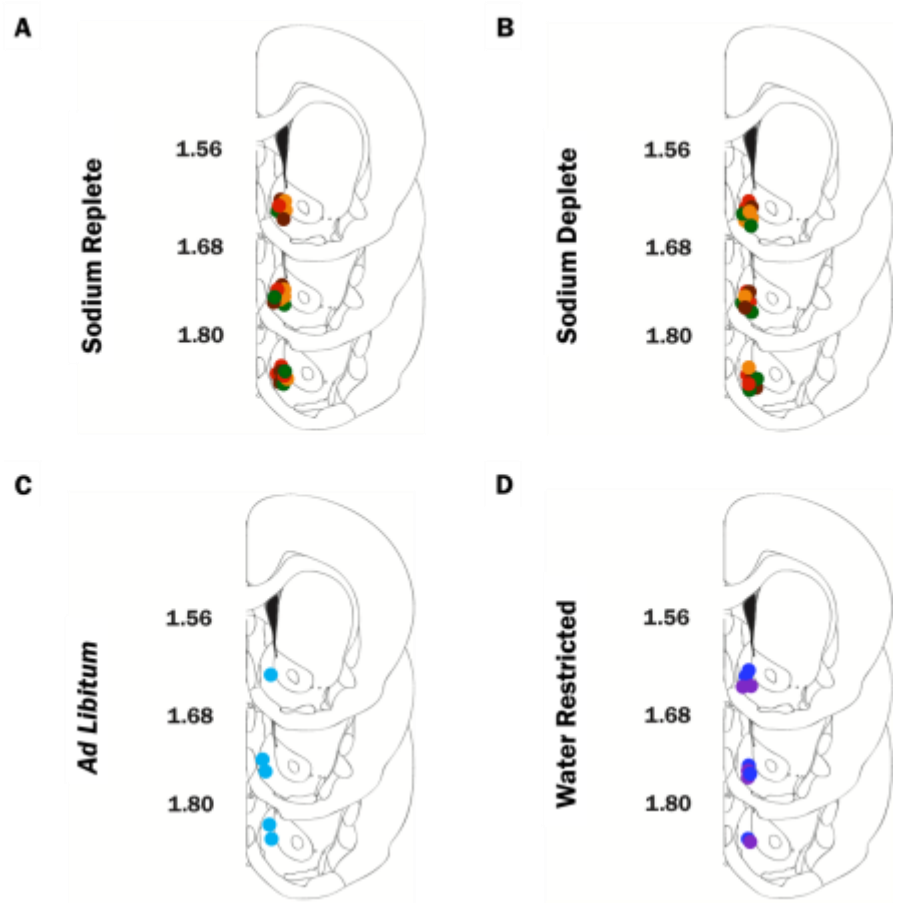

Figure 3.4: Summary of Nac shell recording sites. Approximate sites of FSCV recording are depicted as circles on coronal sections modified from Paxinos and Watson (2007). Numbers to the left indicate approximate distance from bregma. **A,** Recording locations from Sodium Replete (*n*=23) rats infused with 0.15M NaCl [red circles (*n*=6)], KCl [brown circles (*n*=6)], LiCl [orange circles (*n*=6)] or distilled water [green circles (*n*=5)]. *B,* Recording locations from Sodium Deplete (*n*=22) rats infused with 0.15M NaCl [red circles (*n*=6)], KCl [brown circles (*n*=5)], LiCl [orange circles (*n*=5)] or distilled water [green circles (*n*=6)]. ***C,*** Recording locations from *Ad Libitum* watered rats infused with water [light blue circles (*n*=5)]. ***D,*** Recording locations from Water Restricted rats infused with water [royal blue circles (*n*=5)] or Water Restricted rats infused with 0.45M NaCl [purple circles (*n*=5)].

Chapter IV

Furosemide-induced sodium depletion increases c-Fos expression in FoxP2 neurons of the pre-LC and PBel-inner

A. Introduction

Motivated behavior directed at stimuli is modulated by physiological state. Understanding the neurobiological underpinnings of this phenomenon is important, as modulation of physiological state represents a potential way to curb maladaptive motivated behaviors such as obesity and addiction. Progress has been made on this front through investigation of the circuits that support increased drive to consume food during caloric deficit. However, the circuitry is complex and a complete understanding is complicated by the multiple nodes and circuits in the brain that process hormonal and visceral signals related to hunger and satiety. A powerful example of state-dependent motivated behavior is observed during sodium appetite, the drive to seek and consume NaCl under conditions of sodium deficit. Sodium appetite increases the motivation to obtain NaCl to the extent that animals will work for NaCl solutions that are of concentrations which sodium replete animals will actively avoid (Nachman, 1962; Quartermain et al., 1967). Central circuits that process the hormonal and visceral signals related to sodium appetite are perhaps better understood than those that process hunger and satiety. Still, the mechanism that generates motivated behavior observed upon sodium depletion remains uncertain but likely involves the mesolimbic dopamine system. If the dopamine system is involved, it must receive visceral information regarding body fluid homeostasis. The work presented in this chapter seeks to determine a potential circuit by which disruption of sodium balance is sensed and relayed to mesolimbic circuitry.

The visceral sensing of sodium need and consequent sodium appetite is, in part, driven by activation of 11-beta-hydroxysteriod dehydrogenase type 2 (HSD2) neurons (Geerling et al., 2006a; Jarvie and Palmiter, 2016) in the caudal NTS. These neurons represent a rare central population of neurons which sense elevated levels of circulating aldosterone during sodium deficit (Geerling et al., 2006b). Various manipulations of sodium balance which cause elevations in aldosterone all result in elevated c-Fos expression in NTS HSD2 neurons (Geerling et al., 2006b). Sodium- appetite responsive projections from HSD2 neurons include those to two pontine nuclei, the pre-LC and PBel-inner. Both of these sites receive direct glutamatergic projections from HSD2 neurons to a population of neurons cytochemically identified by their expression of the transcription factor FoxP2. The induction of c-Fos by sodium depletion in these FoxP2 containing neuron has been investigated only under the experimental manipulations of dietary sodium restriction. Neuronal activation in these two sites by means other than dietary deprivation has not been explored.

Here, we look to extend the findings of Geerling et al. by investigating c-Fos expression in FoxP2 neurons of the pre-LC and PBel-inner following sodium depletion induced by the diuretic furosemide. The pre-LC and PBel-inner represent two conduits of communication from HSD2 neurons to the VTA (Shin et al., 2011). Demonstrating furosemide-induced c-Fos activation in the pre-LC and Pbel-inner would position these two nuclei as candidates for relaying sodium need to mesolimbic circuitry to differentially drive dopamine signaling and potentially motivated behavior in sodium deplete or replete states.

B. Experimental Methods

1. Subjects

Adult, male Sprague Dawley rats (*n*=8) weighing ~350 g at the time of testing were used. Rats were individually housed in plastic cages with lights on from 7 AM to 7 PM. Prior to sodium depletion, animals were given *ad libitum* access to standard laboratory chow (2010 Teklad global 18% protein diet) and tap water. Details for housing as well as food and water access during the experiment can be found under "Deprivation Protocol". All animals tested were naïve to sodium deprivation or water restriction before the experiments were conducted. Animal care and use was in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* and was approved by the Institutional Animal Care and Use Committee at the University of Illinois at Chicago.

2. Sodium Depletion Protocol

Animals (*n*=4) were made sodium deplete by two, equal volume injections of the diuretic furosemide (20 mg/kg, 0.5 mL/kg, SC, Sigma Aldrich). Animals tested in a replete state (*n*=4) were injected with vehicle (100% DMSO) in place of furosemide. The two injections were spaced one hour apart. Animals were sacrificed 24 hours after the first injection. During the 24-hr depletion period, all animals were housed in hanging wire-bottomed cages to prevent the ingestion of urine.

Prior to injections, food and water were removed and the rats were weighed. Diuresis was evaluated by weighing the animal 2 hours following the first injection. Animals were deemed 'sodium deplete' if they lost at least 15 grams of body weight during the two-hour

period following furosemide injection. At this time, sodium deplete animals were provided *ad libitum* distilled water and sodium deficient diet (Teklad Sodium Deficient Diet). Sodium replete animals were provided with *ad libitum* water and standard laboratory chow.

3. Double Immunohistochemistry Protocol

Following the 24-hr sodium depletion protocol, Sodium Deplete (*n*=4) and Sodium Replete (*n*=4) animals were sacrificed for immunohistochemistry. Rats were deeply anesthetized with sodium pentobarbital (100 mg/kg; Sigma-Aldrich) before being transcardially perfused with 0.9% saline followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer (Sigma-Aldrich, St Louis, MO), pH 7.4. Brains were removed and stored in 4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4 for 24 hours before being transferred to 20% sucrose. Brains were cut on a cryostat (35 μm) into three serial set.

Coronal sections through the pons were processed for immunohistochemical detection of fluorescent FoxP2 and c-Fos. Free floating sections were washed three times with a 0.1 M sodium phosphate buffer. They were next incubated for one hour at room temperature in a blocking solution containing 3% normal donkey serum (Jackson ImmunoResearch) and 0.3% Triton-X (Sigma-Aldrich, St Louis, MO) in 0.1 M sodium phosphate buffer. Sections were then incubated overnight at room temperature in an antibody cocktail [1:2500 sheep anti-FoxP2 (R&D systems) and 1:2000 rabbit anti-c-Fos, (Cell Signaling)] containing 3% normal donkey serum and 0.3% Triton-X.

After the overnight primary antibody incubation, sections were washed three times with 0.1 M sodium phosphate buffer. They were next incubated for four hours at room

temperature in a secondary antibody [1:500 of both Cy3 donkey anti-sheep and Alexa Fluor 488 donkey anti-rabbit (Jackson ImmunoResearch)] solution in 3% normal donkey serum and 0.3% Triton-X. Sections were washed three times with a 0.1 M sodium phosphate buffer before being wetmounted onto Superfrost Plus microscope slides (Fisher Scientific) for imaging. Dry coronal sections were imaged under 200X magnification on a Olympus FV1000 microscope. Separate photographs of were taken of FoxP2 and c-Fos expression under red and green fluorophore excitation using an X-Cite XLED1 light source (Excelitas Technologies).

4. <u>Cell Counts and Data Analysis</u>

Three sections per animal for each region of interest were chosen to count/quantify. Sections were chosen based on the anatomical location of FoxP2 neurons, which cytochemically define the pre-LC and PBel-inner (Geerling et al., 2011). For these sections, Adobe Photoshop was used to merge images taken under red (FoxP2) and green (c-Fos) flurophore excitation. A counting boundary for each region of interest was drawn around the pre-LC and PBel. For the pre-LC, a circle with a diameter of 400 μM was positioned with its lateral edge bordering the most medial region of the mesencephalic trigeminal nucleus. For the PBel-inner, a rectangle (350 μM x 210 μM), was positioned along the ventral border of the superior cerebellar peduncle with its medial edge bordering the most lateral region of the peduncle. The counting boundaries for both the pre-LC and PBel-inner are slightly more restrictive, yet overlap, with previously published reports (Geerling et al., 2011). Cell counts were made for FoxP2, c-Fos and double-labeled ($FoxP2^+/c\text{-}Fos^+$) neurons in both regions of interest (pre-LC and Pbel-inner). Counts were made in Adobe Photoshop and entered into a Microsoft Excel spreadsheet. All

comparisons were made using two-tailed Student's t-tests. Individual data from each animal and group data are show in Table 5.1 A-B. All group data are expressed as mean ± SEM.

C. Results

1. Furosemide-induced sodium depletion increases c-Fos expression within FoxP2 neurons of the pre-LC

Expression of FoxP2 and c-Fos was observed in the pre-LC of both Sodium Replete and Sodium Deplete rats (Table 4.1 A and Fig. 4.1 A). Sodium Replete and Sodium Deplete rats had similar expression profiles of FoxP2 neurons in the pre-LC (134.0 ± 6.45 vs 156.5 ± 17.23 neurons for Sodium Replete vs Depelte rats , resectively; $p>0.05$; Table 4.1 A [$FoxP2^+$ (total)] and Fig. 4.1 A-B). Sodium depletion caused an increase in pre-LC c-Fos expression (100.5 ± 7.66 vs 12.3 ± 3.47 neurons for Sodium Deplete vs Replete rats, respectively; $p<0.001$; Table 4.1 A [c-Fos^+ (total)] and Fig. 4.1 A-B). The increase in c-Fos expression was observed within pre-LC FoxP2 expressing cells such that there was a significantly higher percentage of FoxP2 neurons that co-expressed c-Fos ($FoxP2^+/c\text{-}Fos^+$) in Sodium Deplete rats compared to Sodium Replete rats (63.0 ± 3.14 vs $5.7 \pm 2.11\%$, respectively; $p<0.001$; Fig. 4.1 C). Consistent with previous reports (Geerling et al., 2011), almost all c-Fos expressing neurons of the pre-LC in Sodium Deplete animals were $FoxP2^+$ ($96.8 \pm 0.90\%$).

2. Furosemide-induced sodium depletion increases c-Fos expression in FoxP2 neurons of the PBel-inner

Expression of FoxP2 and c-Fos was observed in the PBel-inner of both Sodium Replete and Sodium Deplete rats (Table 4.1 B and Fig. 4.2 A). Sodium Replete and Sodium Deplete rats had similar expression profiles of FoxP2 neurons in the PBel-inner (138.5 $\pm$ 13.72 vs 168.3 $\pm$ 7.84 neurons for Sodium Repelte vs Depelte rats, respectively; $p>0.05$; Table 4.1 B [$FoxP2^+$ (total)] and Fig. 4.2 A-B). Sodium depletion caused an increase in PBel-inner c-Fos expression (61.5 $\pm$ 4.44 vs 7.3 $\pm$ 2.29 neurons for Sodium Deplete vs Replete rats, respectively; $p<0.001$; Table 4.1 B [$c\text{-}Fos^+$ (total)] and Fig. 4.2 A-B). The increase in c-Fos expression was observed within PBel-inner FoxP2 expressing cells such that there was a significantly higher percentage of FoxP2 neurons that co-expressed c-Fos ($FoxP2^+/c\text{-}Fos^+$) in Sodium Deplete rats compared to Sodium Replete rats (35.4 $\pm$ 3.66 vs 3.13 $\pm$ 1.02% for Sodium Depelte vs Repelte rats, respecitvely; $p<0.001$; Fig. 4.2 C). Consistent with previous reports (Geerling et al., 2011), almost all c-Fos expressing neurons of the PBel-inner in Sodium Deplete animals were $FoxP2^+$ (95.9 $\pm$ 1.48%).

D. Discussion

Here I identify two central populations of sodium appetite responsive neurons that may represent nodes in a circuit by which physiological state modulates dopamine signaling. I restrict my investigation to two pontine cell groups that have been implicated in sodium appetite, the pre-LC and PBel-inner, both immunohistochemically defined by their common marker gene, FoxP2. I use double immunohistochemistry for Foxp2 and c-Fos to demonstrate that furosemide-induced sodium depletion results in neuronal activation of almost all $Foxp2^+$ pre-LC and PBel-inner cells.

Previous work of Geerling and colleagues has shown that within the dorsolateral pons, there are two populations of cells that are cytochemically defined by the expression of the transcription factor FoxP2 (Geerling et al., 2011). These cells are anatomically restricted to the pre-LC and PBel-inner. Using immunohistochemistry for FoxP2, I was able to label these cell populations in both Sodium Replete and Sodium Deplete rats (Fig. 4.1 A and 4.2 A). As FoxP2 is a constitutively active transcription factor, I did not expect body fluid homeostasis to effect expression of FoxP2. Indeed, Sodium Replete and Deplete rats had similar FoxP2 expression within both areas of interest (Fig. 4.1 B and 4.2 B). Successful identification of $FoxP2^{+}$ cells suggests that FoxP2 is a reliable marker for phenotypically specific neurons within the pons and allows for further examination of their responsivity to furosemide-induced sodium depletion.

Both pre-LC and PBel-inner $Foxp2^{+}$ neurons have been shown to be sodium appetite responsive under conditions of dietary-induced sodium depletion (Geerling et al., 2011). Eight days of dietary sodium restriction is one of many ways to induce a sodium appetite in rats. However, chronic sodium deprivation is associated with behavioral plasticity and changes in a variety of brain regions (Na et al., 2007; Sakai et al., 1989; Roitman et al., 2002). Neural changes resulting from extended sodium deprivation may have implications for systems beyond those that are involved in generating motivation. A more rapid method of sodium depletion that has been used to induce a sodium appetite is use of the diuretic furosemide. However, furosemide-induced sodium depletion has not been used previously to investigate FoxP2 neuron activation by sodium depletion. Here, I extend the findings on Geerling and collegues by showing that furosemide-induced sodium depletion activates pre-LC and PBel-inner $Foxp2^{+}$ neurons. I demonstrated this using expression of the early immediate gene c-Fos, which was successfully

labeled within the pre-LC and PBel-inner (Fig. 4.1 A and 4.2 A). Within both areas of interest, there were drastically different expression levels of c-Fos between Sodium Replete and Sodium Deplete groups of rats. Sodium Deplete rats had significantly more c-Fos labeling (Fig. 4.1 B and 4.2 B). No Sodium Replete animal displayed a cluster of c-Fos activated cells within either nucleus of the pons. Expression of c-Fos in both groups, although scant in Sodium Replete rats, was highly restricted to FoxP2^{+} neurons, indicated by the limited number of FoxP2^{-}/ c-Fos^{+} expressing neurons (Table 4.1). The percentage of c-Fos expressing neurons that also express FoxP2 was 96.8% within the pre-LC and 95.9% within the PBel-inner for Sodium Deplete rats. Similar results (86.3 and 91.0% for the pre-LC and PBel-inner, respectively) have been reported when sodium appetite was induced with dietary sodium restriction (Geerling et al., 2011). While dietary sodium depletion may have more ecological validity, the similar c-Fos expression profiles between the two models of deprivation suggests that furosemide is activating similar circuits as dietary sodium depletion. My findings support acute sodium depletion by furosemide as an acceptable method of sodium depletion for neuroanatomical investigations.

In order to determine if sodium depletion affects the neuronal activation of pre-LC and PBel-inner cells, chemically defined by their FoxP2 expression, I analyzed the percentage of FoxP2^{+} cells that were FoxP2^{+} /c-Fos^{+}. Within the population of co-labeled (FoxP2^{+} /c-Fos^{+}) cells, there was a marked difference between treatment groups in both the PBel-inner and pre-LC (Table 4.1). Sodium Depletion significantly increased the expression of c-Fos within the FoxP2^{+}population in both brain regions (Fig. 4.1 C and 4.2 C). The percentages obtained for this analysis were also comparable to those found in Geerling's work using dietary induced sodium depletion. The similar co-expression profiles revealed by two very different methods of sodium

deprivation suggests that both furosemide and dietary-induced sodium depletion have physiologic consequences that ultimately activate similar neural pathways. Tracing studies have revealed inputs, the majority of which arise from NTS HSD2 neurons, and forebrain outputs of FoxP2 expressing neurons in both the pre-LC and PBel-inner. Further work is necessary to determine the functional relevance of this circuit.

This work positions furosemide is a suitable experimental means for inducing a sodium appetite in future work investigating the neuroanatomy of sodium appetite. Additionally, the findings highlight two nodes, the pre-LC and PBel-inner, in a circuit that responds to disruptions of sodium balance. Identifying these centers is the first step towards investigating a circuit that is relays information regarding body fluid homeostasis to mesolimbic circuitry. This circuit is currently unknown and represents an entry point to understanding the influence of physiological state on motivated behavior. Future immunohistochemical and tracing work will be required to investigate if PBel-inner and pre-LC Foxp2 neurons project directly to the VTA. If direct projections are revealed, the proposed circuit can be experimentally manipulated to model changes in body fluid homeostasis and potentially modulate dopamine signaling underlying sodium appetite as well as other motivated behaviors.

