THE BEHAVIORAL AND PHARMACOLOGICAL EFFECTS OF CAFFEINE ON THE CONSUMPTION AND HEDONIC EVALUATION OF SUCROSE SOLUTIONS

BY

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ABSTRACT

The overconsumption of highly palatable, calorie dense food and drink is an important component in the etiology of obesity. Caffeine is a psychostimulant that is regularly consumed alongside highly palatable, calorie-dense beverages in the form of soft drinks, coffees, and energy drinks. Caffeine has been shown to positively shift the hedonic evaluation of beverages in humans, and increase the appetitive motivation to work for sucrose pellet rewards in rats. However, no animal research has examined the impact that caffeine has on the hedonic evaluation and licking microstructure of sweet solutions. In this thesis, the effect of caffeine on the intake and licking microstructure of rats offered a 10% sucrose solution was examined. In the first experiment, caffeine was added directly to the sucrose solution, whereas the second experiment examined the effects of systemic injections of caffeine on the consumption and licking microstructure of the non-caffeinated sucrose solution across 90-min licking sessions. The dependent variables examined were total solution consumed, mean length of licking bursts, number of licking bursts, and latency to initiate drinking. In the first experiment, the presence of caffeine in the solution increased total consumption across days with a trend towards increased burst length. Caffeine in the solution had no effect on latency or the number of bursts. When caffeine was administered systemically in the second experiment, only the moderate dose increased consumption. Following ten days of systemic administration of the moderate caffeine dose, consumption continued to increase, and additional trends towards an increased number of licking bursts and decreased latency were demonstrated. Taken together, these findings suggest that caffeine increases the consumption of palatable solutions, and that this is due to an increase in the hedonic evaluation of the solution

when it contains caffeine. However, when administered systemically, these findings suggest that caffeine increases consumption via an increase in appetitive motivation.

INTRODUCTION

One of the most pertinent health problems plaguing the United States and many other developed nations today is the obesity epidemic. According to the World Health Organization (WHO), obesity is defined as an accumulation of fat beyond what is considered a healthy norm for the body. One way in which this norm is defined is through the utilization of a measure called body mass index (BMI), which utilizes both weight and height in its calculation. A BMI level over 30 designates obesity, and a BMI between 25 and 30 is simply considered overweight. Obesity increases one's risk of developing many life-threatening diseases such as diabetes and high blood pressure, both of which can symptomatically manifest themselves in mortality (Reilly et al., 2003). Additionally, medical expenses related to obesity cost the United States a fortune each year. These costs reached the level of $78.5 billion in the year 1998 alone, and have risen steadily since (Finkelstein et al., 2009). The annual medical expenses incurred related to obesity in the U.S. are projected to increase to $344 billion by 2018 (Thorpe et al., 2004). Thus the problem of obesity is twofold: it endangers the physical health of those afflicted, and it demands extensive financial resources.

The obesity epidemic stems from a variety of causal factors, a prominent one being the fact that people are regularly consuming more calories than is necessary. Ample research has shown that overconsumption plays a critical role in influencing obesity outcomes. Studies utilizing longitudinal consumption and health data have demonstrated that in the United States alone, the percentage of obesity rose from approximately 20% to 30% between 1993 and 2003, and is continuing to steadily rise (Bleich et al., 2008). Compatible with this is the finding that daily calorie consumption is increasing over time,

with daily per capita measures in the U.S. increasing by 12% (300 more calories per day) between 1985 and 2000. Additional longitudinal data analysis has demonstrated that behaviors that dramatically increase energy input from food calories consumed, such as snacking and eating out, have increased over time (McCrory et al., 2002). Specifically, daily snacking can increase total energy intake by 25%, and eating out regularly, as in over a dozen times per month, increases energy intake by 31.5% compared to eating out only once per week. Taken together, all of these findings illustrate the notion that certain eating habits that have increased in prevalence over time are leading to an increase in caloric consumption, and that this overconsumption is occurring in tandem with an increase in the number of obese individuals.