Table 4.1

A

	pre-LC				
	FoxP$^+$ (total)	c-Fos$^+$ (total)	FoxP2$^+$ c-Fos$^+$	FoxP2$^+$ c-Fos$^-$	FoxP2$^-$ c-Fos$^+$
Replete 1	131	20	10	121	10
Replete 2	153	16	16	137	0
Replete 3	127	8	5	122	3
Replete 4	125	5	1	124	4
Mean:	**134.0**	**12.3**	**8.0**	**126.0**	**4.3**
SEM:	**6.45**	**3.47**	**3.24**	**3.72**	**2.10**
Deplete 1	197	110	107	90	3
Deplete 2	136	89	85	51	4
Deplete 3	121	86	82	39	4
Deplete 4	172	117	116	56	1
Mean:	**156.5**	**100.5**	**97.5**	**59.0**	**3.0**
SEM:	**17.23**	**7.66**	**8.31**	**10.93**	**0.71**

B

	PBel-inner				
	FoxP$^+$ (total)	c-Fos$^+$ (total)	FoxP2$^+$ c-Fos$^+$	FoxP2$^+$ c-Fos$^-$	FoxP2$^-$ c-Fos$^+$
Replete 1	104	6	3	101	3
Replete 2	166	14	10	156	4
Replete 3	130	5	3	127	2
Replete 4	154	4	2	152	2
Mean:	**138.5**	**7.3**	**4.5**	**134.0**	**2.8**
SEM:	**13.72**	**2.29**	**1.85**	**12.73**	**0.48**
Deplete 1	188	59	58	130	1
Deplete 2	170	50	47	123	3
Deplete 3	150	69	64	86	5
Deplete 4	165	68	67	98	1
Mean:	**168.3**	**61.5**	**59.0**	**109.3**	**2.5**
SEM:	**7.84**	**4.44**	**4.42**	**10.36**	**0.96**

Table 4.1: Expression of FoxP2 and c-Fos in the pre-LC and PBel-inner of both Sodium Replete and Sodium Deplete rats. **A,** Within the pre-LC, FoxP2 (FoxP2$^+$ total) and c-Fos (c-Fos$^+$ total) immunopositive neurons were quantified in both Sodium Replete (*n*=4) and Sodium Deplete (*n*=4) rats. Individual data along with the mean $\pm$ SEM is presented for each expression profile observed. **B,** Within the PBel-inner, FoxP2 (FoxP2$^+$ total) and c-Fos (c-Fos$^+$ total) immunopositive neurons were quantified in both Sodium Replete (*n*=4) and Sodium Deplete (*n*=4) rats. Individual data along with the mean $\pm$ SEM is presented for each expression profile observed.

Figure 4.1

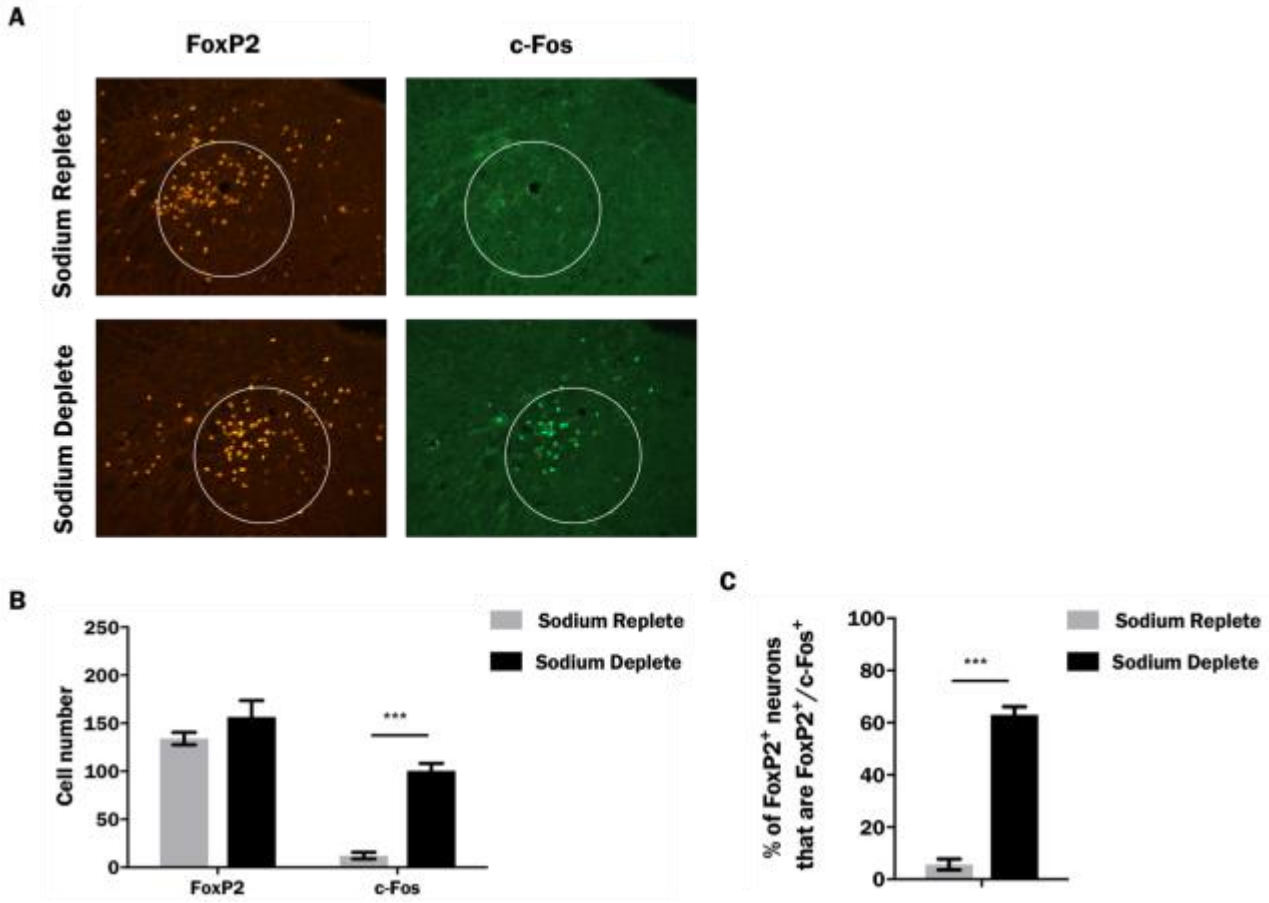

Figure 4.1: Expression of FoxP2 and c-Fos within the pre-LC of Sodium Replete and Sodium Deplete rats. ***A.*** Representative photoimages through the pre-LC of a Sodium Replete (top row) and a Sodium Deplete (bottom row) rat showing FoxP2 (red) and c-Fos (green) immunoreactive neurons. White circles are the defined area of interest selected for quantification within the pre-LC. ***B.*** Average cell counts (mean ± SEM) of FoxP2 and c-Fos expressing neurons in the pre-LC of Sodium Replete (*n*=4) and Sodium Deplete (*n*=4) rats. ***C.*** c-Fos expression within FoxP2 expressing cells of the pre-LC is increased by sodium depletion. Data are expressed as average (mean ± SEM) of the percent of FoxP2 expressing ($FoxP2^+$) neurons that co-express c-Fos ($FoxP2^+/c\text{-}Fos^+$) in the pre-LC of Sodium Replete (*n*=4) and Sodium Deplete (*n*=4) rats. ***p<0.001.

Figure 4.2

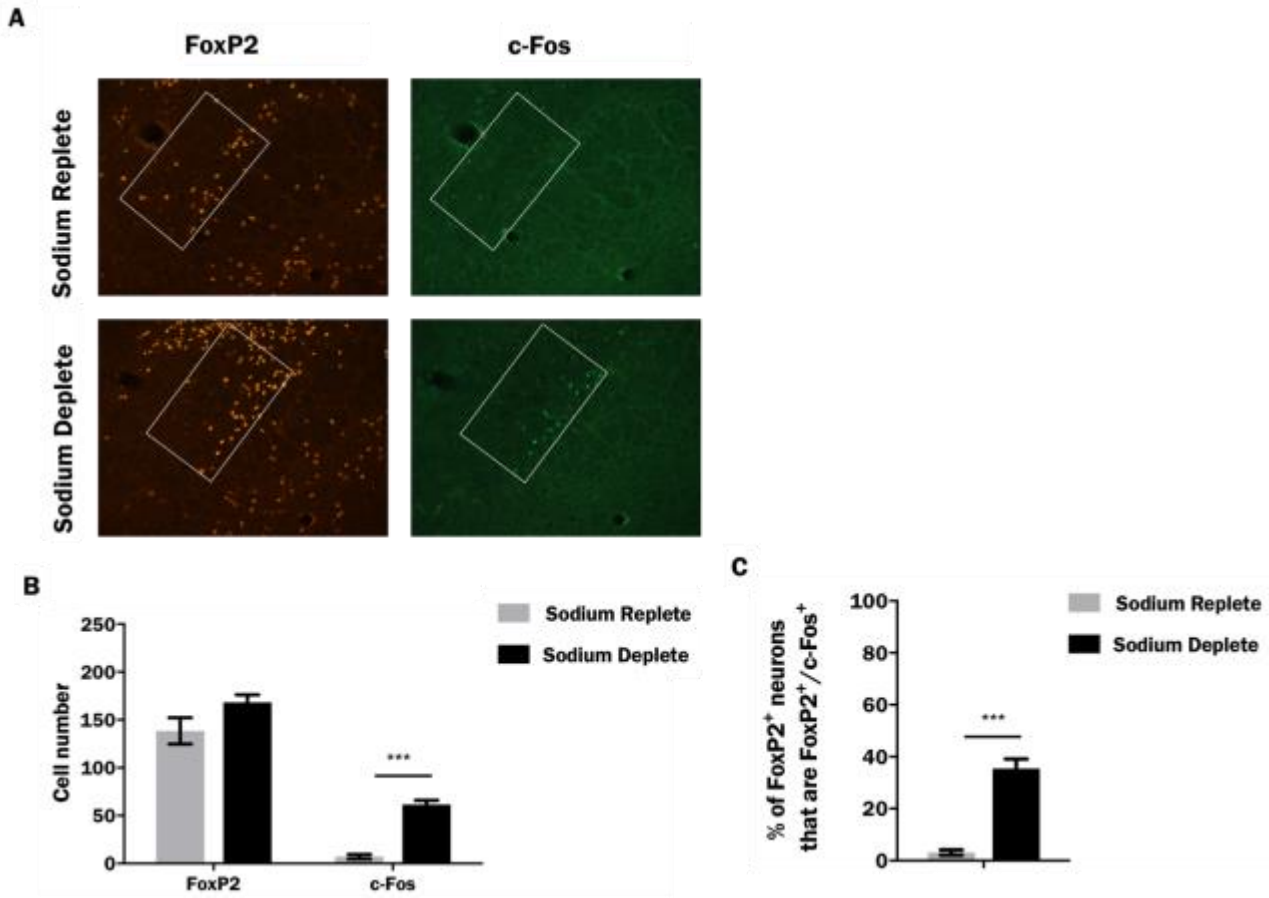

Figure 4.2: Expression of FoxP2 and c-Fos within the PBel-inner of Sodium Replete and Sodium Deplete rats. ***A.*** Representative photoimages through the PBel-inner of a Sodium Replete (top row) and a Sodium Deplete (bottom row) rat showing FoxP2 (red) and c-Fos (green) immunoreactive neurons. White circles are the defined area of interest selected for quantification within the PBel-inner. ***B.*** Average cell counts (mean ± SEM) of FoxP2 and c-Fos expressing neurons in the PBel-inner of Sodium Replete (*n*=4) and Sodium Deplete (*n*=4) rats. ***C.*** c-Fos expression within FoxP2 expressing cells of the PBel-inner is increased by sodium depletion. Data are expressed as average (mean ± SEM) of the percent of FoxP2 expressing ($FoxP2^+$) neurons that co-express c-Fos ($FoxP2^+/c\text{-}Fos^+$) in the PBel-inner of Sodium Replete (*n*=4) and Sodium Deplete (*n*=4) rats. ***p<0.001.

Chapter V

FoxP2^{+}/ c-Fos^{+} neurons of the pre-LC and PBel-inner project to the VTA

A. Introduction

The motivational value of rewards (i.e. food or drugs) can be modulated by changes in physiological state [see (Fulton, 2010; Engel and Jerlhag, 2014) for review]. Understanding how motivated behavior is shaped by physiological state requires an anatomical framework that identifies neuronal populations of cells that are modulated by homeostasis and project to sites implicated in driving motivated behavior. Phasic dopamine release in the NAc has been implicated in reward and reinforcement behaviors. Furthermore, it has been postulated that changes in motivation modulate the excitability of VTA dopamine neuron cell bodies (Abizaid et al., 2006; Branch et al., 2013; Hommel et al., 2006) and consequent dopamine release in the NAc (Krügel et al., 2003). Some sites which communicate physiological state to mesolimbic circuitry have been identified (Alhadeff et al., 2012), but this remains an understudied area of research. As such, these experiments are aimed at identifying sites which monitor body fluid homeostasis and project to the VTA.

I chose to use sodium appetite, the drive to seek and consume NaCl under conditions of sodium deficit, as the manipulation of homeostasis. Sodium appetite causes an innate drive to consume sodium chloride that is state dependent and taste specific (Nachman, 1962; Handal, 1965; Epstein and Stellar, 1955). Sodium appetite provides an ideal platform to study potential pathways by which VTA dopamine neuron excitability is modulated by physiological state.

The visceral sensing of sodium need and consequent sodium appetite is, in part, driven by activation of 11-beta-hydroxysteriod dehydrogenase type 2 (HSD2) neurons (Geerling et al., 2006a) in the caudal NTS. HSD2 neurons sense elevated levels of circulating aldosterone during sodium deficit, at which time they become c-Fos activated (Geerling et al., 2006b). Two pontine nuclei, the pre-LC and Pbel-inner receive glutamatergic input from HSD2 neurons and also become c-Fos activated during sodium depletion (Geerling et al., 2011). These sites, cytochemically defined by the expression of the transcription factor FoxP2 (Geerling et al., 2011), represent two potential conduits by which HSD2 neurons transmit visceral information regarding body fluid homeostasis to the VTA to modulate dopamine neuron excitability. Indeed, both the pre-LC and PBel-inner FoxP2^{+} neurons send direct projections to the VTA (Shin et al., 2011). Whether these projections communicate sodium balance is currently unknown and is the focus of these experiments. In the previous chapter, I found that the pre-LC and PBel-inner are responsive to furosemide-induced sodium depletion. Here, I seek to determine if depletion-responsive neurons in the pre-LC and PBel-inner send monosynaptic projections to the VTA. To compare Sodium Deplete vs Sodium Replete c-Fos expression in FoxP2 neurons that project to the VTA, I will inject the retrograde tracer Fluorogold (FG) into the VTA and use triple immunohistochemistry to stain for Fluorogold, FoxP2 and c-Fos. I hypothesize that both sites will show enhanced c-Fos expression within the population of interest (FoxP2^{+}/FG^{+}), and will therefore represent candidates for relaying sodium need to mesolimbic circuitry to differentially drive dopamine signaling in sodium deplete and replete states.

B. Experimental Methods

1. Subjects

Adult, male Sprague Dawley rats (*n*=7) weighing ~350 g at the time of testing were used. Rats were individually housed in plastic cages with lights on from 7 AM to 7 PM. Prior to sodium depletion, animals were given *ad libitum* access to standard laboratory chow (2010 Teklad global 18% protein diet) and tap water. Details for housing as well as food and water access during the experiment can be found under "Deprivation Protocol". All animals tested were naïve to sodium deprivation or water restriction before the experiments were conducted. Animal care and use was in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* and was approved by the Institutional Animal Care and Use Committee at the University of Illinois at Chicago.

2. Surgery

Animals were deeply anesthetized with intraperitoneal (IP) ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (20 mg/kg). A trephine drill bit was used to remove the skull over the VTA (AP: -5.6 mm, ML: -0.5 mm). Iontophoresis was used to inject the retrograde tracer Fluorogold (Fluorochrome) so as to minimize the spread of the tracer. A custom injector was used to iontophoretically (5 μA, 7 s on/7 s off, 10 min) deliver 2% Fluorogold to the VTA (DV: 8 mm from skull) using a current pump (Digital Midgard Precision Current Source, Stoelting Co). Injectors were made by using a glass puller to create a taper on a capillary tube (1.0 mm x 0.5 mm, 4", A-M Systems). The taper was cut under 10 X magnification using a scalpel such that the diameter of the taper was 20 mm. After the current was terminated, 5 min were allowed

for residual diffusion before the injector was removed from the brain. The scalp of the rat was sutured closed. Following surgery, rats were injected with subcutaneous (SC) meloxicam (1 mg/Kg) . Rats were given 5-7 days of postoperative recovery time. All animals returned to pre-surgical body weight during this time.

3. Sodium Depletion Protocol

Animals (*n*=3) were made sodium deplete by two, equal volume injections of the diuretic furosemide (20 mg/kg, 0.5 mL/kg, SC, Sigma Aldrich). Animals tested in a replete state (*n*=4) were injected with vehicle (100% DMSO) in place of furosemide. The two injections were spaced one hour apart. Animals were sacrificed 24 hours after the first injection. During the 24-hr depletion period, all animals were housed in hanging wire-bottomed cages to prevent the ingestion of urine.

Prior to injections, food and water were removed and the rats were weighed. Diuresis was evaluated by weighing the animal 2 hours following the first injection. Animals were deemed 'sodium deplete' if they lost at least 15 grams of body weight during the two-hour period following furosemide injection. At this time, sodium deplete animals were provided *ad libitum* distilled water and sodium deficient diet (Teklad Sodium Deficient Diet). Sodium replete animals were provided with *ad libitum* water and standard laboratory chow.

4. Triple Immunohistochemistry Protocol

Following the 24-hr sodium depletion protocol, Sodium Deplete (*n*=3) and Sodium Replete (*n*=4) animals were sacrificed for immunohistochemistry. Rats were deeply anesthetized with sodium pentobarbital (100 mg/kg; Sigma-Aldrich) and then transcardially perfused with 0.9% saline followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer (Sigma-Aldrich), pH 7.4. Brains were removed and stored in 4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4 for 24 hours before being transferred to 20% sucrose. Brains were cut on a cryostat (35 μm) into three serial sets.

Coronal sections through the VTA were processed for native Flurogold fluorescence. Only rats with Fluorogold injection sites restricted to the VTA were included in subsequent analysis. Coronal sections through the pons were processed for immunohistochemical detection of fluorescent Fluorogold, FoxP2 and c-Fos. Free floating sections were washed three times with a 0.1 M sodium phosphate buffer. They were next incubated for one hour at room temperature in a blocking solution containing 3% normal donkey serum (Jackson ImmunoResearch) and 0.3% Triton-X (Sigma-Aldrich) in 0.1 M sodium phosphate buffer. Sections were then incubated overnight at room temperature in an antibody cocktail [1:2500 sheep anti-FoxP2 (R&D systems) and 1:2000 rabbit anti-c-Fos, (Cell Signaling)] containing 3% normal donkey serum and 0.3% Triton-X. Native fluorescence of Fluorogold (Fluorochrome) was examined. Antibodies for Fluorogold were, therefore, not utilized.

After the overnight primary antibody incubation, sections were washed three times with 0.1 M sodium phosphate buffer. They were next incubated for four hours at room temperature in a secondary antibody [1:500 of both Cy3 donkey anti-sheep and Alexa Fluor 488 donkey anti-rabbit (Jackson ImmunoResearch)] solution in 3% normal donkey serum and

0.3% Triton-X. Sections were washed three times with a 0.1 M sodium phosphate buffer before being wet mounted onto Superfrost Plus microscope slides (Fisher Scientific) for imaging. Dry coronal sections were imaged under 200X magnification on a Olympus FV1000 microscope. Separate photographs of were taken of Fluorogold, FoxP2 and c-Fos expression under blue, red and green fluorophore excitation, respectively, using an X-Cite XLED1 light source (Excelitas Technologies).

5. <u>Cell Counts and Data Analysis</u>

Three sections per animal for each region of interest were chosen to count/quantify. Sections were chosen based on the anatomical location of FoxP2 neurons, which cytochemically define the pre-LC and PBel-inner (Geerling et al., 2011). For these sections, Adobe Photoshop was used to merge images taken under blue (Fluorogold), red (FoxP2) and green (c-Fos) flurophore excitation. A counting boundary for each regions of interest was drawn around the pre-LC and PBel. For the pre-LC, a circle with a diameter of 400 μm was positioned with its lateral edge bordering the most medial region of the mesencephalic trigeminal nucleus. For the PBel-inner, a rectangle (350 μm x 210 μm), was positioned along the ventral border of the superior cerebellar peduncle with its medial edge bordering the most lateral region of the peduncle. The counting boundaries for both the pre-LC and PBel-inner are slightly more restrictive, yet overlap, with previously published reports (Geerling et al., 2011). Cell counts were made for FoxP2, c-Fos and double-labeled ($FoxP2^{+}$/$c\text{-}Fos^{+}$) neurons in both regions of interest (pre-LC and Pbel-inner). Counts were made in Adobe Photoshop and entered into a Microsoft Excel spreadsheet. All comparisons were made using two-tailed Student's t-tests.