People are more inclined to overeat when foods are highly palatable, and this is at least in part due to the fact that ingestion of palatable foods is promoted by reward-processing neural circuitry, which both conveys the hedonic value of food and increases the motivation to feed (Berthoud, 2012; Kelley et al., 2005; Pecina & Berridge, 2005). For example, dopamine signaling in the dorsal, lateral, and ventromedial striatum, which has been associated with the promotion of food reward and motivation, undergoes major alterations in obese individuals (Guo et al., 2014). Specifically, positron emission tomography (PET) imaging of individuals with elevated BMIs demonstrated increased dopamine D2 receptor binding potential in the dorsal and lateral striatum and decreased potential in the ventromedial striatum compared to those with lower BMIs. Unfortunately, the foods that stimulate this circuitry also tend to have a disproportionate amount of calories, fat, and sugar in them compared to other less palatable foods, thus making their overconsumption problematic.

Many regularly consumed palatable beverages take the form of highly sweetened, caffeinated soft drinks, energy drinks, and coffees. Consumption of soft drinks has been positively associated with increased incidence of obesity and diabetes across the world (Basu et al., 2013; Funtikova et al., 2015). Due to its presence in these drinks, caffeine is a common component of many people's diets. In addition, caffeine is the most commonly used psychostimulant and exerts its effects in the brain by blocking adenosine receptors (Fisone et al., 2004). Caffeine's psychostimulant effects exhibit sensitization that is dependent upon both dosage and reinforcement schedule, just like many recreationally abused psychostimulants such as nicotine, amphetamine, and cocaine (Sheppard et al., 2012). The prevalence of this drug in many palatable, high calorie beverages demands that research be done to examine any potential effect caffeine is having on the consumption and hedonic evaluation of those beverages. To date, any role that caffeine may play in the overconsumption of these palatable beverages has received only scant research attention. The goals of this thesis will be to examine the impact that caffeine has on the consumption and hedonic evaluation of sucrose solutions. However, in order to understand any impact that caffeine may have, it is first essential to parse apart the different components of motivation, as proposed by prominent motivational theorists. As such, the next section will establish different ways to think about motivation and how to apply different motivational theories to food-related behavior.

Motivational Theory

In its simplest form, motivation seeks to understand why a creature, human or otherwise, engages in a particular behavior. Examples of high motivation can be examined in a wide variety of circumstances, such as a child eagerly completing their

homework in order to be able to play outside, or a rat consistently licking a spout for a sucrose solution in an operant chamber. Motivation can also provide useful information regarding the "goal directed" nature of behavior, as well as the learning that occurs (Toates, 1986). As such, motivation does not simply constitute a "drive" to engage in a particular behavior, but incorporates external information and learned associations between the behavior and past outcomes and stimuli. Theorist Frederick Toates incorporates all of these attributes in his conceptualization of a motivational system. Toates' system is highly applicable to this paper's aims since it can readily be applied to explain obesity-related aspects of feeding behavior, as will be discussed below.

In Toates' motivational model, external factors that modulate motivation, such as the palatability of a particular food, are often referred to as "incentives" (Toates, 1986). Internal factors that influence motivation, such as hunger or thirst, are described as internal drive states, and changes in either these or incentives will exert an influence over an individual's motivation to carry out a particular behavior (Toates, 1986). Additionally, and important to note, incentives and drive need not both be present in full force in order to promote an organism's engagement in a particular behavior. In other words, the behavior can still occur if drive is low, if the incentive value of an external factor is low and vice versa. For example, after finishing dinner you may no longer be hungry (low drive) but you may very well eat a savory dessert (high incentive value). Of noteworthy importance is that both drive and incentive must be present in some capacity; you likely wouldn't opt for dessert if it was made from spoiled ingredients. This aspect of Toates' theory has major implications for understanding the etiology of obesity: in spite of

limited hunger, people could be motivated to consume highly palatable foods, which have high incentive value and contribute to the energy imbalance evident in obesity.

In the wake of Toates' theory regarding the building blocks of motivated behavior, other motivational theorists have expanded upon or reframed it. One example of this has been laid out by Kent Berridge and his colleagues over the past several years. His theory delineates reward learning theory into two components ("liking" and "wanting"), which determine the motivation to pursue a particular food substance. However, it is important to note that his ideas are applicable to more than just food, and can be relevant to nearly any process involving reward learning. These two components serve to expand upon the concept of "incentives" laid out by Toates. Recall that Toates used the term "incentive" to describe the external factors modifying motivation, such as the taste of a particular food. As such, incentives represent learned associations between a reward and its properties, such as the discovery of how palatable ice cream tastes, and encompass two sub-processes, which are Berridge's theory's components (Berridge, 2004).