Individual data from each animal and group data are show in Table 5.1 A-B. All group data are expressed as mean ± SEM.

C. Results

1. Furosemide-induced sodium depletion increases c-Fos expression in FoxP2 neurons of the pre-LC and PBel-inner

Furosemide-induced sodium depletion increased c-Fos expression within $FoxP2^+$ neurons of both the pre-LC and PBel-inner, replicating results described in the previous chapter. Expression of FoxP2 and c-Fos was observed in the pre-LC of both Sodium Replete and Sodium Deplete rats (Table 5.1 A and Fig. 5.2 A). Sodium Replete and Sodium Deplete rats had similar expression profiles of FoxP2 neurons [$FoxP2^+$ (total)] in the pre-LC (153.8 $\pm$ 12.35 vs 192.0 $\pm$ 27.02 neurons for Sodium Replete vs Deplete rats, respectively; $p>0.05$; Table 5.1 A and Fig. 5.2 A-B). Sodium depletion caused an increase in c-Fos expression [$c\text{-}Fos^+$ (total)] in the pre-LC (142.0 $\pm$ 11.24 vs 8.3 $\pm$ 3.40 neurons for Sodium Deplete vs Replete rats, respectively; $p<0.001$; Table 5.1 A and Fig. 5.2 A-B). The increase in c-Fos expression was observed within pre-LC FoxP2 expressing cells such that there was a significantly higher percentage of FoxP2 neurons that co-expressed c-Fos ($FoxP2^+/c\text{-}Fos^+$) in Sodium Deplete rats compared to Sodium Replete rats (71.7 $\pm$ 3.22 vs 3.61 $\pm$ 1.64% for Sodium Deplete vs Replete rats, respectively; $p<0.001$; Fig. 5.2 C). Consistent with previous reports (Geerling et al., 2011), almost all c-Fos expressing neurons of the pre-LC in Sodium Deplete animals were $FoxP2^+$ (95.4 $\pm$ 2.44%).

Expression of FoxP2 and c-Fos was observed in the PBel-inner of both Sodium Replete and Sodium Deplete rats (Table 5.1 B and Fig. 5.3 A). Sodium Replete and Sodium Deplete rats

had similar expression profiles of FoxP2 neurons [FoxP2$^+$ (total)] in the PBel-inner (132.5 $\pm$ 22.01 vs 142.0 $\pm$ 19.50 neurons for Sodium Replete and Deplete rats, respectively; p>0.05; Table 5.1 B and Fig. 5.3 A-B). Sodium depletion caused an increase in c-Fos expression [c-Fos$^+$ (total)] in the PBel-inner (69.7 $\pm$ 5.90 vs 10.8 $\pm$ 3.12 neurons for Sodium Deplete vs Replete rats, respectively, p<0.001; Table 5.1 A and Fig. 5.3 A-B). The increase in c-Fos expression was observed within PBel-inner FoxP2 expressing cells such that there was a significantly higher percentage of FoxP2 neurons that co-expressed c-Fos (FoxP2$^+$/c-Fos$^+$) in Sodium Deplete rats compared to Sodium Replete rats (49.1 $\pm$ 7.07 vs 5.6 $\pm$ 0.94% for Sodium Deplete vs Replete rats, respectively; p<0.001; Fig. 5.3 C). Consistent with previous reports (Geerling 2011), almost all c-Fos expressing neurons of the PBel-inner in Sodium Deplete animals were FoxP2$^+$ (97.3 $\pm$ 2.11%).

2. <u>FoxP2 neurons of the pre-LC and PBel-inner project to VTA</u>

Iontophoretically delivered Fluorogold within the VTA of Sodium Replete (*n*=4) and Sodium Deplete (*n*=3) rats (Fig. 5.1 A-B) successfully labeled cell bodies within the pre-LC and PBel-inner (Table 5.1A-B, Fig. 5.2, Fig. 5.3). There was no difference in the number of Fluorogold labeled cells between Sodium Replete and Sodium Deplete rats in either the pre-LC or PBel-inner (68.0 $\pm$ 4.26 vs 85.0 $\pm$ 10.26 neurons and 48.8 $\pm$ 7.36 vs 45.0 $\pm$ 3.79 neurons for Sodium Replete vs Deplete, respectively; p>0.05; Table 5.1, Fig. 5.2 B, Fig. 5.3 B). Similarly, there were no differences between Sodium Replete and Sodium Deplete rats in the percentage of Fluorogold expressing neurons that co-express FoxP2 in either the pre-LC or PBel-inner (67.2 $\pm$ 7.43 vs 86.4 $\pm$ 1.76% and 50.0 $\pm$ 16.68 vs 61.7 $\pm$ 25.89% for Sodium Replete vs Depelte rats, respectively; p>0.05; Fig. 5.4).

3. Furosemide-induced sodium depletion increases c-Fos expression in FoxP2 neurons of the pre-LC and PBel-inner that project to the VTA

Sodium depletion increases the percentage of Fluorogold and FoxP2 co-localized neurons ($FG^+/FoxP2^+$) that express c-Fos within both the pre-LC (88.6 ± 1.27 vs 6.8 ± 4.11% for Sodium Deplete vs Replete rats, respectively; $p<0.001$) and PBel-inner (75.2 ± 0.23 vs 25.6 ± 5.89% for Sodium Deplete vs Replete rats, respectively; $p<0.001$; Fig. 5.5).

D. Discussion

Previous work by our lab (unpublished, Chapter 4) and others (Geerling et al., 2011) has demonstrated that both the pre-LC and PBel-inner contain neuron that become c-Fos activated under conditions of sodium depletion. Here, I replicated the augmented c-Fos activation of neurons in these two areas of the brain, cytochemically defined by expression of FoxP2, by furosemide-induced sodium depletion using double immunohistochemistry (Fig. 5.2 C and 5.3 C). I continued on to find that the VTA is a projection target of the FoxP2 pre-LC and PBel-inner neurons (Fig. 5.4) and that projections from these neurons to the VTA are responsive to sodium deprivation (Fig. 5.5). Collectively, the studies described here identify the pre-LC and PBel-inner as relay nodes for the transmission of sodium-need viscerosensory information to the VTA. While further investigation is necessary, it is possible that the pre-LC and PBel-inner are part of a circuit that uses sodium balance to modulate dopamine neuron responsivity to taste stimuli (Fig. 5.6).

The focused investigation of the pre-LC and PBel-inner conducted here is supported by work which highlights these two areas of the brain as being highly implicated in sodium homeostasis. Both the pre-LC and PBel-inner become c-Fos activated following sodium depletion [(Geerling et al., 2011) and Fig. 5.2 and 5.3). Nearly all of the c-Fos neurons that are sodium appetite responsive also express FoxP2 (Table 5.1), a transcription factor which is constitutively active to the same degree in both Sodium Deplete and Replete animals (Fig. 5.2 B and 5.3 B). Therefore, expression of FoxP2 is a useful marker for the identification of pre-LC and PBel-inner neurons involved in detection of furosemide- induced sodium appetite.

Efferent connections between the pre-LC and PBel-inner were analyzed using the retrograde tracer Fluorogold. Iontophoretic injection of Fluorogold allowed for restricted infusion into the VTA of Sodium Replete and Deplete animals (Fig. 5.1). Only rats with VTA Fluorogold infusion were included in these studies (Fig. 5.1 B). Successful retrograde tracing from the VTA was indicated by expression of Flurogold labeled cell bodies within both the pre-LC (Fig. 5.2) and PBel-inner (Fig. 5.3). Colocalization of Fluorogold and FoxP2 indicated that the relevant populations of interest investigated here projected to the VTA (Table 5.1). Importantly, there were no differences in pre-LC or PBel-inner expression of FoxP2 within Fluorogold immunopositive cells of Sodium Replete and Sodium Deplete rats (Fig. 5.4). In line with my finding, previous reports have indicated the presence of $FoxP2^{+}$ pre-LC and PBel-inner projections to the VTA (Shin et al., 2011). However, this study was based on retrograde tracing results obtained from a single rat with optimal retrograde tracer injection. Furthermore, this study did not examine whether or not the two projections to the VTA were sodium appetite responsive.

I next examined the sodium appetite responsivity of the pre-LC and PBel-inner FoxP2 neuron projections to the VTA using triple immunohistochemistry for Fluorogold, FoxP2 and c-Fos. In both the pre-LC and PBel-inner, sodium depletion significantly increases the expression of c-Fos within Fluorogold and FoxP2 immunopositive cells (Fig. 5.5). The identified projections from the pre-LC and PBel-inner to the VTA are therefore functionally relevant in that they respond to sodium deprivation. Given these projections and the known inputs from HSD2 neurons to pre-LC and PBel-inner FoxP2 neurons (Geerling and Loewy, 2006a), I have proposed a theoretical circuit-level diagram by which motivated behavior is modulated by sodium balance (Fig. 5.6). This model rests on the assumption that motivated behavior is generated by dopamine responses in the NAc. In my model, NAc dopamine concentration increases to sodium taste stimuli are selectively evoked in a sodium deplete state. Previous unpublished work in the NAc shell (Chapter 2) as well as published work in the NAc core (Cone et al., 2016) has revealed the state-dependency of dopamine signaling. This model proposes that the pre-LC and PBel-inner are the nodes that modulate dopamine signaling through input from the NTS and projections to the VTA. Projections from the pre-LC and PBel-inner to the VTA potentially modulate the excitability of the VTA such that dopamine concentration increases to taste stimuli are enhanced in a sodium deplete state. The triple immunohistochemistry approach that was used here should be applied to other nuclei to investigate their potential as both sodium appetite monitoring and dopamine regulatory sites. Candidate nuclei could be identified by a whole-brain analysis of c-Fos activated cell body location following sodium depletion in VTA Fluorogold injected rats. Such a thorough investigation has not yet been conducted. Following identification of sodium appetite responsive projections to the VTA,

causal work is necessary to determine if modulation of these projections has any behavioral relevance. Given the state-dependent projections to the VTA identified here and the role of VTA dopamine neuron excitability in driving motivated behaviors, future work manipulating VTA projecting sodium-appetite responsive sites hold promise for understanding the neurobiological basis of motivated behaviors.

Table 5.1

A

pre-LC

	FG^+ (total)	$FoxP2^+$ (total)	$c\text{-}Fos^+$ (total)	FG^+ $FoxP2^+$ $c\text{-}Fos^+$	FG^+ $FoxP2^+$ $c\text{-}Fos^-$	FG^+ $FoxP2^-$ $c\text{-}Fos^-$	FG^+ $FoxP2^-$ $c\text{-}Fos^+$	FG^- $FoxP2^+$ $c\text{-}Fos^+$	FG^- $FoxP2^+$ $c\text{-}Fos^-$	FG^- $FoxP2^-$ $c\text{-}Fos^+$
Replete A	75	156	13	6	52	15	2	4	94	1
Replete B	73	157	15	10	50	12	1	0	97	4
Replete C	68	121	1	0	39	28	1	0	82	0
Replete D	56	181	4	0	29	27	0	3	149	1
Mean:	**68.0**	**153.8**	**8.3**	**4.0**	**42.5**	**20.5**	**1.0**	**1.8**	**105.5**	**1.5**
SEM:	**4.26**	**12.35**	**3.40**	**2.45**	**5.33**	**4.09**	**0.41**	**1.03**	**14.86**	**0.87**
Deplete A	65	141	120	50	5	5	5	60	26	5
Deplete B	99	233	157	77	12	10	0	80	64	0
Deplete C	91	202	149	68	9	9	5	73	52	3
Mean:	**85.0**	**192.0**	**142.0**	**65.0**	**8.7**	**8.0**	**3.3**	**71.0**	**47.3**	**2.7**
SEM:	**10.26**	**27.02**	**11.24**	**7.94**	**2.03**	**1.53**	**1.67**	**5.86**	**11.22**	**1.45**

B

PBel-inner

	FG^+ (total)	$FoxP2^+$ (total)	$c\text{-}Fos^+$ (total)	FG^+ $FoxP2^+$ $c\text{-}Fos^+$	FG^+ $FoxP2^+$ $c\text{-}Fos^-$	FG^+ $FoxP2^-$ $c\text{-}Fos^-$	FG^+ $FoxP2^-$ $c\text{-}Fos^+$	FG^- $FoxP2^+$ $c\text{-}Fos^+$	FG^- $FoxP2^+$ $c\text{-}Fos^-$	FG^- $FoxP2^-$ $c\text{-}Fos^+$
Replete A	37	110	19	8	16	11	2	1	85	8
Replete B	36	188	12	4	28	3	1	4	152	3
Replete C	66	145	7	4	17	44	1	2	122	0
Replete D	56	87	5	3	5	48	0	2	77	0
Mean:	**48.8**	**132.5**	**10.8**	**4.8**	**16.5**	**26.5**	**1.0**	**2.3**	**109.0**	**2.8**
SEM:	**7.36**	**22.01**	**3.12**	**1.11**	**4.70**	**11.41**	**0.41**	**0.63**	**17.36**	**1.89**
Deplete A	38	123	58	3	1	34	0	51	68	4
Deplete B	46	181	74	28	9	9	0	45	99	1
Deplete C	51	122	77	36	12	3	0	41	33	0
Mean:	**45.0**	**142.0**	**69.7**	**22.3**	**7.3**	**15.3**	**0.0**	**45.7**	**66.7**	**1.7**
SEM:	**3.79**	**19.50**	**5.90**	**9.94**	**3.28**	**9.49**	**0.00**	**2.91**	**19.06**	**1.20**

Table 5.1: Expression of Fluorogold, FoxP2 and c-Fos in the pre-LC and PBel-inner of both Sodium Replete and Sodium Deplete rats. **A,** Within the pre-LC, Fluorogold (FG^+ total), FoxP2 ($FoxP2^+$ total) and c-Fos ($c\text{-}Fos^+$ total) immunopositive neurons were quantified in both Sodium Replete (*n*=4) and Sodium Deplete (*n*=3) rats. Individual data along with the mean ± SEM is presented for each expression profile observed. **B,** Within the PBel-inner, Fluorogold (FG^+ total), FoxP2 ($FoxP2^+$ total) and c-Fos ($c\text{-}Fos^+$ total) immunopositive neurons were quantified in both Sodium Replete (*n*=4) and Sodium Deplete (*n*=3) rats. Individual data along with the mean ± SEM is presented for each expression profile observed.

Figure 5.1

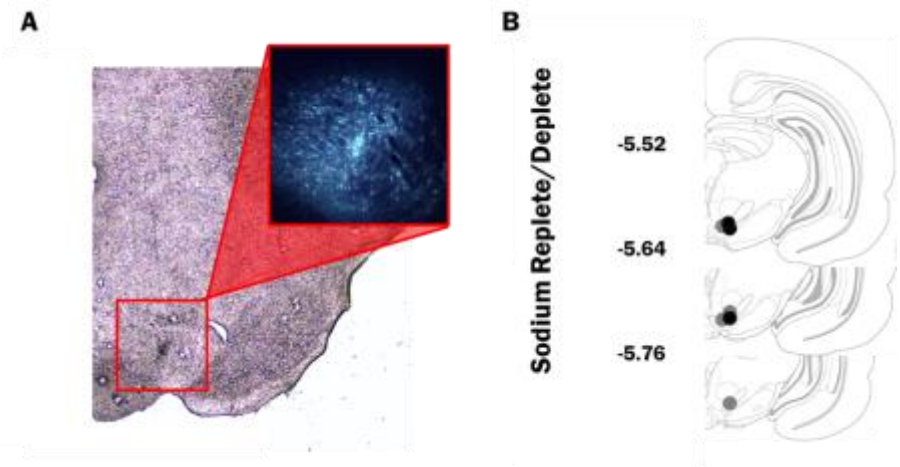

Figure 5.1: Summary of VTA Fluorogold injection sites. ***A,*** Representative unilateral-VTA injection site of iontophoretically injected Fluorogold. Brightfield image was taken under 200x magnification. The fluorescent image was taken in the blue channel to capture native Fluorogold fluroescence. ***B,*** Approximate injection sites of Fluorogold in Sodium Replete [grey circles (*n*=4)] and Sodium Deplete [black circles (*n*=3)] rats. Injection sites are depicted as circles on coronal sections modified from Paxinos and Watson (2007). Numbers to the left indicate approximate distance from bregma.

Figure 5.2

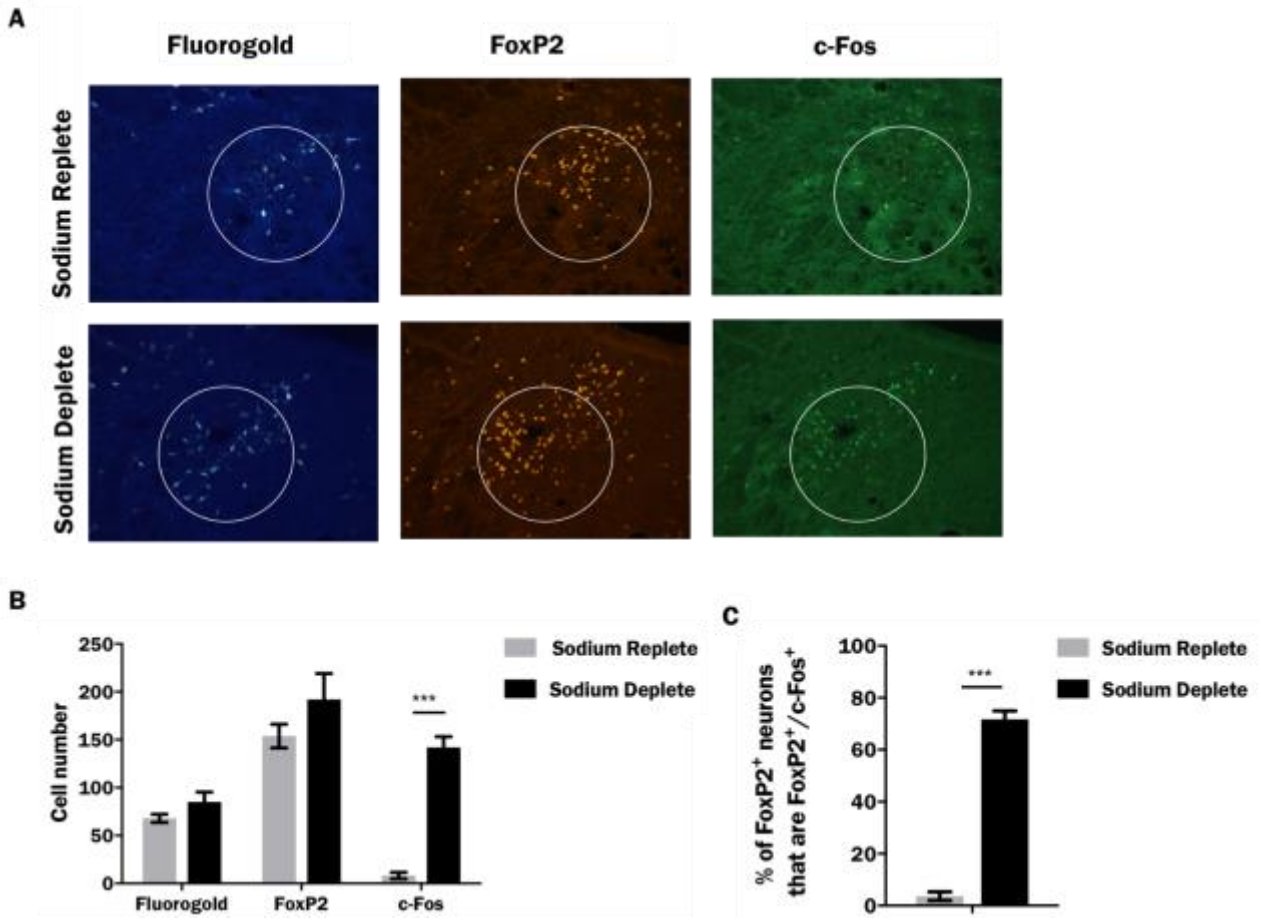

Figure 5.2: Expression of Flurogold, FoxP2 and c-Fos within the pre-LC of Sodium Replete and Sodium Deplete rats. ***A.*** Representative photoimages through the pre-LC of a Sodium Replete (top row) and a Sodium Deplete (bottom row) rat showing Fluorogold (blue), FoxP2 (red) and c-Fos (green) immunoreactive neurons. White circles are the defined area of interest selected for quantification within the pre-LC. ***B.*** Average cell counts (mean ± SEM) of Fluroogold, FoxP2 and c-Fos expressing neurons in the pre-LC of Sodium Replete (*n*=4) and Sodium Deplete (*n*=3) rats. ***C.*** c-Fos expression within FoxP2 expressing cells of the pre-LC is increased by sodium depletion. Data are expressed as average (mean ± SEM) of the percent of FoxP2 expressing ($FoxP2^+$) neurons that co-express c-Fos ($FoxP2^+/c\text{-}Fos^+$) in the pre-LC of Sodium Replete (*n*=4) and Sodium Deplete (*n*=3) rats. ***p<0.001.

Figure 5.3

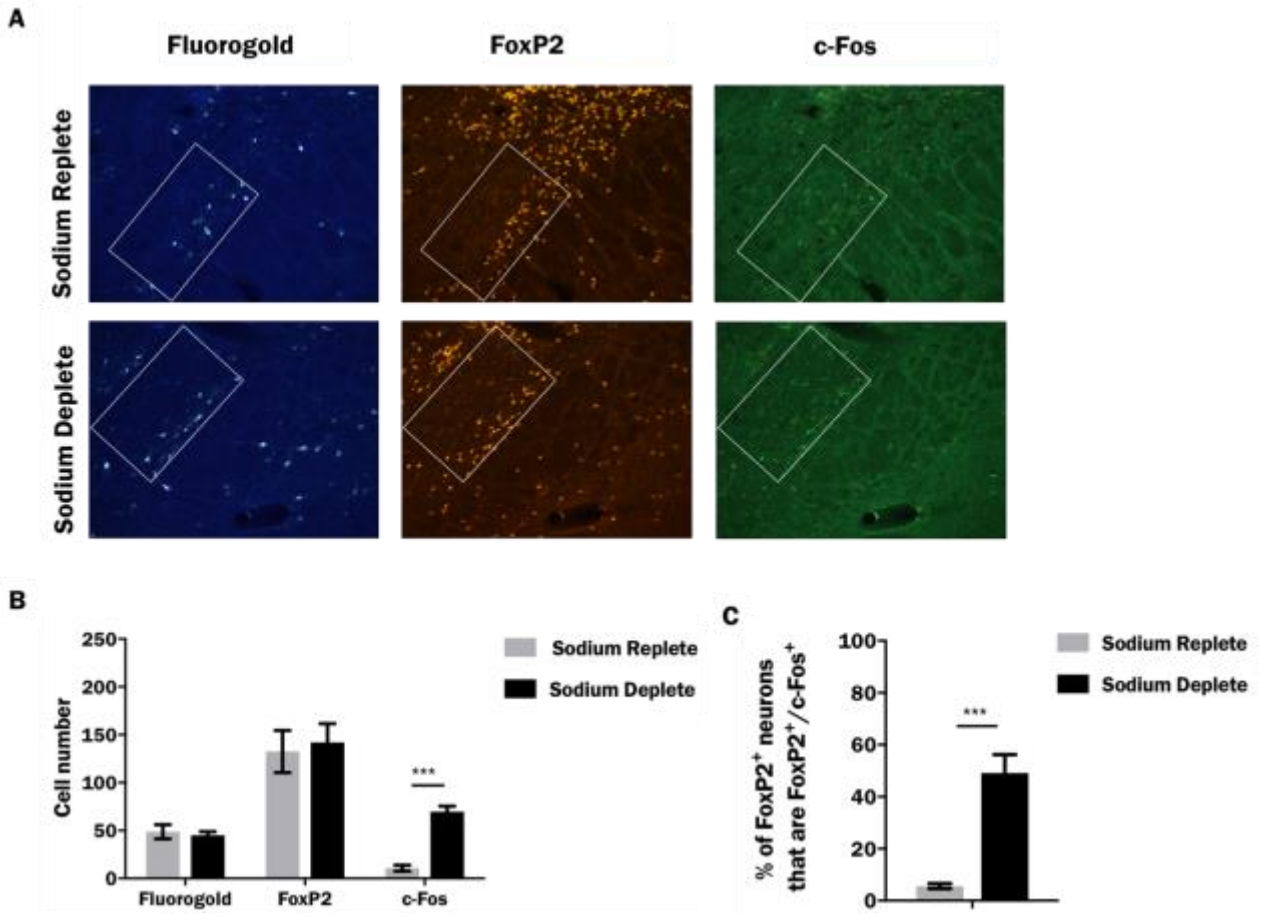

Figure 5.3: Expression of Flurogold, FoxP2 and c-Fos within the PBel-inner of Sodium Replete and Sodium Deplete rats. ***A.*** Representative photoimages through the PBel-inner of a Sodium Replete (top row) and a Sodium Deplete (bottom row) rat showing Fluorogold (blue), FoxP2 (red) and c-Fos (green) immunoreactive neurons. White circles are the defined area of interest selected for quantification within the PBel-inner. ***B.*** Average cell counts (mean ± SEM) of Flurorogold, FoxP2 and c-Fos expressing neurons in the PBel-inner of Sodium Replete (*n*=4) and Sodium Deplete (*n*=3) rats. ***C.*** c-Fos expression within FoxP2 expressing cells of the PBel-inner is increased by sodium depletion. Data are expressed as average (mean ± SEM) of the percent of FoxP2 expressing ($FoxP2^+$) neurons that co-express c-Fos ($FoxP2^+/c\text{-}Fos^+$) in the PBel-inner of Sodium Replete (*n*=4) and Sodium Deplete (*n*=3) rats. ***p<0.001.

Figure 5.4

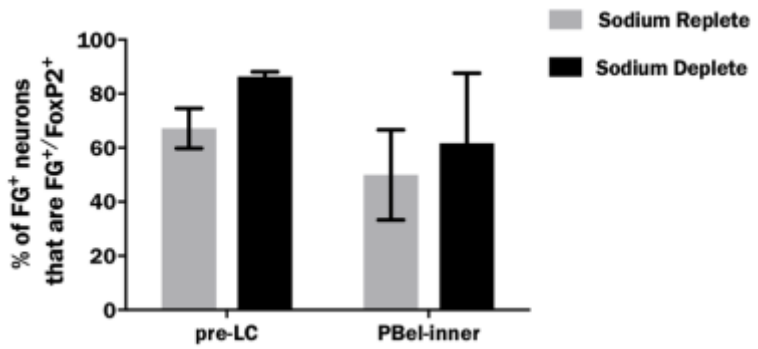

Figure 5.4: Expression of FoxP2 within Fluorogold immunopositive cells is similar between Sodium Replete and Sodium Deplete rats. In Sodium Deplete (*n*=3) rats, the pre-LC and PBel-inner have a similar ($p>0.05$) percentage of Fluorogold expressing neurons (FG^+) that co-express FoxP2 ($FG^+/FoxP2^+$), relative to Sodium Replete (*n*=4) rats.

Figure 5.5

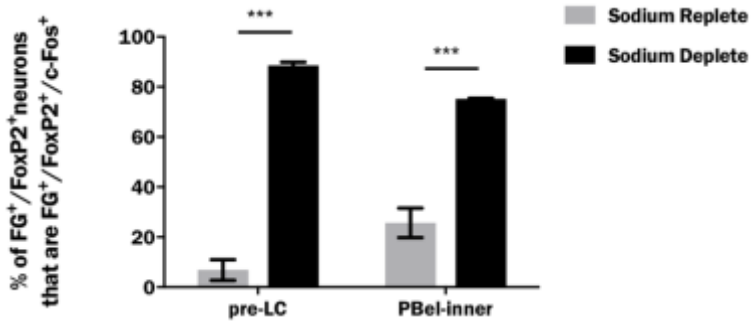

Figure 5.5: Expression of c-Fos within Fluorogold and FoxP2 immunopositive cells of the pre-LC and PBel-inner is increased by sodium depletion. In both brain regions, Sodium Deplete rats (*n*=3) have a significantly increased percentage of Fluorogold and FoxP2 expressing neurons ($FG^+/FoxP2^+$) that also express c-Fos ($FG^+/FoxP2^+/c\text{-}Fos^+$) relative to Sodium Replete (*n*=4) rats. ***p<0.001.

Figure 5.6

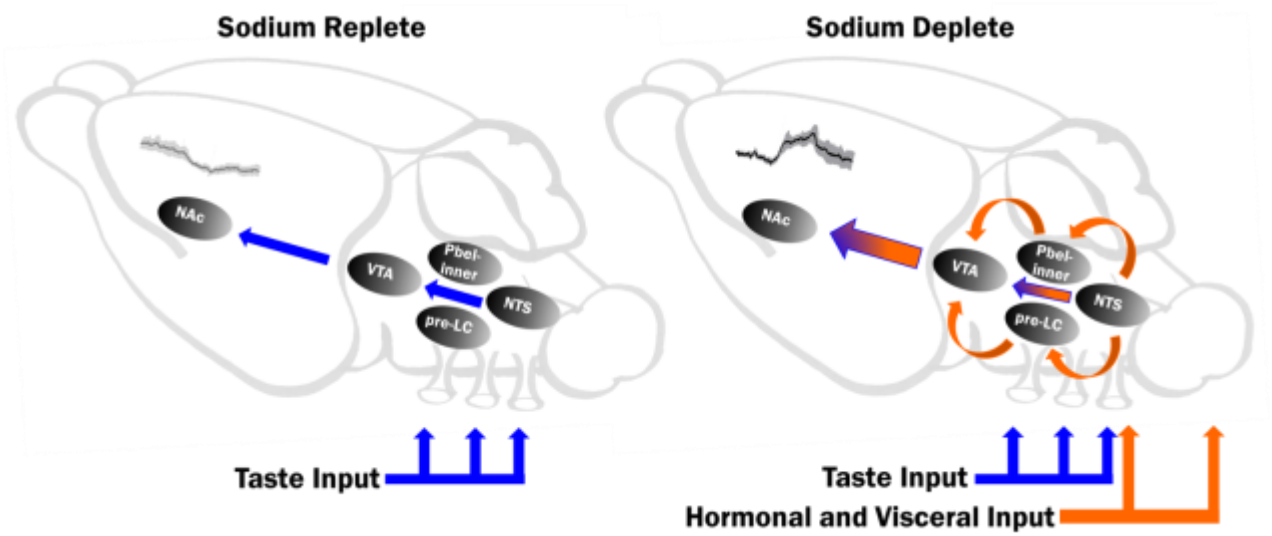

Figure 5.6 Proposed circuit-level diagram by which dopamine responses in the NAc are influenced by sodium depletion. (Left) Under Sodium Replete conditions, the taste on intraoral NaCl does not evoke an increase in dopamine concentration in the NAc (grey concentration trace). Blue arrows represent a potential route for gustatory information to travel from the oral cavity to the VTA to influence dopamine signaling. It is unclear if there are any gustatory inputs to the VTA from the NTS. (Right) Under Sodium Deplete conditions, the taste of NaCl (blue arrows) converges at the level of the VTA with hormonal and visceral input (orange arrows). Hormonal and visceral input is communicated to the NTS via the circulatory system and vagus nerve. The NTS then transmits information regarding body fluid homeostasis to the pre-LC and PBel-inner, both of which send direct projections to the VTA to influence excitability of VTA dopamine neurons. An increase in VTA dopamine neuron excitability underlies the increase in dopamine concentration observed in the NAc following intraoral NaCl infusion under deplete conditions (black concentration trace).

Chapter VI

General Discussion

Survival requires maintenance of homeostasis, achieved through many diverse internal processes and external behaviors. When prompted by physiological need, motivated behaviors emerge; driving animals to seek and consume specifically those stimuli which defend homeostasis. The idea that behaviors exist to satisfy need states has been around since the ancient Greeks (Stellar and Stellar, 1985). Within the past 80 years there has been an explosion of psychological theories to explain the generation of motivated behaviors (Hull and Krantz, 1943). However, the neural basis of motivation and the state and stimulus specificity that is characteristic of motivated behaviors, is still not well understood.

As dopamine signaling has been suggested to be involved various aspects of reinforcement and behaviors requiring drive and motivation (Tsai et al., 2009; Witten et al., 2011; Steinberg et al., 2014; Ilango et al., 2014; Salamone et al., 2016; Hamid et al., 2016; Syed et al., 2015; Berridge and Robinson, 1998b; Schultz, 1997), I hypothesized that dopamine signaling evoked by motivating stimuli is also state-dependent and stimulus-specific. I chose to examine the state and taste-specificity of phasic dopamine signaling under the motivated states of sodium appetite and thirst. Both perturbations of body fluid homeostasis give rise to robust state and stimulus-specific motivated states. The studies described here use FSCV to compare dopamine responses evoked by intraoral delivery of physiologically restorative stimuli under the normal as well as sodium deplete and water restricted conditions. Similarly to sodium appetite and thirst-evoked behaviors, observed dopamine signaling was state-dependent and taste-specific. Increases in phasic dopamine release in the NAc shell were observed only under

conditions of physiological need (sodium deprivation or water restriction) and only when the intraorally infused stimulus satisfied the need state of the animal. Therefore, the taste of sodium and water differentially drive dopamine signaling based on physiological state. I conclude my studies by using tracing and immunohistochemistry to identify projections to the VTA from the pre-LC and PBel-inner. I propose that these inputs to the VTA communicate sodium appetite to mesolimbic circuitry to increase responsivity of the dopamine neurons, thereby facilitating NAc dopamine release during sodium appetite.

A. Central circuits that modulate state and taste-dependent dopamine signaling

As demonstrated by this thesis, dopamine signaling is state and taste-dependent. Mesolimbic circuitry is somehow receiving information regarding body fluid homeostasis and taste in order to generate state-and taste-specific dopamine responses. However, the circuits that communicate state and taste to mesolimbic circuitry are not known.

I have proposed a circuit which allows for sodium balance to tune mesolimbic signaling (Fig. 5.6). My work reveals direct projections from FoxP2 pre-LC and PBel-inner neurons to the VTA that are sodium-deprivation responsive. While I did not include the tracing studies necessary to confirm the origin of the inputs to the FoxP2 neurons, it has been shown that FoxP2 neurons are targets of caudal NTS aldosterone-sensitive HSD2 neurons (Geerling and Loewy, 2008). The excitatory input from HSD2 neurons (Geerling et al., 2008) likely contributes to the sodium-deprivation responsivity of pre-LC and PBel-inner Foxp2 neurons. Sodium deprivation results in c-Fos activation in all three populations of cells (Geerling and Loewy, 2007). My proposed circuits include the pre-LC and PBel-inner as relay nodes between HSD2

neurons and the VTA (Fig. 5.6). The circuit is straightforward, as it proposes that two excitatory projections are necessary for hormonal action (aldosterone) to be communicated to mesolimbic circuitry. While the excitatory nature of the NTS to pre-LC or PBel-inner projection is supported (Geerling et al., 2008), future testing is necessary to characterize the pre-LC or PBel-inner to VTA projection. Direct axonal projections from HSD2 neurons to the VTA are also possible, however the traced projections are minor (Geerling and Loewy, 2006a). The ability of HSD2 projections, directly to the VTA or through relay nuclei, to alter dopamine responses remains to be tested. Future studies which pair FSCV with optogenetics or chemogenetics are described below.

Dopamine modulation through projections of the pre-LC and PBel-inner to the VTA is contingent upon VTA dopamine neuron excitability being modulated by sodium deprivation. It is not currently known if the projections from the pre-LC and PBel-inner synapse on dopamine neurons and impact their excitability. Electrophysiolgical experiments examining sodium deprivation-evoked changes in dopamine neuron mEPSC frequency and amplitude are necessary. Such studies have not yet been conducted. Increased excitability of the VTA following sodium deprivation would support my proposed circuits, but does not exclude the possibility of other modulatory inputs to the VTA beyond the pre-LC and PBel-inner (Shin et al., 2011). The lateral hypothalamus and orbitofrontal cortex (OFC), for example, are known to send dense projections to VTA dopamine neurons (Watabe-Uchida et al., 2012). Furthermore, the firing rate of lateral hypothalamus and OFC neurons response to rewards is sensitive to physiological state (Burton et al., 1976; Rolls et al., 1986, 1990). Whether changes in motivated behavior that arise from the influence of physiological state on neurons in the pre-LC, PBEl-

inner, lateral hypothalamus or OFC are a consequence of VTA modulatory projections requires further investigation.

If future electrophysiology studies find that VTA dopamine neuron excitability is not augmented by sodium depletion, the function of the sodium deprivation responsive pre-LC/PBel-inner to VTA projections that this thesis describes should be explored further. The necessity of the VTA in sodium appetite has been questioned (Wolf, 1967; Shibata et al., 2009) and it is possible that the projections that were traced in this thesis were fibers of passage destined for other regions of the brain like the hypothalamus. If the VTA is indeed not involved with the generation of state dependent dopamine responses, other mechanism by which sodium appetite can modulate NAc dopamine release should be explored. For example, the rate of dopamine reuptake via the DAT and dopamine transporter binding are decreased in sodium deplete animals or sodium replete animals treated with aldosterone or DOCA (Roitman et al., 1999a; Lucas et al., 2000; Figlewicz et al., 1999). A decrease in DAT number or function would contribute to increased dopamine exposure time and diffusion upon release (Cragg and Rice, 2004; Sulzer et al., 2016), effectively increasing extracellular dopamine release over longer periods of time, an effect which has been shown to enhance motivation and goal-directed behavior (Niv, 2007). Other potential changes within the NAc by sodium deprivation include increased dopamine production (Grafe and Flanagan-Cato, 2016) or the regulation of NAc neuropeptide production (Lucas et al., 2007). Lastly, inputs to the NAc may alter phasic dopamine release independently of VTA afferent firing rate. An example of this type of modulation was observed with basolateral amygdala (BLA) projections onto NAc dopamine terminals (Floresco et al., 1998; Jones et al., 2010). The BLA is necessary for the full expression

of a sodium appetite (Nachman and Ashe, 1974). Importantly, the BLA regulates reward seeing (Stuber et al., 2011) and modulates phasic dopamine release evoked by rewards (Jones et al., 2010). While HSD2 neurons do not directly project to the NAc (Geerling and Loewy, 2006a), a multisynaptic pathway has been described (Shekhtman et al., 2007). Whether or not this pathway can modulate phasic dopamine release is unknown.

The circuit that I proposed as a potential mechanism (Fig. 5.6) for the state- dependency of phasic dopamine signaling during sodium appetite involves modulation of dopamine signaling by aldosterone through a multisynaptic pathway. Aldosterone activates NTS HSD2 neurons which project to pre-LC and PBel-inner FoxP2 neurons. In my model, it is the activation of FoxP2 neurons which modulates the excitability of VTA dopamine neurons via direct glutamatergic projections (Fig. 5.6) such that the taste of sodium evokes dopamine release only under sodium deplete conditions. While aldosterone signaling has been shown to contribute to the expression of a sodium appetite, it is certainly not the only hormone implicated in the generation of sodium intake behavior. Hormones including Ang-II, vasopressin and oxytocin have all been implicated in sodium appetite (Stellar and Epstein, 1991; Schulkin, 1992; Zhang et al., 1984). Future studies should aim to explore alternative central circuits that are activated by hormones beyond aldosterone. It is likely that they also influence mesolimbic neurotransmission.

While a circuit by which dopamine responses in the NAc are influenced by water restriction was not a focus of this thesis, one that involves Ang-II action within the SFO, should certainly be explored. The SFO is a well-established structure for eliciting drinking behavior under the conditions of thirst (Simpson and Routtenberg, 1973). Recent work has shown that

SFO neurons are well positioned to respond to sodium and water stimuli in a state- and stimulus specific manner, as they integrate inputs from the oral cavity with information about blood composition (osmolarity and changes in plasma volume and pressure, communicated via Ang-II) (Zimmerman et al., 2016). Further studies are necessary to determine a circuit by which the SFO regulates sodium appetite and thirst. A serotonin-mediated mechanism has recently been suggested, as serotonin levels in the SFO are modulated by manipulations of body fluid homeostasis as well as the intake of water and sodium (Takahashi and Tanaka, 2016). Efferent projections from sodium appetite and thirst-promoting neurons of the SFO, perhaps those that are responsive to serotonin, to mesolimbic circuitry should be explored further.