The first component he establishes is "liking", which focuses on the hedonic impact of a reward as it is consciously experienced by the individual (Berridge, 2001). The neurological and psychological processes responsible for conveying objective pleasure are responsible for determining how much an individual "likes" a particular reward (Berridge et al., 2009). When you take a bite out of a candy bar and savor the sweet taste, you are experiencing "liking". The second component is termed "wanting", and refers to the incentive salience of a reward. Processes responsible for influencing "wanting" do so by promoting or inhibiting pursuit of a particular reward instead of

focusing on the actual immediate, conscious experience of the pleasure inherent in the reward (Berridge et al., 2009). Past experience has taught you that spoiled milk tastes very unpleasant, and as such it possesses low incentive salience, leading to the inhibition of your pursuit of it. This is an example of experiencing low "wanting". Though these two components typically go hand-in-hand, there are isolated examples in which one can occur without the other. For example, rats with lesions of dopamine projections from the midbrain to the forebrain exhibit a lack in motivation to pursue palatable food reward (no "wanting"), but demonstrate positive facial reactions when consuming these foods indicating intact "liking" (Berridge, 2001). "Wanting" in the absence of "liking" can occur as well, and is often exhibited in instances of drug addiction. Individuals experiencing addiction may crave a particular drug and go to great lengths to obtain it (high "wanting"), all while feeling an absence of pleasurable "liking" when using the drug because of the accumulation of tolerance to its previously euphoric effects (Berridge, 2001).

Motivation has also been described in other ways, including one concept that has traditionally divided motivated behavior into two phases. The first is termed "appetitive," and represents the variable "searching" and pursuit behaviors that lead to a later behavior sequence which comprises the second phase, termed "consummatory" (Ball and Balthazart, 2008). Appetitive motivation is what causes people to seek food when they are hungry or are experiencing a food-craving. Leaving one's home to drive to the nearest fast food restaurant to eat would be an example of a behavior mediated by this component. The second phase is referred to as "consummatory," and refers to the stereotyped behaviors that represent "consumption" of the pursued reward, up to behavior

termination (Ball and Balthazart, 2008). For food specifically, consummatory motivation refers to the act of eating and continuing to consume food once one has access to it. It is this component that, for example, determines how much you will eat when visiting an all-you-can eat college dining hall.

The advantage to this conceptualization, and the reason for its inception, is that its division of motivated behavior into different phases allows for reconciliation of the presence of both stereotyped repetitive behaviors and the more variable ones in organisms (Ball & Balthazart, 2008). However, on its own it doesn't directly address the role that hedonics play in motivated behavior in the manner that Berridge's expansion on Toates' model does. Over time researchers have come to acknowledge that motivated behavior must be accompanied by an affective reaction to the reward/goal (Berridge 2004). This particular type of reaction is an indicator, physiological or otherwise, of the hedonic value of the reward. This "hedonic evaluative" component of motivation has been relatively unexplored, but no doubt plays an important role in the etiology of obesity, since, as discussed previously, overconsumption of palatable foods is a primary factor. The hedonic evaluative component of motivation would, for example, influence your decision to get a second helping of dessert instead of a salad due to the dessert's markedly greater perceived palatability.

Despite how crucial a role hedonics would appear to play in motivated behavior surrounding food rewards, the behaviors responsible for seeking out and eventually consuming food are likely to be critical as well. Toates' framework does allow for the palatability of food to influence feeding through increased incentive value. However, this ensures hedonic value is viewed solely as a property of the reward itself, and doesn't

allow for variation in the organism's own hedonic evaluation of the reward. Berridge's theory expands upon Toates' idea of the role of external incentives in motivated behavior, and fleshes out the role of hedonics. Others have examined food motivated behavior in terms of separate appetitive and consummatory phases. As will be discussed next, these different aspects of motivation possess distinct neurological frameworks as well. Caffeine is a pharmacological agent that directly exerts an influence over neural circuitry via the antagonism of adenosine receptors. As such, caffeine is likely affecting the neurological representations of the various motivational constructs discussed in the preceding paragraphs. The way in which caffeine does this is not understood, but an assessment of the instantiation of motivation in the brain is a necessary starting point.