The circuit proposed in this thesis suggests a mechanism by which a hormone that communicates sodium balance (aldosterone) alters VTA dopamine neuron excitability in response to taste stimuli. Future work will aim to identify central nodes that receive and integrate both state *and* taste information to alter dopamine neuron responses during sodium appetite. It is possible that the integration of state and taste signaling occurs at very early stages of neural processing. For example, numerous studies have shown modulation of taste receptor cell transduction by neuropeptides and steroid hormones implicated in energy balance regulation (Herness et al., 2002; Kawai et al., 2000). Indeed, responses of amiloride-sensitive sodium channels have been shown to be altered by hormones that control body fluid maintenance (i.e. aldosterone, vasopressin) (Herness, 1992; Gilbertson et al., 1993). Furthermore, both chorda tympani nerve and NTS taste neurons are modulated by sodium depletion (Contreras, 1977; Contreras and Frank, 1979; Jacobs et al., 1988). Future work is necessary to characterize the impact of gustatory drive changes resulting from sodium balance.

It is also possible that circuits processing taste and state converge at a single node, which projects to mesolimbic circuitry to modulate dopamine signaling. However, identification of this node in the context of sodium appetite is challenging, given that the circuits communicating sodium balance and taste are, at least initially, separate. The sodium need and gustatory pathways originate from different subnuclei within the NTS. Sodium-sensing projections from caudal NTS neurons target primarily the pre-LC and PBel-inner neurons, which in turn project to sites including the midline and intralaminar thalamic nuclei, VTA and multiple sites within the hypothalamus. Conversely, sodium taste responsive neurons of the rostral NTS project to different subdivisions within the parabrachial nucleus (medial, waist, dorsal lateral) (Geerling et al., 2006a; Geerling and Loewy, 2007; Yamamoto et al., 1993, 2009). While ascending gustatory projections from the PBN to the gustatory thalamus (ventral posterior medial thalamic nuclei) do not overlap with the sodium need projection system, both gustatory and sodium need circuits include the parasubthalamic nucleus (PSTN) (Karimnamazi and Travers, 1998). Whether the PSTN integrates state and taste information and a mechanism by which the PSTN could influence mesolimbic circuitry remains to be explored.

B. The role of phasic dopamine signaling in motivated behavior

The involvement of phasic dopamine signaling has been proposed across multiple aspects of motivated behavior. Roles for dopamine signaling include promoting behavioral reinforcement (Tsai et al., 2009; Witten et al., 2011; Steinberg et al., 2014) and motivation (Ilango et al., 2014), enhancing the salience of reward-predictive cues (Berridge and Robinson, 1998a), promoting effortful responses to obtain rewards (Salamone et al., 2016; Syed et al.,

2015; Hamid et al., 2016) and functioning as a teaching signal during associative learning (Schultz et al., 1997; Steinberg et al., 2013; Hamid et al., 2016). However, a mechanism by which dopamine supports appetitive and consummatory behaviors remains unclear. One prevailing hypothesis is that dopamine impacts the generation of motor behaviors by modulating the activity of the NAc (Nicola et al., 2000). The NAc has been termed a 'limbic-motor interface' (Mogenson et al., 1980) due to its cortical, limbic and thalamic afferents (Meredith et al., 2008) and motor-related efferents [i.e. ventral pallidum, SN pars compacta, SN pars reticulate, retrorubral area (Groenewegen et al., 1999)]. Normal functioning of the NAc is required for various aspects of goal-directed behavior (Salamone et al., 2003; de Borchgrave et al., 2002). The NAc is therefore well positioned to convert motivation into action and modulation of NAc excitability represents a means to shape motivated behavior.

Neurons of the NAc are almost exclusively (90-95%) GABAergic projection neurons called medium spiny neurons (MSNs). Single unit electrophysiological studies have revealed that hedonic value is differentially encoded by MSNs, with rewarding stimuli reducing and aversive stimuli increasing neuronal activity [see (Carlezon and Thomas, 2009; Volman et al., 2013) for review] . MSNs of the NAc are GABAergic (Meredith, 1999) and tonically inhibit downstream motor output nuclei (Chuhma et al., 2011). Consequently, pauses in NAc neuron activity evoked by rewarding stimuli may promote reward-directed behavior (Krause et al., 2010; Taha and Fields, 2006) by removing motor disinhibition. Indeed, pharmacological suppression of NAc neural activity increases appetitive oral-facial responses to palatable taste stimuli (Peciña and Berridge, 2000, 2005; Mahler et al., 2007) and the promotes the initiation of reward-directed behaviors (Kelley, 2004). Importantly, dopamine modulates the activity of

MSNs through action on MSN dopamine receptors (Geldwert et al., 2006). As dopamine responses to rewarding and aversive stimuli are also opposing, with rewarding stimuli evoking increases and aversive stimuli evoking pauses in NAc dopamine release (Roitman et al., 2005, 2008), it is likely that the high concentration increases in phasic dopamine release that are evoked by primary rewarding taste stimuli contribute to the pauses in MSN firing rate that these same stimuli evoke (Roitman et al., 2005, 2008). Neuromodulation of MSN activity represents a mechanism by which dopamine impacts the generation of behaviors.

I postulate that behaviors directed towards sodium or water in a state of physiological need are reinforced through a circuit involving dopaminergic modulation of NAc output. Here, dopamine recordings were within the medial shell of the NAc, an area identified as a "hedonic hotspot" for its observed encoding of hedonically rewarding stimuli (Peciña et al., 2006). Dopamine release was evoked by state-satisfying stimuli that, indeed, elicit positive hedonic responses under conditions of homeostatic need (Berridge et al., 1984; Grill and Miselis, 1981). It is likely that all taste stimuli which evoke NAc dopamine release (NaCl and LiCl under sodium deprivation and water under water restriction) also evoke pauses in NAc firing rate, however, only pauses to NaCl under conditions of sodium depletion have been confirmed to date (Loriaux et al., 2011). The NAc motor output nuclei that are affected by pauses in NAc shell firing rate following NaCl stimuli, potentially driving intake behavior, are unknown. A potential target is the ventral pallidum. Pharmacological manipulations which remove VP inhibition increase feeding and enhance appetitive taste reactivity (Smith and Berridge, 2007). Additionally, increases in VP neural activity are observed following sodium ingestion in sodium deplete rats

(Tindell et al., 2006). Whether or not dopamine modulation of the NAc can affect VP activity to the extent that it affects intake behavior remains to be investigated.

The mesolimbic dopamine signaling undoubtedly plays some role in the generation of motivated behavior, and accordingly, the behavioral expression of sodium appetite and thirst. However, mesolimbic dopamine signaling is not necessary for all aspects of motivated behavior. Lesions of the VTA do not cause impairments of sodium appetite following multiple methods of sodium deprivation (Wolf, 1967). Furthermore, neither systemic nor intra-NAc dopamine antagonists have been shown to alter the expression of a sodium appetite (Roitman et al., 1997; Lucas et al., 2007). These results are consistent with the feeding literature in which NAc dopamine depletion and antagonism does not impair food intake (Koob et al., 1978; Ungerstedt, 1971; Baldo et al., 2002). If NAc dopamine release is not necessary for the expression of a sodium appetite, what role in this specific behavior does it play?

I propose that NAc dopamine subserves reinforcement learning under conditions of sodium depletion. However, the facilitation of NaCl intake behavior by dopamine may be restricted to the first instance of sodium depletion. While the first episode of sodium depletion evokes dopamine release in the forebrain following NaCl consumption, subsequent depletion episodes do not (Frankmann et al., 1994). It is important to note that the analysis of dopamine release used by Frankmann et al. is based on rate of dopamine metabolism. Using a dopamine metabolite/ dopamine ratio is a crude measure of dopamine release that requires many assumptions about dopamine kinetics including a constant rate of reuptake by DAT. While Frankmann did not observe an increase in dopamine metabolism after the first depletion, multiple depletions are characterized by enhanced sodium intake (Sakai et al., 1987)

(sensitization). Some dopamine-independent mechanism must, therefore, be influencing sodium intake in these later depletion sessions. Accompanying sensitization of sodium appetite are morphological changes associated with changes in NAc plasticity (increased dendritic branches and spines) (Roitman et al., 2002), which are similarly observed during amphetamine sensitization (Robinson and Kolb, 1999). It is possible that the initial sodium depletion requires dopamine signaling to induce plasticity within the NAc that underlies reinforcement such that increased intake is promoted during subsequent encounters with NaCl in a deficient state.

A causal role for dopamine signaling in driving sodium and water intake during sodium appetite and thirst should be explored. My tracing and immunohistochemical studies were aimed at taking the initial steps towards the goal of establishing a causal role for dopamine signaling in sodium appetite. I identified projections that potentially modify dopamine signaling under conditions of sodium depletion. I found that both the pre-LC and PBel-inner are responsive to sodium deprivation and project to the VTA. Now that projections have been proposed, optogenetic and chemogenetic methods should be used to selectively modulate them. Fortunately, pre-LC and PBel-inner neurons share a common molecular marker, Foxp2. Expression of FoxP2 within the neuronal population of interest facilitates genetic access to these neurons and application of optogenetics or chemogenetics for their selective modulation. Future work should test 1) the ability of selective activation of pre-LC/PBel-inner to VTA projections to evoke NAc dopamine release following NaCl infusion in a sodium replete state 2) the ability of selective inhibition of pre-LC/PBel-inner to VTA projections to suppress NAc dopamine release evoked by NaCl in a sodium deplete state.

After the proposed circuit is tested for its ability to modulate dopamine signaling, similar methods should be used to probe the functionality of the circuit. Support for dopamine playing a causal role in motivation would arise from the induction or suppression of behavior through excitation or inhibition of pre-LC/ PBel-inner to VTA projections. Multiple sodium appetite-induced behaviors should be tested in an attempt to shed light on a role for dopamine signaling in motivated behavior. For example, both passive intake of NaCl and progressive ratio responding should be investigated, as passive intake has been shown to be dopamine independent, while progressive ratio responding is reportedly dopamine dependent (Salamone and Correa, 2012). The advent of optogenetics and chemogenetics has contributed to our current understanding of phasic dopamine signaling's role in reinforcement (Tsai et al., 2009; Witten et al., 2011; Steinberg et al., 2014). Demonstrating a causal role for dopamine signaling in the motivated behavior evoked by sodium appetite and confirming dopamine regulatory inputs (i.e. pre-LC or PBel-inner) requires similar application of optogenetic and chemogenetic methods.

C. Implications of current studies

Motivated behaviors are adaptive behaviors that serve to defend against homeostatic perturbation by driving animals to seek and consume physiologically required stimuli. However, motivated behaviors can also be maladaptive if they are dysregulated (i.e. overconsumption of food) or are directed at harmful stimuli (i.e. drugs of abuse). Diseases and disorders that are deeply rooted in maladaptive motivated behaviors (obesity, drug addiction) represent public health concerns for which we currently have few medical interventions.

Recent work has explored the possibility of using the endogenous systems that regulate adaptive drive states to curb maladaptive motivated behaviors. Physiological state is a powerful regulator of adaptive motivated behaviors, and indeed, maladaptive behaviors as well. For example, manipulations of energy balance have been repeatedly shown to modulate behaviors directed not only at food, but also drugs of abuse [see (Hayes and Schmidt, 2016) for review]. However, the shared neural substrates of adaptive and maladaptive motivated behaviors which are affected by physiological state are less clear.

This thesis uses manipulations of body fluid homeostasis to illustrate the profound impact of physiological state on mesolimbic dopamine signaling. Here, the induction of a sodium appetite or thirst is sufficient to evoke NAc shell dopamine responses to stimuli that otherwise cause no change in dopamine signaling. Manipulations of body fluid homeostasis, therefore, provide an ideal entry point to ask basic science questions about the influence of physiological state on dopamine signaling. An obvious question, and one which I have examined here, involves the circuits by which physiological state are communicated to mesolimbic circuitry. This question is important to fully understand the neurobiological underpinnings of motivated behavior. Furthermore, as mesolimbic dopamine signaling plays a key role in reinforcement processing, my findings support the possibility of using manipulations of physiology its endogenous hormone systems to alter motivated behaviors- both adaptive and, importantly, maladaptive. After a causal role for dopamine signaling in the generation of state-dependent motivated behaviors is established, exploring therapeutic interventions for obesity and drug addiction that target the dopamine system will follow.

CITATIONS

Abizaid, A., Liu, Z.-W., Andrews, Z. B., Shanabrough, M., Borok, E., Elsworth, J. D., Roth, R. H., Sleeman, M. W., Picciotto, M. R., Tschöp, M. H., et al. 2006. Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *The Journal of clinical investigation* 116:3229–39.

Alhadeff, A. L., Rupprecht, L. E., and Hayes, M. R. 2012. GLP-1 neurons in the nucleus of the solitary tract project directly to the ventral tegmental area and nucleus accumbens to control for food intake. *Endocrinology* 153:647–58.

Amelung, D., Hubener, H., Roka, L., and Meyerheim, G. 1953. Conversion of cortisone to compound F. *The Journal of Clinical Endocrinology & Metabolism* 13:1125–1126.

Ames, M. K., Atkins, C. E., Lantis, A. C., and zum Brunnen, J. 2016. Evaluation of subacute change in RAAS activity (as indicated by urinary aldosterone:creatinine, after pharmacologic provocation) and the response to ACE inhibition. *Journal of the Renin-Angiotensin-Aldosterone System* 17:147032031663389.

Anderson, B. 1977. Regulation of Body Fluids. *Annual Review of Physiology* 39:185–200.

Andersson, B., and McCann, S. 1955. Hypothalamic control of water intake. *The Journal of physiology* 129:44P.

Antunes-Rodrigues, J., de Castro, M., Elias, L. L. K., Valença, M. M., and McCann, S. M. 2004. Neuroendocrine Control of Body Fluid Metabolism. *Physiological Reviews* 84:169–208.

Aragona, B. J., Cleaveland, N. A., Stuber, G. D., Day, J. J., Carelli, R. M., and Wightman, R. M. 2008. Preferential enhancement of dopamine transmission within the nucleus accumbens shell by cocaine is attributable to a direct increase in phasic dopamine release events. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 28:8821–31.

de Araujo, I. E. T., Kringelbach, M. L., Rolls, E. T., and McGlone, F. 2003. Human Cortical Responses to Water in the Mouth, and the Effects of Thirst. *Journal of Neurophysiology* 90.

Arriza, J. L., Simerly, R. B., Swanson, L. W., Evans, R. M., Fortin, M., Philibert, D., Gustafsson, J.-Å., Yamamoto, K. R., Evans, R. M., and Gustafsson, J.-Å. 1988. The neuronal mineralocorticoid eeceptor as a mediator of glucocorticoid response. *Neuron* 1:887–900.

Arsenijevic, Y., and Baertschi, A. J. 1985. Activation of the hypothalamo-neurohypophysial system by hypertonic superfusion of the rat mesentery. *Brain research* 347:169–72.

Avenet, P., and Lindemann, B. 1988. Amiloride-blockable sodium currents in isolated taste receptor cells. *The Journal of membrane biology* 105:245–55.

Avrith, DB, Wiselka, MJ, Fitzsimons, J. 1980. Increased Sodium Appetite in Adrenalectomized or Hypophysectomized Rats After Intracranial Injections of Renin or Angiotensin II. *J Endocrinol* 87:109–112.

Baldo, B. A., Sadeghian, K., Basso, A. M., and Kelley, A. E. 2002. Effects of selective dopamine D1 or D2 receptor blockade within nucleus accumbens subregions on ingestive behavior and associated motor activity. *Behavioural brain research* 137:165–77.

Beidler, L. M. 1953. Properties of chemoreceptors of tongue of rat. *Journal of neurophysiology* 16:595–607.

Bernstein, I. L., and Hennessy, C. J. 1987. Amiloride-sensitive sodium channels and expression of sodium appetite in rats. *The American journal of physiology* 253:R371-4.

Berridge, K. C., Flynn, F. W., Schulkin, J., and Grill, H. J. 1984. Sodium depletion enhances salt

palatability in rats. *Behavioral neuroscience* 98:652–60.
Berridge, K. C., and Robinson, T. E. 1998a. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain research. Brain research reviews* 28:309–69.
Berridge, K. C., and Robinson, T. E. 1998b. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Research Reviews* 28:309–369.
Bianchetti, M. G., Simonetti, G. D., and Bettinelli, A. 2009. Body fluids and salt metabolism - Part I. *Italian journal of pediatrics* 35:36.
Birmingham, M. K., Stumpf, W. E., and Sar, M. 1979. Nuclear localization of aldosterone in rat brain cells assessed by autoradiography. *Experientia* 35:1240–1.
de Borchgrave, R., Rawlins, J. N. P., Dickinson, A., and Balleine, B. W. 2002. Effects of cytotoxic nucleus accumbens lesions on instrumental conditioning in rats. *Experimental Brain Research* 144:50–68.
Bourque, C. W., Voisin, D. L., and Chakfe, Y. 2002. Stretch-inactivated cation channels: cellular targets for modulation of osmosensitivity in supraoptic neurons. *Progress in brain research* 139:85–94.
Bowell, R. J., Warren, A., and Redmond, I. 1996. Formation of cave salts and utilization by elephants in the Mount Elgon region, Kenya. *Geological Society, London, Special Publications* 113:63–79.
Branch, S. Y., Goertz, R. B., Sharpe, A. L., Pierce, J., Roy, S., Ko, D., Paladini, C. A., and Beckstead, M. J. 2013. Food restriction increases glutamate receptor-mediated burst firing of dopamine neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 33:13861–72.
Breslin, P. A., Spector, A. C., and Grill, H. J. 1993. Chorda tympani section decreases the cation specificity of depletion-induced sodium appetite in rats. *The American journal of physiology* 264:R319-23.
Breslin, P. A., Spector, A. C., and Grill, H. J. 1995. Sodium specificity of salt appetite in Fischer-344 and Wistar rats is impaired by chorda tympani nerve transection. *The American journal of physiology* 269:R350-6.
Broadwell, R. D., and Sofroniew, M. V. 1993. Serum Proteins Bypass the Blood-Brain Fluid Barriers for Extracellular Entry to the Central Nervous System. *Experimental Neurology* 120:245–263.
Brot, M. D., Watson, C. H., and Bernstein, I. L. Amiloride-sensitive signals and NaCl preference and appetite: a lick-rate analysis.
Buggy, J., and Fisher, A. E. 1974. Evidence for a dual central role for angiotensin in water and sodium intake. *Nature* 250:733–735.
Burton, M. J., Rolls, E. T., and Mora, F. 1976. Effects of hunger on the responses of neurons in the lateral hypothalamus to the sight and taste of food. *Experimental Neurology* 51:668–677.
Carlezon, W. A., and Thomas, M. J. 2009. Biological substrates of reward and aversion: A nucleus accumbens activity hypothesis. *Neuropharmacology* 56:122–132.
Chandrashekar, J., Hoon, M. A., Ryba, N. J. P., and Zuker, C. S. 2006. The receptors and cells for mammalian taste. *Nature* 444:288–294.
Choi-Kwon, S., and Baertschi, A. J. 1991. Splanchnic osmosensation and vasopressin:

mechanisms and neural pathways. *The American journal of physiology* 261:E18-25.

Chuhma, N., Tanaka, K. F., Hen, R., and Rayport, S. 2011. Functional connectome of the striatal medium spiny neuron. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 31:1183–92.

Clark, J. J., and Bernstein, I. L. 2006. Sensitization of salt appetite is associated with increased "wanting" but not "liking" of a salt reward in the sodium-deplete rat. *Behavioral neuroscience* 120:206–10.

Cohen, M. J., Hagiwara, S., and Zotterman, Y. 1955. The Response Speetrum of Taste Fibres in the Cat: A Single Fibre Analysis. *Acta Physiologica Scandinavica* 33:316–332.

Cone, J. J., Fortin, S. M., McHenry, J. A., Stuber, G. D., McCutcheon, J. E., and Roitman, M. F. 2016. Physiological state gates acquisition and expression of mesolimbic reward prediction signals. *Proceedings of the National Academy of Sciences* 113:1943–1948.

Cone, J. J., McCutcheon, J. E., and Roitman, M. F. 2014. Ghrelin acts as an interface between physiological state and phasic dopamine signaling. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 34:4905–13.

Cone, J. J., Roitman, J. D., and Roitman, M. F. 2015. Ghrelin regulates phasic dopamine and nucleus accumbens signaling evoked by food-predictive stimuli. *Journal of neurochemistry* 133:844–56.

Contreras, R. J. 1977. Changes in gustatory nerve discharges with sodium deficiency: a single unit analysis. *Brain research* 121:373–8.

Contreras, R. J., and Frank, M. 1979. Sodium deprivation alters neural responses to gustatory stimuli. *The Journal of general physiology* 73:569–94.

Cox, J. R., Cruz, C. E., and Ruger, J. 1978. Effect of total amygdalectomy upon regulation of salt intake in rats. *Brain research bulletin* 3:431–5.

Cragg, S. J., and Rice, M. E. 2004. DAncing past the DAT at a DA synapse. *Trends in neurosciences* 27:270–7.

Daniels, D., and Fluharty, S. J. 2004. Salt appetite: a neurohormonal viewpoint. *Physiology & Behavior* 81:319–337.

Davis, J. O., Urquhart, J., Higgins, J. T., and Jr. 1963. The effects of alteration of plasma sodium and potassium concentration on aldosterone secretion. *The Journal of clinical investigation* 42:597–609.

Denton, D. A. 1965. Evolutionary Aspects of the Emergence of Aldosterone Secretion and Salt Appetite. *Physiological Reviews* 45.

Doolin, R. E., and Gilbertson, T. A. 1996. Distribution and characterization of functional amiloride-sensitive sodium channels in rat tongue. *The Journal of general physiology* 107:545–54.

Dunkley, P. R., Bobrovskaya, L., Graham, M. E., Von Nagy-Felsobuki, E. I., and Dickson, P. W. 2004. Tyrosine hydroxylase phosphorylation: regulation and consequences. *Journal of Neurochemistry* 91:1025–1043.

Eckel, L. A., and Ossenkopp, K.-P. 1995. Cholecystokinin reduces ingestive taste reactivity responses to water in fluid-replete but not fluid-deprived rats.

Egecioglu, E., Engel, J. A., and Jerlhag, E. 2013. The glucagon-like peptide 1 analogue Exendin-4 attenuates the nicotine-induced locomotor stimulation, accumbal dopamine release, conditioned place preference as well as the expression of locomotor sensitization in mice.

PloS one 8:e77284.
Elliott, E. J., and Simon, S. A. 1990. The anion in salt taste: a possible role for paracellular pathways. *Brain research* 535:9–17.
Engel, J. A., and Jerlhag, E. 2014. Role of Appetite-Regulating Peptides in the Pathophysiology of Addiction: Implications for Pharmacotherapy. *CNS Drugs* 28:875–886.
Epstein, A., Fitzsimons, J. T., and Rolls, B. J. 1970. Drinking induced by injection of antiotensin into the brain of the rat. 210:457–474.
Epstein, A. N. 1982. Mineralocorticoids and cerebral angiotensin may act together to produce sodium appetite. *Peptides* 3:493–4.
Epstein, A. N., and Stellar, E. 1955. The control of salt preference in the adrenalectomized rat. *Journal of comparative and physiological psychology* 48:167–72.
Falk, L., and Young, P. T. 1956. The relative acceptability of sodium chloride solutions as a function of concentration and water need. *Journal of comparative and physiological psychology* 49:569–75.
Figlewicz, D. P., Patterson, T. A., Zavosh, A., Brot, M. D., Roitman, M., and Szot, P. 1999. Neurotransmitter transporters: target for endocrine regulation. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et métabolisme* 31:335–9.
Fitzsimons, J. T. 1998. Angiotensin, thirst, and sodium appetite. *Physiological reviews* 78:583–686.
Floresco, S. B., Yang, C. R., Phillips, A. G., and Blaha, C. D. 1998. Association Basolateral amygdala stimulation evokes glutamate receptor-dependent dopamine efflux in the nucleus accumbens of the anaesthetized rat. *European Journal of Neuroscience* 10:1241–1251.
Fluharty, SJ and Sakai, R. 1996. Behavioral and cellular analysis of adrenal steroid and angiotensin interactions mediating salt appetite., 16th ed. Academic Press, San Diego.
Flynn, F. W., Grill, H. J., Schulkin, J., and Norgren, R. 1991. Central gustatory lesions: II. Effects on sodium appetite, taste aversion learning, and feeding behaviors. *Behavioral Neuroscience* 105:944–954.
Fortin, S. M., Chartoff, E. H., and Roitman, M. F. 2016. The Aversive Agent Lithium Chloride Suppresses Phasic Dopamine Release Through Central GLP-1 Receptors. *Neuropsychopharmacology* 41:906–915.
Fortin, S. M., Cone, J. J., Ng-Evans, S., McCutcheon, J. E., and Roitman, M. F. 2015. Sampling phasic dopamine signaling with fast-scan cyclic voltammetry in awake, behaving rats. *Current protocols in neuroscience / editorial board, Jacqueline N. Crawley ... [et al.]* 70:7.25.1-7.25.20.
Frank, M. E., Contreras, R. J., and Hettinger, T. P. 1983. Nerve fibers sensitive to ionic taste stimuli in chorda tympani of the rat. *Journal of neurophysiology* 50:941–60.
Frankmann, S. P., Broder, L., Dokko, J. H., and Smith, G. P. 1994. Differential changes in central monoaminergic metabolism during first and multiple sodium depletions in rats. *Pharmacology, biochemistry, and behavior* 47:617–24.
Fulton, S. 2010. Appetite and reward. *Frontiers in Neuroendocrinology* 31:85–103.
Fulwiler, C. E., and Saper, C. B. 1984. Subnuclear organization of the efferent connections of the parabrachial nucleus in the rat. *Brain research* 319:229–59.
Funder, J., Pearce, P., Smith, R., and Smith, A. 1988. Mineralocorticoid action: target tissue

specificity is enzyme, not receptor, mediated. *Science* 242.
Garcia, J., Kimeldorf, D. J., and Koelling, R. A. 1955. Conditioned Aversion to Saccharin Resulting from Exposure to Gamma Radiation. *Science* 122.
Garty, H., and Palmer, L. G. 1997. Epithelial sodium channels: function, structure, and regulation. *Physiological reviews* 77:359–96.
Geerling, J. C., Chimenti, P. C., and Loewy, A. D. 2008. Phox2b expression in the aldosterone-sensitive HSD2 neurons of the NTS. *Brain research* 1226:82–8.
Geerling, J. C., Engeland, W. C., Kawata, M., and Loewy, A. D. 2006a. Aldosterone Target Neurons in the Nucleus Tractus Solitarius Drive Sodium Appetite. *Journal of Neuroscience* 26:411–417.
Geerling, J. C., Kawata, M., and Loewy, A. D. 2006b. Aldosterone-sensitive neurons in the rat central nervous system. *The Journal of Comparative Neurology* 494:515–527.
Geerling, J. C., and Loewy, A. D. 2006a. Aldosterone-sensitive neurons in the nucleus of the solitary: efferent projections. *The Journal of comparative neurology* 498:223–50.
Geerling, J. C., and Loewy, A. D. 2006b. Aldosterone-sensitive neurons in the nucleus of the solitary tract: bidirectional connections with the central nucleus of the amygdala. *The Journal of comparative neurology* 497:646–57.
Geerling, J. C., and Loewy, A. D. 2006c. Aldosterone-sensitive NTS neurons are inhibited by saline ingestion during chronic mineralocorticoid treatment. *Brain Research* 1115:54–64.
Geerling, J. C., and Loewy, A. D. 2008. Central regulation of sodium appetite. *Experimental physiology* 93:177–209.
Geerling, J. C., and Loewy, A. D. 2006d. Sodium depletion activates the aldosterone-sensitive neurons in the NTS independently of thirst. *AJP: Regulatory, Integrative and Comparative Physiology* 292:R1338–R1348.
Geerling, J. C., and Loewy, A. D. 2007. Sodium deprivation and salt intake activate separate neuronal subpopulations in the nucleus of the solitary tract and the parabrachial complex. *The Journal of Comparative Neurology* 504:379–403.
Geerling, J. C., Shin, J.-W., Chimenti, P. C., and Loewy, A. D. 2010. Paraventricular hypothalamic nucleus: axonal projections to the brainstem. *The Journal of comparative neurology* 518:1460–99.
Geerling, J. C., Stein, M. K., Miller, R. L., Shin, J.-W., Gray, P. A., and Loewy, A. D. 2011. FoxP2 expression defines dorsolateral pontine neurons activated by sodium deprivation. *Brain research* 1375:19–27.
Geldwert, D., Norris, J. M., Feldman, I. G., Schulman, J. J., Joyce, M. P., and Rayport, S. 2006. Dopamine presynaptically and heterogeneously modulates nucleus accumbens medium-spiny neuron GABA synapses in vitro. *BMC neuroscience* 7:53.
Geran, L. C., and Spector, A. C. 2000a. Amiloride increases sodium chloride taste detection threshold in rats. *Behavioral neuroscience* 114:623–34.
Geran, L. C., and Spector, A. C. 2004. Anion size does not compromise sodium recognition by rats after acute sodium depletion. *Behavioral neuroscience* 118:178–83.
Geran, L. C., and Spector, A. C. 2000b. Sodium taste detectability in rats is dependent of anion size: The psychophysical characteristics of the transcellular sodium taste transduction pathway. *Behavioral Neuroscience* 114:1229–1238.
Gilbertson, T. A. 2002. Hypoosmotic stimuli activate a chloride conductance in rat taste cells.

Chemical senses 27:383–94.
Gilbertson, T. A., Baquero, A. F., and Spray-Watson, K. J. 2006. Water taste: the importance of osmotic sensing in the oral cavity. *Journal of water and health* 4 Suppl 1:35–40.
Gilbertson, T. A., Roper, S. D., and Kinnamon, S. C. 1993. Proton currents through amiloride-sensitive Na+ channels in isolated hamster taste cells: enhancement by vasopressin and cAMP. *Neuron* 10:931–42.
Grafe, L. A., and Flanagan-Cato, L. M. 2016. Differential effects of mineralocorticoid and angiotensin II on incentive and mesolimbic activity. *Hormones and behavior* 79:28–36.
Grill, H. J., and Miselis, R. R. 1981. Lack of ingestive compensation to osmotic stimuli in chronic decerebrate rats. *The American journal of physiology* 240:R81-6.
Grill, H. J., and Norgren, R. 1978. The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats. *Brain research* 143:263–79.
Grill, H. J., Schulkin, J., and Flynn, F. W. 1986. Sodium homeostasis in chronic decerebrate rats. *Behavioral neuroscience* 100:536–43.
Groenewegen, H. J., Wright, C. I., Beijer, A. V, and Voorn, P. 1999. Convergence and segregation of ventral striatal inputs and outputs. *Annals of the New York Academy of Sciences* 877:49–63.
Gross, P. M., Wall, K. M., Pang, J. J., Shaver, S. W., and Wainman, D. S. 1990. Microvascular specializations promoting rapid interstitial solute dispersion in nucleus tractus solitarius. *The American journal of physiology* 259:R1131-8.
Grossman, S. 1990. Thirst and Sodium Appetite : Physiological Basis. Elsevier Science.
Hall, J. E., and Guyton, A. C. 2011. Guyton and Hall Textbook of Medical Physiology.
Hamid, A. A., Pettibone, J. R., Mabrouk, O. S., Hetrick, V. L., Schmidt, R., Vander Weele, C. M., Kennedy, R. T., Aragona, B. J., and Berke, J. D. 2016. Mesolimbic dopamine signals the value of work. *Nature neuroscience* 19:117–26.
Hamilton, R. B., and Norgren, R. 1984. Central projections of gustatory nerves in the rat. *The Journal of comparative neurology* 222:560–77.
Handal, P. J. 1965. Immediate acceptance of sodium salts by sodium deficient rats. *Psychonomic Science* 3:315–316.
Hayes, M. R., and Schmidt, H. D. 2016. GLP-1 influences food and drug reward. *Current Opinion in Behavioral Sciences* 9:66–70.
Heck, G. L., Mierson, S., and DeSimone, J. A. 1984. Salt taste transduction occurs through an amiloride-sensitive sodium transport pathway. *Science (New York, N.Y.)* 223:403–5.
Heien, M. L. A. V, Johnson, M. A., and Wightman, R. M. 2004. Resolving neurotransmitters detected by fast-scan cyclic voltammetry. *Analytical chemistry* 76:5697–704.
Herness, M. S. 1992. Aldosterone increases the amiloride-sensitivity of the rat gustatory neural response to NaCl. *Comparative biochemistry and physiology. Comparative physiology* 103:269–73.
Herness, S., Zhao, F.-L., Lu, S., Kaya, N., and Shen, T. 2002. Expression and physiological actions of cholecystokinin in rat taste receptor cells. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 22:10018–29.
Hoebel, B. G., Hernandez, L., Schwartz, D. H., MARK, G. P., and Hunter, G. A. 1989. Microdialysis Studies of Brain Norepinephrine, Serotonin, and Dopamine Release During Ingestive Behavior Theoretical and Clinical Implications. *Annals of the New York Academy of Sciences*

575:171–193.
Hommel, J. D., Trinko, R., Sears, R. M., Georgescu, D., Liu, Z.-W., Gao, X.-B., Thurmon, J. J., Marinelli, M., and DiLeone, R. J. 2006. Leptin receptor signaling in midbrain dopamine neurons regulates feeding. *Neuron* 51:801–10.
Huang, T., and Yan, J. 2008. Dietary sodium deprivation reduces gustatory neural responses of the parabrachial nucleus in rats. *Neuroscience letters* 432:170–3.
Hughes, J. E., Amyx, H., Howard, J. L., Nanry, K. P., and Pollard, G. T. 1994. Health effects of water restriction to motivate lever-pressing in rats. *Laboratory animal science* 44:135–40.
Hull, C. L., and Krantz, D. L. 1943. Hull's Principles of Behavior and Psychology's Unity A review of Principles of Behavior: An Introduction to Behavior Theory. 422.
Ilango, A., Kesner, A. J., Broker, C. J., Wang, D. V, and Ikemoto, S. 2014. Phasic excitation of ventral tegmental dopamine neurons potentiates the initiation of conditioned approach behavior: parametric and reinforcement-schedule analyses. *Frontiers in behavioral neuroscience* 8:155.
Izzo, J. L., Sica, D. A., Black, H. R. (Henry R., and Council for High Blood Pressure Research (American Heart Association) 2008. Hypertension primer : [the essentials of high blood pressure : basic science, population science, and clinical management]. Lippincott Williams & Wilkins.
Jacobs, K. M., Mark, G. P., and Scott, T. R. 1988. Taste responses in the nucleus tractus solitarius of sodium-deprived rats. *The Journal of physiology* 406:393–410.
Jarvie, B. C., and Palmiter, R. D. 2016. HSD2 neurons in the hindbrain drive sodium appetite. *Nature Neuroscience* 20:167–169.
Jerlhag, E., Egecioglu, E., Dickson, S. L., Andersson, M., Svensson, L., and Engel, J. A. 2006. Ghrelin stimulates locomotor activity and accumbal dopamine-overflow via central cholinergic systems in mice: implications for its involvement in brain reward. *Addiction biology* 11:45–54.
Jhamandas, J. H., and Renaud, L. P. 1986. A gamma-aminobutyric-acid-mediated baroreceptor input to supraoptic vasopressin neurones in the rat. *The Journal of physiology* 381:595–606.
Johnson, A. K., and Fisher, A. E. 1973. Taste preferences for sucrose solutions and water under cholinergic and deprivation thirst. *Physiology & behavior* 10:607–12.
Johnson, A. K., and Thunhorst, R. L. 1997. The Neuroendocrinology of Thirst and Salt Appetite: Visceral Sensory Signals and Mechanisms of Central Integration. *Frontiers in Neuroendocrinology* 18:292–353.
Johnson, J. A., Zehr, J. E., and Moore, W. W. 1970. Effects of separate and concurrent osmotic and volume stimuli on plasma ADH in sheep. *The American journal of physiology* 218:1273–80.
Jones, J. L., Day, J. J., Aragona, B. J., Wheeler, R. A., Wightman, R. M., and Carelli, R. M. 2010. Basolateral Amygdala Modulates Terminal Dopamine Release in the Nucleus Accumbens and Conditioned Responding. *Biological Psychiatry* 67:737–744.
Kang, B. J., Chang, D. A., MacKay, D. D., West, G. H., Moreira, T. S., Takakura, A. C., Gwilt, J. M., Guyenet, P. G., and Stornetta, R. L. 2007. Central nervous system distribution of the transcription factor Phox2b in the adult rat. *The Journal of Comparative Neurology* 503:627–641.

Karimnamazi, H., and Travers, J. B. 1998. Differential projections from gustatory responsive regions of the parabrachial nucleus to the medulla and forebrain. *Brain Research* 813:283–302.

Kawai, K., Sugimoto, K., Nakashima, K., Miura, H., and Ninomiya, Y. 2000. Leptin as a modulator of sweet taste sensitivities in mice. *Proceedings of the National Academy of Sciences* 97:11044–11049.

Kellenberger, S., Gautschi, I., and Schild, L. 1999. A single point mutation in the pore region of the epithelial Na+ channel changes ion selectivity by modifying molecular sieving. *Proceedings of the National Academy of Sciences of the United States of America* 96:4170–5.

Kelley, A. E. 2004. Ventral striatal control of appetitive motivation: role in ingestive behavior and reward-related learning. *Neuroscience & Biobehavioral Reviews* 27:765–776.

Khavari, K. A., and Russell, R. W. 1966. Acquisition, retention, and extinction under conditions of water deprivation and of central cholinergic stimulation. *Journal of comparative and physiological psychology* 61:339–45.

Kimura, T., Funyu, T., Ohta, M., Yamamoto, T., Ota, K., Shoji, M., Inoue, M., Sato, K., and Abe, K. 1994. The role of GABA in the central regulation of AVP and ANP release and blood pressure due to angiotensin and carbachol, and central GABA release due to blood pressure changes. *Journal of the autonomic nervous system* 50:21–9.

Koob, G. F., Riley, S. J., Smith, S. C., and Robbins, T. W. 1978. Effects of 6-hydroxydopamine lesions of the nucleus accumbens septi and olfactory tubercle on feeding, locomotor activity, and amphetamine anorexia in the rat. *Journal of comparative and physiological psychology* 92:917–27.

Krause, M., German, P. W., Taha, S. A., and Fields, H. L. 2010. A pause in nucleus accumbens neuron firing is required to initiate and maintain feeding. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 30:4746–56.

Krieckhaus, E. E., and Wolf, G. 1968. Acquisition of sodium by rats: interaction of innate mechanisms and latent learning. *Journal of comparative and physiological psychology* 65:197–201.

Krout, K. E., and Loewy, A. D. 2000. Parabrachial nucleus projections to midline and intralaminar thalamic nuclei of the rat. *The Journal of Comparative Neurology* 428:475–494.

Krügel, U., Schraft, T., Kittner, H., Kiess, W., and Illes, P. 2003. Basal and feeding-evoked dopamine release in the rat nucleus accumbens is depressed by leptin. *European journal of pharmacology* 482:185–7.

Labouèbe, G., Liu, S., Dias, C., Zou, H., Wong, J. C. Y., Karunakaran, S., Clee, S. M., Phillips, A. G., Boutrel, B., and Borgland, S. L. 2013. Insulin induces long-term depression of ventral tegmental area dopamine neurons via endocannabinoids. *Nature neuroscience* 16:300–8.

Leib, D. E., Zimmerman, C. A., and Knight, Z. A. 2016. Thirst. *Current Biology* 26:R1260–R1265.

Liu, S., and Borgland, S. L. 2015. Regulation of the mesolimbic dopamine circuit by feeding peptides. *Neuroscience* 289:19–42.

Loriaux, A. L., Roitman, J. D., and Roitman, M. F. 2011. Nucleus accumbens shell, but not core, tracks motivational value of salt. *Journal of neurophysiology* 106:1537–44.

Lucas, L. R., Grillo, C. A., and McEwen, B. S. 2007. Salt appetite in sodium-depleted or sodium-replete conditions: possible role of opioid receptors. *Neuroendocrinology* 85:139–47.

Lucas, L. R., Pompei, P., and McEwen, B. S. 2000. Salt appetite in salt-replete rats: involvement of mesolimbic structures in deoxycorticosterone-induced salt craving behavior. *Neuroendocrinology* 71:386–95.

Mahler, S. V, Smith, K. S., and Berridge, K. C. 2007. Endocannabinoid Hedonic Hotspot for Sensory Pleasure: Anandamide in Nucleus Accumbens Shell Enhances ?Liking? of a Sweet Reward. *Neuropsychopharmacology* 32:2267–2278.

Marinelli, M., Rudick, C. N., Hu, X.-T., and White, F. J. 2006. Excitability of dopamine neurons: modulation and physiological consequences. *CNS & neurological disorders drug targets* 5:79–97.

Markison, S., St John, S. J., and Spector, A. C. 1995. Glossopharyngeal nerve transection does not compromise the specificity of taste-guided sodium appetite in rats. *The American journal of physiology* 269:R215-21.

McCutcheon, J. E., Ebner, S. R., Loriaux, A. L., and Roitman, M. F. 2012. Encoding of aversion by dopamine and the nucleus accumbens. *Frontiers in neuroscience* 6:137.

McEwen, B. S., Lambdin, L. T., Rainbow, T. C., and De Nicola, A. F. 1986. Aldosterone effects on salt appetite in adrenalectomized rats. *Neuroendocrinology* 43:38–43.

McKinley, M. J., Denton, D. A., Nelson, J. F., and Weisinger, R. S. 1983. Dehydration induces sodium depletion in rats, rabbits, and sheep. *The American journal of physiology* 245:R287-92.

Meredith, G. E. 1999. The synaptic framework for chemical signaling in nucleus accumbens. *Annals of the New York Academy of Sciences* 877:140–56.

Meredith, G. E., Baldo, B. A., Andrezjewski, M. E., and Kelley, A. E. 2008. The structural basis for mapping behavior onto the ventral striatum and its subdivisions. *Brain Structure and Function* 213:17–27.

Miller, I. 1977. Gustatory receptors of the palate. *Food Intake and Chemical Senses*:173–185.

Mogenson, G. J., Jones, D. L., and Yim, C. Y. 1980. From motivation to action: functional interface between the limbic system and the motor system. *Progress in neurobiology* 14:69–97.

Mook, D. G., and Wagner, S. 1988. Sham drinking of glucose solutions in rats: some effects of hydration. *Appetite* 10:71–87.

Morris, M. J., Na, E. S., Grippo, A. J., and Johnson, A. K. 2006. The effects of deoxycorticosterone-induced sodium appetite on hedonic behaviors in the rat. *Behavioral Neuroscience* 120:571–579.

Na, E. S., Morris, M. J., Johnson, R. F., Beltz, T. G., and Johnson, A. K. 2007. The neural substrates of enhanced salt appetite after repeated sodium depletions. *Brain research* 1171:104–10.

Nachman, M. 1963a. Learned aversion to the taste of lithium chloride and generalization to other salts. *Journal of comparative and physiological psychology* 56:343–9.

Nachman, M. 1963b. Taste preferences for lithium chloride by adrenalectomized rats. *The American journal of physiology* 205:219–21.

Nachman, M. 1962. Taste preferences for sodium salts by adrenalectomized rats. *Journal of comparative and physiological psychology* 55:1124–9.

Nachman, M., and Ashe, J. H. 1974. EFFECTS OF BASOLATERAL AMYGDALA LESIONS ON NEOPHOBIA, LEARNED TASTE AVERSIONS, AND SODIUM APPETITE IN RATS. *Journal of*

Comparative and Physiological Psychology 1074:622–643.

Nakamura, K., and Norgren, R. 1991. Gustatory responses of neurons in the nucleus of the solitary tract of behaving rats. *Journal of Neurophysiology* 66.

Nicola, S. M., Surmeier, J., and Malenka, R. C. 2000. Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. *Annual review of neuroscience* 23:185–215.

Nishijo, H., and Norgren, R. 1990. Responses from parabrachial gustatory neurons in behaving rats. *Journal of Neurophysiology* 63.

Niv, Y. 2007. Cost, benefit, tonic, phasic: what do response rates tell us about dopamine and motivation? *Annals of the New York Academy of Sciences* 1104:357–76.

Norgren, R. 1978. Projections from the nucleus of the solitary tract in the rat. *Neuroscience* 3:207–18.

Parker, L. A. 1984. Behavioral conditioned responses across multiple conditioning/testing trials elicited by lithium- and amphetamine-paired flavors. *Behavioral and neural biology* 41:190–9.

Paxinos, G., and Watson, C. 2007. The rat brain in stereotaxic coordinates.

Peciña, S., and Berridge, K. C. 2005. Hedonic Hot Spot in Nucleus Accumbens Shell: Where Do μ-Opioids Cause Increased Hedonic Impact of Sweetness? *Journal of Neuroscience* 25.

Peciña, S., and Berridge, K. C. 2000. Opioid site in nucleus accumbens shell mediates eating and hedonic “liking” for food: map based on microinjection Fos plumes. *Brain Research* 863:71–86.

Peciña, S., Smith, K. S., and Berridge, K. C. 2006. Hedonic hot spots in the brain. *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry* 12:500–11.

Pfaffmann, C. 1955. Gustatory nerve impulses in rat, cat and rabbit. *Journal of Neurophysiology* 18.

Pfaffmann, C., Norgren, R., and Grill, H. J. 1977. Sensory affect and motivation. *Annals of the New York Academy of Sciences* 290:18–34.

Phillips, P. E. M., Stuber, G. D., Heien, M. L. A. V, Wightman, R. M., and Carelli, R. M. 2003. Subsecond dopamine release promotes cocaine seeking. *Nature* 422:614–8.

Quartermain, D., Miller, N. E., and Wolf, G. 1967. Role of experience in relationship between sodium deficiency and rate of bar pressing for salt. *Journal of Comparative and Physiological Psychology* 63:417–420.

Radomski, J. L., Fuyat, H. N., Nelson, A. A., and Smith, P. K. 1950. The toxic effects, excretion and distribution of lithium chloride. *The Journal of pharmacology and experimental therapeutics* 100:429–44.

Reilly, J. J., Maki, R., Nardozzi, J., and Schulkin, J. 1994. The effects of lesions of the bed nucleus of the stria terminalis on sodium appetite. *Acta neurobiologiae experimentalis* 54:253–7.

Richter, C. 1947. Biology of drives. *Journal of Comparative and Physiological Psychology* 40:129–134.

Richter, C. 1936. Increased salt appetite in adrenalectomized rats. *American Journal of Physiology* 115:155–161.

Robinson, M. J. F., and Berridge, K. C. 2013. Instant transformation of learned repulsion into motivational "wanting". *Current biology : CB* 23:282–9.

Robinson, T. E., and Kolb, B. 1999. Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. *The European journal of neuroscience* 11:1598–604.

Roitman, M. F., and Bernstein, I. L. 1999. Amiloride-sensitive sodium signals and salt appetite: multiple gustatory pathways. *The American journal of physiology* 276:R1732-8.

Roitman, M. F., Na, E., Anderson, G., Jones, T. A., and Bernstein, I. L. 2002. Induction of a salt appetite alters dendritic morphology in nucleus accumbens and sensitizes rats to amphetamine. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 22:RC225.

Roitman, M. F., Patterson, T. A., Sakai, R. R., Bernstein, I. L., and Figlewicz, D. P. 1999a. Sodium depletion and aldosterone decrease dopamine transporter activity in nucleus accumbens but not striatum. *The American journal of physiology* 276:R1339-45.

Roitman, M. F., Patterson, T. A., Sakai, R. R., Bernstein, I. L., Figlewicz, D. P., Ahima, R., Krozowski, Z., Harlan, R., Aldridge, J. W., Berridge, K. C., et al. 1999b. Sodium depletion and aldosterone decrease dopamine transporter activity in nucleus accumbens but not striatum. *The American journal of physiology* 276:R1339-45.

Roitman, M. F., Schafe, G. E., Thiele, T. E., and Bernstein, I. L. 1997. Dopamine and sodium appetite: antagonists suppress sham drinking of NaCl solutions in the rat. *Behavioral neuroscience* 111:606–11.

Roitman, M. F., Stuber, G. D., Phillips, P. E. M., Wightman, R. M., and Carelli, R. M. 2004. Dopamine operates as a subsecond modulator of food seeking. *The Journal of neuroscience* 24:1265–71.

Roitman, M. F., Wheeler, R. A., and Carelli, R. M. 2005. Nucleus accumbens neurons are innately tuned for rewarding and aversive taste stimuli, encode their predictors, and are linked to motor output. *Neuron* 45:587–97.

Roitman, M. F., Wheeler, R. A., Wightman, R. M., and Carelli, R. M. 2008. Real-time chemical responses in the nucleus accumbens differentiate rewarding and aversive stimuli. *Nature neuroscience* 11:1376–7.

Rolls, B. J., Jones, B. P., and Fallows, D. J. 1972. A comparison of the motivational properties of thirst induced by intracranial angiotensin and by water deprivation. *Physiology & Behavior* 9:777–782.

Rolls, B. J., and Rolls, E. T. 1973. Effects of lesions in the basolateral amygdala on fluid intake in the rat. *Journal of comparative and physiological psychology* 83:240–7.

Rolls, B. J., and Rolls, E. T. 1982. Thirst. Cambridge University Press.

Rolls, B. J., Wood, R. J., Rolls, E. T., Lind, H., Lind, W., and Ledingham, J. G. 1980. Thirst following water deprivation in humans. *The American journal of physiology* 239:R476-82.

Rolls, E. T. 2015. Taste, olfactory, and food reward value processing in the brain. *Progress in Neurobiology* 127–128:64–90.

Rolls, E. T. 1999. The brain and emotion. Oxford University Press.

Rolls, E. T., Murzi, E., Yaxley, S., Thorpe, S. J., and Simpson, S. J. 1986. Sensory-specific satiety: Food-specific reduction in responsiveness of ventral forebrain neurons after feeding in the monkey. *Brain Research* 368:79–86.

Rolls, E. T., Yaxley, S., and Sienkiewicz, Z. J. 1990. Gustatory responses of single neurons in the caudolateral orbitofrontal cortex of the macaque monkey. *Journal of neurophysiology*

64:1055–66.
Rosen, A. M., Roussin, A. T., and Di Lorenzo, P. M. 2010. Water as an Independent Taste Modality. *Frontiers in Neuroscience* 4:175.
Rowland, N. E., and Morian, K. R. 1999. Roles of aldosterone and angiotensin in maturation of sodium appetite in furosemide-treated rats. *The American journal of physiology* 276:R1453-60.
Sakaguchi, T., Tamaki, M., Akaishi, T., and Miyaoka, Y. 1989. Responses in discharge of vasopressinergic neurons in the hypothalamic paraventricular nucleus elicited by water application to the pharyngolaryngeal regions in the rat. *Chemical Senses* 14:327–333.
Sakai, R. R., Fine, W. B., Epstein, A. N., and Frankmann, S. P. 1987. Salt appetite is enhanced by one prior episode of sodium depletion in the rat. *Behavioral neuroscience* 101:724–31.
Sakai, R. R., Frankmann, S. P., Fine, W. B., and Epstein, A. N. 1989. Prior episodes of sodium depletion increase the need-free sodium intake of the rat. *Behavioral neuroscience* 103:186–92.
Sakai, R. R., Mcewen, B. S., Fluharty, S. J., and Ma, L. Y. 2000. The amygdala: Site of genomic and nongenomic arousal of aldosterone-induced sodium intake. *Kidney International* 57:1337–1345.
Salamone, J. D., and Correa, M. 2012. The Mysterious Motivational Functions of Mesolimbic Dopamine.
Salamone, J. D., Correa, M., Mingote, S., and Weber, S. M. 2003. Nucleus Accumbens Dopamine and the Regulation of Effort in Food-Seeking Behavior: Implications for Studies of Natural Motivation, Psychiatry, and Drug Abuse. *Journal of Pharmacology and Experimental Therapeutics* 305:1–8.
Salamone, J. D., Pardo, M., Yohn, S. E., López-Cruz, L., SanMiguel, N., and Correa, M. 2016. Mesolimbic Dopamine and the Regulation of Motivated Behavior. *Current topics in behavioral neurosciences* 27:231–57.
Schulkin, J. 1991. Sodium hunger : the search for a salty taste. Cambridge University Press.
Schulkin, J. 1992. Sodium hunger: The search for a salty taste., 1st ed. Cambridge University Press, New York.
Schulkin, J., Arnell, P., and Stellar, E. 1985. Running to the taste of salt in mineralocorticoid-treated rats. *Hormones and behavior* 19:413–25.
Schultz, W. 1997. Dopamine neurons and their role in reward mechanisms. *Current opinion in neurobiology* 7:191–7.
Schultz, W., Dayan, P., and Montague, P. R. 1997. A neural substrate of prediction and reward. *Science (New York, N.Y.)* 275:1593–9.
Sequeira, S. M., Geerling, J. C., and Loewy, A. D. 2006. Local inputs to aldosterone-sensitive neurons of the nucleus tractus solitarius. *Neuroscience* 141:1995–2005.
Shade, R. E., and Share, L. 1975. Volume Control of Plasma Antidiuretic Hormone Concentration Following Acute Blood Volume Expansion n i the Anesthetized Dog. *Endocrinology* 97:1048–1057.
Share, L. 1988. Role of vasopressin in cardiovascular regulation. *Physiological reviews* 68:1248–84.
Shekhtman, E., Geerling, J. C., and Loewy, A. D. 2007. Aldosterone-sensitive neurons of the nucleus of the solitary tract: Multisynaptic pathway to the nucleus accumbens. *The Journal*

of Comparative Neurology 501:274–289.

Sheppard, K. E., and Funder, J. W. 1987. Equivalent affinity of aldosterone and corticosterone for type I receptors in kidney and hippocampus: Direct binding studies. *Journal of Steroid Biochemistry* 28:737–742.

Shibata, R., Kameishi, M., Kondoh, T., and Torii, K. 2009. Bilateral dopaminergic lesions in the ventral tegmental area of rats influence sucrose intake, but not umami and amino acid intake. *Physiology & behavior* 96:667–74.

Shin, J.-W., Geerling, J. C., and Loewy, A. D. 2009. Vagal innervation of the aldosterone-sensitive HSD2 neurons in the NTS. *Brain research* 1249:135–47.

Shin, J.-W., Geerling, J. C., Stein, M. K., Miller, R. L., and Loewy, A. D. 2011. FoxP2 brainstem neurons project to sodium appetite regulatory sites. *Journal of chemical neuroanatomy* 42:1–23.

Shin, J.-W., and Loewy, A. D. 2009. Gastric afferents project to the aldosterone-sensitive HSD2 neurons of the NTS. *Brain research* 1301:34–43.

Shingai, T. 1980. Water fibers in the superior laryngeal nerve of the rat. *The Japanese journal of physiology* 30:305–7.

Simpson, J. B., Epstein, A. N., and Camardo, J. S. 1978. Localization of receptors for the dipsogenic action of angiotensin II in the subfornical organ of rat. *Journal of comparative and physiological psychology* 92:581–601.

Simpson, J. B., and Routtenberg, A. 1973. Subfornical organ: site of drinking elicitation by angiotensin II. *Science (New York, N.Y.)* 181:1172–5.

Simpson, S. J., Sword, G. A., Lorch, P. D., and Couzin, I. D. 2006. Cannibal crickets on a forced march for protein and salt. *Proceedings of the National Academy of Sciences of the United States of America* 103:4152–6.

Sinkala, E., McCutcheon, J. E., Schuck, M. J., Schmidt, E., Roitman, M. F., and Eddington, D. T. 2012. Electrode calibration with a microfluidic flow cell for fast-scan cyclic voltammetry. *Lab on a chip* 12:2403–8.

Smith, K. S., and Berridge, K. C. 2007. Opioid Limbic Circuit for Reward: Interaction between Hedonic Hotspots of Nucleus Accumbens and Ventral Pallidum. *Journal of Neuroscience* 27.

Smith, M. H., Holman, G. L., and Fortune, K. H. 1968. Sodium need and sodium consumption. *Journal of comparative and physiological psychology* 65:33–7.

Sossi, V., and Ruth, T. J. 2005. Micropet imaging: in vivo biochemistry in small animals. *Journal of neural transmission* 112:319–30.

Spector, A. C., and Grill, H. J. 1992. Salt taste discrimination after bilateral section of the chorda tympani or glossopharyngeal nerves. *The American journal of physiology* 263:R169-76.

Spector, A. C., Guagliardo, N. A., and St John, S. J. 1996. Amiloride disrupts NaCl versus KCl discrimination performance: implications for salt taste coding in rats. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 16:8115–22.

St John, S. J., Markison, S., Guagliardo, N. A., Hackenberg, T. D., and Spector, A. C. 1997. Chorda tympani transection and selective desalivation differentially disrupt two-lever salt discrimination performance in rats. *Behavioral neuroscience* 111:450–9.

Starr, L. J., and Rowland, N. E. 2006. Characteristics of salt appetite in chronically sodium-depleted rats using a progressive ratio schedule of procurement. *Physiology & Behavior* 88:433–442.

Stein, M. K., and Loewy, A. D. 2010. Area postrema projects to FoxP2 neurons of the pre-locus coeruleus and parabrachial nuclei: brainstem sites implicated in sodium appetite regulation. *Brain research* 1359:116–27.

Steinberg, E. E., Boivin, J. R., Saunders, B. T., Witten, I. B., Deisseroth, K., and Janak, P. H. 2014. Positive Reinforcement Mediated by Midbrain Dopamine Neurons Requires D1 and D2 Receptor Activation in the Nucleus Accumbens. *PLoS ONE* 9:e94771.

Steinberg, E. E., Keiflin, R., Boivin, J. R., Witten, I. B., Deisseroth, K., and Janak, P. H. 2013. A causal link between prediction errors, dopamine neurons and learning. *Nature neuroscience* 16:966–73.

Stellar, E. 1980. Brain mechanisms and hedonic processes. *Acta neurobiologiae experimentalis* 40:313–24.

Stellar, E. 1993. Salt appetite: its neuroendocrine basis. *Acta neurobiologiae experimentalis* 53:475–84.

Stellar, E., and Epstein, A. N. 1991. Neuroendocrine factors in salt appetite. *Journal of physiology and pharmacology : an official journal of the Polish Physiological Society* 42:345–55.

Stellar, E., and Hill, J. H. THE RAT'S RATE OF DRINKING AS A FUNCTION OF WATER DEPRIVATION.

Stellar, J. R., and Stellar, E. 1985. The Neurobiology of Motivation and Reward. Springer New York, New York, NY.

Stornetta, R. L., Moreira, T. S., Takakura, A. C., Kang, B. J., Chang, D. A., West, G. H., Brunet, J. F., Mulkey, D. K., Bayliss, D. A., and Guyenet, P. G. 2006. Expression of Phox2b by Brainstem Neurons Involved in Chemosensory Integration in the Adult Rat. *Journal of Neuroscience* 26.

Stouffer, M. A., Woods, C. A., Patel, J. C., Lee, C. R., Witkovsky, P., Bao, L., Machold, R. P., Jones, K. T., de Vaca, S. C., Reith, M. E. A., et al. 2015. Insulin enhances striatal dopamine release by activating cholinergic interneurons and thereby signals reward. *Nature communications* 6:8543.

Stricker, E. M. 1981. Thirst and sodium appetite after colloid treatment in rats. *Journal of Comparative and Physiological Psychology* 95:1–25.

Stricker, E. M., Thiels, E., and Verbalis, J. G. 1991. Sodium appetite in rats after prolonged dietary sodium deprivation: a sexually dimorphic phenomenon. *The American journal of physiology* 260:R1082-8.

Stuber, G. D., Sparta, D. R., Stamatakis, A. M., van Leeuwen, W. A., Hardjoprajitno, J. E., Cho, S., Tye, K. M., Kempadoo, K. A., Zhang, F., Deisseroth, K., et al. 2011. Excitatory transmission from the amygdala to nucleus accumbens facilitates reward seeking. *Nature* 475:377–80.

Sulzer, D., Cragg, S. J., and Rice, M. E. 2016. Striatal dopamine neurotransmission: regulation of release and uptake. *Basal ganglia* 6:123–148.

Syed, E. C. J., Grima, L. L., Magill, P. J., Bogacz, R., Brown, P., and Walton, M. E. 2015. Action initiation shapes mesolimbic dopamine encoding of future rewards. *Nature Neuroscience* 19:34–36.

Taha, S. A., and Fields, H. L. 2006. Inhibitions of nucleus accumbens neurons encode a gating signal for reward-directed behavior. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 26:217–22.

Takahashi, M., and Tanaka, J. 2016. Serotonin release in the subfornical organ area induced by sodium and water intake in the rat. *Physiology & Behavior* 164:123–128.

Takamata, A., Mack, G. W., Gillen, C. M., and Nadel, E. R. 1994. Sodium appetite, thirst, and body fluid regulation in humans during rehydration without sodium replacement. *The American journal of physiology* 266:R1493-502.

Tindell, A. J., Smith, K. S., Peciña, S., Berridge, K. C., and Aldridge, J. W. 2006. Ventral Pallidum Firing Codes Hedonic Reward: When a Bad Taste Turns Good. *Journal of Neurophysiology* 96.

Trowill JA, Panksepp J, G. R. 1969. An incentive model of rewarding brain stimulation. *Psychol Rev.* 76:264–81.

Tsai, H.-C., Zhang, F., Adamantidis, A., Stuber, G. D., Bonci, A., de Lecea, L., and Deisseroth, K. 2009. Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. *Science (New York, N.Y.)* 324:1080–4.

Ungerstedt, U. 1971. Adipsia and Aphagia after 6-Hydroxydopamine Induced Degeneration of the Nigro-striatal Dopamine System. *Acta Physiologica Scandinavica* 82:95–122.

Verhagen, J. V., Giza, B. K., and Scott, T. R. 2003. Responses to Taste Stimulation in the Ventroposteromedial Nucleus of the Thalamus in Rats. *Journal of Neurophysiology* 89.

Verney, E. B. 1947. The antidiuretic hormone and the factors which determine its release. *Proceedings of the Royal Society of London. Series B, Biological sciences* 135:25–106.

Volman, S. F., Lammel, S., Margolis, E. B., Kim, Y., Richard, J. M., Roitman, M. F., and Lobo, M. K. 2013. New insights into the specificity and plasticity of reward and aversion encoding in the mesolimbic system. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 33:17569–76.

Vonder, A. J., and Carlson, J. Mechanism of the Effects of Furosemide on Renin Secretion in Anesthetized Dogs.

Wagman, W. 1963. Sodium chloride deprivation: development of sodium chloride as a reinforcement. *Science (New York, N.Y.)* 140:1403–4.

Warden, C. 1931. Animal motivation: experimental studies on the albino rat,. Columbia University Press, New York.

Watabe-Uchida, M., Zhu, L., Ogawa, S. K., Vamanrao, A., and Uchida, N. 2012. Whole-Brain Mapping of Direct Inputs to Midbrain Dopamine Neurons. *Neuron* 74:858–873.

Watson, C. J., Venton, B. J., and Kennedy, R. T. 2006. In vivo measurements of neurotransmitters by microdialysis sampling. *Analytical chemistry* 78:1391–9.

Watson, K. J., Kim, I., Baquero, A. F., Burks, C. A., Liu, L., and Gilbertson, T. A. Expression of Aquaporin Water Channels in Rat Taste Buds.

Weisinger, R. S., Denton, D. A., McKinley, M. J., and Nelson, J. F. 1985. Dehydration-induced sodium appetite in rats. *Physiology & behavior* 34:45–50.

Wise, R. A. 2004. Dopamine, learning and motivation. *Nature reviews. Neuroscience* 5:483–94.

Wise, R. A., Spindler, J., deWit, H., and Gerberg, G. J. 1978. Neuroleptic-induced "anhedonia" in rats: pimozide blocks reward quality of food. *Science (New York, N.Y.)* 201:262–4.

Witten, I. B., Steinberg, E. E., Lee, S. Y., Davidson, T. J., Zalocusky, K. A., Brodsky, M., Yizhar, O., Cho, S. L., Gong, S., Ramakrishnan, C., et al. 2011. Recombinase-driver rat lines: tools, techniques, and optogenetic application to dopamine-mediated reinforcement. *Neuron*

72:721–33.
Wolf, G. 1967. Hypothalamic regulation of sodium intake: relations to preoptic and tegmental function. *The American journal of physiology* 213:1433–8.
Wolf, G., Dicara, L. V, and Braun, J. J. 1970. Sodium appetite in rats after neocortical ablation. *Physiology & behavior* 5:1265–9.
Wolf, G., and Quartermain, D. 1967. Sodium chloride intake of adrenalectomized rats with lateral hypothalamic lesions. *The American journal of physiology* 212:113–8.
Wood, R. J., Rolls, E. T., and Rolls, B. J. 1982. Physiological mechanisms for thirst in the nonhuman primate. *The American journal of physiology* 242:R423-8.
Yamada, H., Louie, K., and Glimcher, P. W. 2010. Controlled water intake: A method for objectively evaluating thirst and hydration state in monkeys by the measurement of blood osmolality. *Journal of Neuroscience Methods* 191:83–89.
Yamamoto, T., Shimura, T., Sako, N., Sakai, N., Tanimizu, T., and Wakisaka, S. 1993. c-Fos expression in the parabrachial nucleus after ingestion of sodium chloride in the rat. *Neuroreport* 4:1223–6.
Yamamoto, T., Takemura, M., Inui, T., Torii, K., Maeda, N., Ohmoto, M., Matsumoto, I., and Abe, K. 2009. Functional Organization of the Rodent Parabrachial Nucleus. *Annals of the New York Academy of Sciences* 1170:378–382.
Zardetto-Smith, A. M., Beltz, T. G., and Johnson, A. K. 1994. Role of the central nucleus of the amygdala and bed nucleus of the stria terminalis in experimentally-induced salt appetite. *Brain research* 645:123–34.
Zhang, D.-M., Stellar, E., and Epstein, A. N. 1984. Together intracranial angiotensin and systemic mineralocorticoid produce avidity for salt in the rat. *Physiology & Behavior* 32:677–681.
Zimmerman, C. A., Lin, Y.-C., Leib, D. E., Guo, L., Huey, E. L., Daly, G. E., Chen, Y., and Knight, Z. A. 2016. Thirst neurons anticipate the homeostatic consequences of eating and drinking. *Nature* 537:680–684.
Zocchi, D., Wennemuth, G., and Oka, Y. 2017. The cellular mechanism for water detection in the mammalian taste system. *Nature neuroscience*.
Zotterman, Y. 1956. Species Differences in the Water Taste. *Acta Physiologica Scandinavica* 37:60–70.

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Education

University of Illinois at Chicago | Chicago, IL| 2012- present
PhD candidate in Neuroscience |GPA: 3.90
Anticipated date for dissertation defense: July 2017

University of Pennsylvania |Philadelphia, PA|2010-2011
Non-degree studies|GPA: 3.5

Ursinus College|Collegeville, PA | 2006-2010
B.S. in Biology and Spanish |GPA: 3.55 |Cum Laude |Honors in Biology

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Research Experience

Doctoral Candidate in Neuroscience | University of Illinois, Graduate Program in Neuroscience| 2012-present
Laboratory of Dr. Mitchell Roitman
Focus: The influence of physiological state on motivated behavior and underlying dopamine neurotransmission

Techniques: Fast-scan cyclic voltammetry, DREADD technology, optogenetics, cocaine self-administration, intracranial self-stimulation, survival rodent surgery (stereotaxic, intravenous, intraoral), immunohistochemistry

Research Technician | University of Pennsylvania, Department of Psychology | 2010-2012
Laboratory of Dr. Harvey Grill
Focus: Central and peripheral neural circuits regulating feeding behavior and energy expenditure

Techniques: phenotyping of animal behavior (conditioned taste avoidance, conditioned place preference, contextual fear conditioning, operant conditioning, spatial maze learning, and passive avoidance), central/peripheral pharmacological manipulations, survival rodent surgery (stereotaxic, gastrointestinal), Western Blots, ELISA

Honors Undergraduate Researcher | Ursinus College, Department of Biology | 2009-2010
Laboratory of Dr. Rebecca Lyczak
Focus: The role of the centrosome in establishing anterior-posterior axis polarity in *C. elegans* embryos

Techniques: *C. elegans* genetic crossing, RNAi, and confocal laser-scanning microscopy

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Peer-Reviewed Publications

1. **Fortin SM** and Roitman MF. Challenges to body fluid homeostasis recruit mesolimbic signaling in a need-specific manner. *In Preparation.*
2. **Fortin SM** and Roitman MF. Central GLP-1 receptor activation modulates cocaine-evoked phasic dopamine release in the nucleus accumbens core. *Physiology and Behavior*. 2017 March 16. Epub ahead of print..
3. **Fortin SM** and Roitman MF. Physiological state tunes mesolimbic signaling: lessons from sodium appetite and inspiration from Randall R. Sakai. *Physiology and Behavior*. 2016 Nov 19. Epub ahead of print.
4. Cone JJ, **Fortin SM**, McHenry JA, Stuber GD, McCutcheon JE, Roitman MF. Physiological state gates acquisition and expression of mesolimbic reward prediction signals. *PNAS*. 2016 Feb 16; 113(7):1943-8.
5. **Fortin SM**, Chartoff EH, Roitman MF. The Aversive Agent Lithium Chloride Suppresses Phasic Dopamine Release Through Central GLP-1 Receptors. *Neuropsychopharamacology.* 2016 Feb; 41(3):906-15.
6. Kanoski SE, Ong ZY, **Fortin SM,** Schlessinger ES, Grill HJ. Liraglutide, leptin and their combined effects on feeding: additive intake reduction through common intracellular signaling mechanism. *Diabetes Obes Metab.* 2015 Mar;17(3):285-93.
7. **Fortin SM**, Cone JJ, Ng-Evans S, McCutcheon JE, Roitman MF. Sampling phasic dopamine signaling with fast-scan cyclic voltammetry in awake, behaving rats. *Curr Protoc Neurosci*. 2015 Jan 5; 70:7.25.1-7.25.20.
8. McCutcheon JE, Cone JJ, Sinon CG, **Fortin SM,** Kantak PA, Witten IB, Deisseroth K, Stuber GD, Roitman MF. Optical Suppression of drug-evoked phasic dopamine release. *Front. Neural Circuits*. 2014 Sept 17;8:114.
9. Kanoksi SE, Alhadeff AL, **Fortin SM**, Gilbert JR, Grill HJ. Leptin signaling in the medial nucleus tractus solitaries reduces food seeking and willingness to work for food. *Neuropsychopharmacology*. 2014 Feb;39(3):605-13.
10. Kanoski SE, **Fortin SM**, Ricks KM, Grill HJ. Ghrelin signaling in the ventral hippocampus stimulates learned and motivational aspects of feeding via PI3K-Akt signaling. *Biol Psychiatry*. 2013 May 1;73(9):915-23.
11. Kanoski, SE, Rupprecht L, **Fortin SM**, De Jonghe BC, Hayes MR. Body weight and food intake suppression by glucagon-like-peptide-1 receptor agonists, exendin-4 and liraglutide, may require nausea. *Neuropharmacology.* 2012 Apr; 62(5-6):1916-27.
12. Hayes MR, Kanoski SE, De Jonghe BC, Leichner TM, Alhadeff AL, **Fortin SM**, Arnold M, Langhans W, Grill HJ. The common hepatic branch of the vagus is not required to mediate the glycemic and food intake suppressive effects of glucagon-like-peptide-1. *Am J Physiol Regul Integr Comp Physiol.* 2011 Nov; 301(5):R1479-85.
13. Kanoski SE, **Fortin SM**, Arnold M, Grill HJ, Hayes MR. Peripheral and central GLP-1 receptor populations mediate the anorectic effects of peripherally administered GLP-1 receptor agonists, liraglutide and exendin-4. *Endocrinology.* 2011 Aug; 152(8):3103-12.

14. Kanoski SE, Hayes MR, Greenwald HS, **Fortin SM**, Gianessi CA, Gilbert JR, Grill HJ. Hippocampal leptin signaling reduces food intake and modulates food-related memory processing. *Neuropsychopharmacology.* 2011 Aug; 36(9):1859-70.
15. **Fortin SM**, Marshall SL, Jaeger EC, Greene PE, Brady LK, Isaac RE, Schrandt JC, Brooks DR, Lyczak R. The PAM-1 aminopeptidase regulates centrosome positioning to ensure anterior-posterior axis specification in one-cell C. elegans embryos. *Dev Biol.* 2010 Aug 15;344(2):992-1000.

Conference and Symposia Presentations

1-2. **Fortin, SM** and Roitman MF. Physiological state modulated real time dopamine responses to taste stimuli.
- Chicago SFN | Chicago, IL | 2017
- Graduate Program in Neuroscience Student Symposium | Chicago, IL | 2017

3. **Fortin, SM** and Roitman MF. Nucleus accumbens dopamine responds to fluid balance-restoring stimuli in a state-dependent manner.
- Society for Neuroscience | San Diego, CA | 2016

4-6. **Fortin, SM** and Roitman MF. Challenges to body fluid homeostasis recruit mesolimbic signaling to fluid ingestion in a need-specific manner.
- Society for the Study of Ingestive Behavior | Porto, Portugal | 2016
- Society for Neuroscience | San Diego, CA | 2016
- Graduate Program in Neuroscience Student Symposium | Chicago, IL | 2016

7. **Fortin SM** and Roitman MF. Central GLP-1 receptor activation mediates cocaine-evoked phasic dopamine release in the nucleus accumbens core.
- Brain Research Foundation Neuroscience Day | Chicago, IL | 2016

8-12. **Fortin SM**, Cone JJ, Roitman MF. Central glucagon-like peptide-1 receptor activation mediates lithium chloride-induced dopamine suppression in the nucleus accumbens.
- Graduate Program in Neuroscience Student Symposium | Chicago, IL | 2015
- Invited Talk at Loyola University | Chicago, IL | 2015
- Brain Research Foundation Neuroscience Day | Chicago, IL | 2015
- Chicago Brain Bee| Chicago, IL | 2015
- Society for Neuroscience | Washington DC | 2014

13-16**. Fortin SM**, Roitman MF. GLP-1 receptor activation mediates LiCl induced dopamine suppression in the nucleus accumbens.
- Society for the Study of Ingestive Behavior | Seattle, WA | 2014
- Brain Research Foundation Neuroscience Day | Chicago, IL | 2014
- Chicago Brain Bee| Chicago, IL | 2014, 2015
- Biology and Control of Nausea and Vomiting | Pittsburgh, PA | 2013

17**. Fortin SM**, Kanoski SE, Grill HJ. Lepting signaling in the nucleus tractus solitarius suppresses motivation to obtain rewarding food.
- Society for the Study of Ingestive Behavior | Zurich, Switzerland | 2012

18-22. **Fortin SM**, Marshall SL, Jaeger EC, Greene PE, Brady LK, Isaac RE, Schrandt JC, Brooks DR, Lyczak R. The PAM-1 aminopeptidase regulates centrosome positioning to ensure anterior-posterior axis specification in one-cell C. elegans embryos.
- Honors talk | Ursinus College Celebration of Student Achievement | Collegeville, PA |2010
- American Society for Cell Biology Meeting | San Diego, CA | 2009
- Undergraduate Research Symposium at Haverford College | Haverford, PA | 2009
- 20th Annual Sigma Xi Student Research Symposium at St. Joseph's University | Philadelphia, PA| 2009
- Ursinus College Celebration of Student Achievement | Collegeville, PA | 2009

Conference and Symposia Abstracts

1. Conway, SM, Alhadeff, AL, **Fortin, SM**, Grill, HJ, Rotiman, MF. Leptin differentially modulates the affective response to food restriction depending on the behavioral paradigm.
   - Society for the Study of Ingestive Behavior | Porto, Portugal | 2016
2. Gerth AI, **Fortin SM**, Cone JJ, Grill HJ, Roitman MF. Cocaine delivery to the fourth ventricle reveals evidence for hindbrain modulation of phasic mesolimbic dopamine signaling.
   - The Society for Neuroscience | Washington, DC | 2014
3. Conway SM, **Fortin SM**, Sinon CG, McCutcheon JE, Roitman MF. Chemogenic inhibition of mesolimbic dopamine is insufficient to condition taste aversion.
   - The Society for Neuroscience | Washington, DC | 2014
4. Cone JJ, **Fortin SM**, McCutcheon JE, Roitman MF. Phasic dopamine signaling tracks the rewarding value of sodium based on physiological state
   - The Society for Neuroscience | Washington, DC | 2014
5. Cone JJ, **Fortin SM**, Palmer BA, McCutcheon JE, Roitman MF. Phasic dopamine signaling tracks the hedonic value of sodium based on physiological state
   - Society for the Study of Ingestive Behavior | Seattle, WA| 2013
6. McCutcheon JE, Cone JJ, **Fortin SM**, Sinon CG, Conway S, Witten IB, Deisseroth K, Stuber GD, Roitman MF. Optical suppression of phasic dopamine spikes evoked by presynaptic release modulators.
   - The Society for Neuroscience | San Diego, CA | 2013
7. Kanoski SE, **Fortin SM**, Ricks KM, Grill HJ. Ghrelin signaling in the ventral hippocampus stimulates learned and motivational aspects of feeding via PI3K-Akt signaling.
   - Society for the Study of Ingestive Behavior | Zurich, Switzerland| 2012
8. Kanoski SE, Rupprecht L, **Fortin SM**, De Jonghe BC, Hayes MR. Body weight and food intake suppression by glucagon-like-peptide-1 receptor agonists, exendin-4 and liraglutide, may require nausea.
   - The Obesity Society Annual Meeting | Orlando, FL | 2011
9. Hayes MR, Kanoski SE, De Jonghe BC, Leichner TM, Alhadeff AL, **Fortin SM**, Arnold M, Langhans W, Grill HJ. The common hepatic branch of the vagus is not required to mediate the glycemic and food intake suppressive effects of glucagon-like-peptide-1.
   - The Obesity Society Annual Meeting | Orlando, FL | 2011

Awards/Honors

UIC Neuroscience Graduate Student Symposium, 1st Place| University of Illinois at Chicago | Chicago, IL |2017 | $350
Graduate Student Symposium, 2nd Place| The Chicago Chapter of SFN | Chicago, IL |2017 | $100
Randal R. Sakai New Investigator Travel Award | Society for the Study of Ingestive Behavior, Porto, Portugal |2016 | $1000
Dean's Scholar Fellowship | University of Illinois at Chicago | 2016-2017 | 12 months stipend + tuition and fee waiver
Provost Award for Graduate Research | University of Illinois at Chicago | 2013 | $1000
Chancellors Graduate Research Award| University of Illinois at Chicago | 2013, 2014 | $4000
LAS PhD Student Travel Award | University of Illinois at Chicago |2013, 2014 | $500
GSC Student Travel Award | University of Illinois at Chicago| 2013, 2014 | $275
Biology and Control of Nausea and Vomiting Travel Award | Pittsburgh, PA | 2012 | $400
University Fellowship | University of Illinois at Chicago | 2012 | 24 months of stipend + tuition and fee waivers
Ursinus College Outstanding Student Research in Biology Award | Ursinus College | 2010
Honors in Biology | Ursinus College | Advisor: Dr. Rebecca Lyczak | 2010
Dean's Honor List| Ursinus College | 2006-2010
Beta Beta Beta National Biological Honor Society | 2007-2010, Treasurer (2010)

Teaching/Mentoring Experiences

Seminar Coordinator
University of Illinois at Chicago | Spring 2017 | Undergraduate Seminar in Neurobiology
Guest Lecturer
University of Illinois at Chicago | Spring, 2015-2017 | Undergraduate Seminar in Neurobiology
Teaching Assistant
Laboratory in Behavioral Neuroscience | University of Illinois at Chicago | Spring 2014, Fall 2014, Spring 2015
Behavioral Neuroscience | University of Illinois at Chicago | Fall 2013
Direct Supervisor of Undergraduate Student Researchers
University of Illinois at Chicago | 2013-2016 | 5 students | 1 recipient of The College of Liberal Arts and Sciences Undergraduate Research Initiative Award
University of Pennsylvania | 2010-2012 | 8 students
Trainer of Research Technicians |University of Pennsylvania | 2011-2012 | 2 technicians
Supplemental Biology Instructor | Ursinus College | 2009-2010
Peer Advisor | Ursinus College | 2009-2010
On Campus Tutor | Ursinus College | 2007-2010
Mosaic International Institute Intern | Madrid, Spain | 2008

Volunteer Work

Graduate Program in Neuroscience Executive Committee | University of Illinois at Chicago | 2016-2017
Spring Symposium in Neuroscience, Organizing Committee | University of Illinois at Chicago | 2016-2017
Expanding Your Horizons (EYH) Organizing Committee | Chicago Chapter | 2016- 2017
Neuroscience Symposium Data Blitz Facilitator | University of Illinois at Chicago | 2016
Non-academic Career Day, Organizing Committee | University of Illinois at Chicago | 2016
Chicago Brain Bee | University of Illinois at Chicago | 2014-2016
Expanding Your Horizons (EYH) | Chicago Chapter | 2014-2017
Society for Neuroscience "Dopamine Dinner" Organizing Committee | 2016
El Paseo Community Garden | Chicago, IL | 2016
Graduate Program in Neuroscience Recruitment Interviews | University of Illinois at Chicago | 2014-2016
Brain Awareness Week Invited Speaker | Ludwig van Beethoven Elementary School, Chicago, IL | 2013
Graduate Student Council Representative | University of Illinois at Chicago | 2013-present