The biological underpinnings of motivation. Interestingly enough, years of research have suggested that certain aspects of motivation are separable with respect to neural circuitry. Thus, the neural instantiation of these components offers a way for them to be studied so that the motivational processes surrounding obesity can be better understood. One particular neurotransmitter system that is involved is the dopaminergic system. Dopamine is a neurotransmitter in the catecholamine family, and has been shown to be a critical component of the brain's reward system. It is released in neuroanatomical areas such as the nucleus accumbens in the presence of natural obtainable rewards (i.e. food, water, etc.) and is essential for facilitating learned associations between unconditioned stimuli and reward (Wise, 2004). The nucleus accumbens (NAc) is a region of the basal forebrain that contains dopaminergic innervations from the ventral tegmental area (VTA). Dopamine release in the NAc has been shown to be critical for processing drug rewards, with administration of opiates, alcohol, and barbiturates in rats

stimulating dopamine release in the NAc in correspondence with their behavioral effects (Di Chiara & Imperato 1986).

To explore the role these systems play with respect to food-relevant motivation and reward, researchers have examined the impact blocking dopamine receptors in the NAc has on this motivation. A blockade of this nature has been shown to reduce incentive motivation to work for food in rats, but leaves consummatory motivation and the hedonic evaluation of food intact (Berridge, 2007; Kelley et al., 2005). Further, when given intermittent unlimited access to a highly palatable diet high in both sucrose and fat, rats consumed no less when given dopamine receptor antagonists compared to rats with intact dopaminergic projections (Lardeux et al., 2015). These findings provide further support for the notion that dopamine transmission in the NAc is not critical for consummatory motivation nor hedonic evaluation since the palatable diet was consumed to the same extent regardless. The dopamine signaling that has been described is essential for learning associations between reward and their cues are critical for appetitive motivation since that is the component involving reward-seeking. In rats trained to lever press for sucrose in the presence of a tone-cue, dopamine antagonism in the NAc reduced cue-evoked excitation (du Hoffmann & Nicola, 2014). These findings imply a disruption in appetitive motivation, and a potential disabling of reward learning. Taken together, all of these findings suggest the dopamine system in the NAc possesses specificity with regards to which motivational component it is related to. Future research needs to be done to determine what role other neural structures innervated by dopamine projections play in influencing these motivational components.

Systemic manipulations of dopamine D1 and D2 receptors have yielded valuable information regarding dopamine's role in regulating both the appetitive and hedonic evaluative components of motivation as well. Blocking dopamine D1 receptors in rats with the selective antagonist ecopipam reduces the motivation to work for a highly palatable sucrose pellet reward, and shifts feeding preference towards simultaneously freely available rat chow in a food choice task (Yohn et al., 2015). Additionally, the same study demonstrated that D1 antagonism also decreased rats' motivation to obtain a larger, but "high effort" food option compared to an easily accessible, smaller food reward in a t-maze. These findings suggest that dopamine D1 antagonism decreases appetitive motivation related to rewarding food stimuli. In another variation of a feeding-choice task, rats were freely able to choose between consuming a highly concentrated, palatable 5% sucrose solution, or a less palatable 0.3% sucrose concentration (Pardo et al., 2015). Following dopamine D1 and D2 receptor antagonism, rats preferred the less concentrated sucrose solution. These findings further support a role for dopamine D1 receptors (and additionally D2 receptors) in regulating the hedonic component of motivation. Additionally, these findings assessed changes in hedonic evaluation separate from appetitive changes, as both concentrations required the same amount of "work" or effort to be obtained. It would appear that dopamine plays a diverse widespread role in regulating the various components of food-relevant motivation. Further research is needed in order to better understand how the dopaminergic system not only influences these components on its own, but perhaps to ascertain whether or not its diverse roles may be due to interactions with other neurotransmitter systems.

Yet another applicable neurotransmitter system that exerts an influence over these motivational components is the opioid system. Opioids are endogenously produced by the body, though several drugs of abuse, such as morphine and codeine, mimic them in structure and pharmacology. They bind to opioid receptors, which are widely dispersed throughout the brain, and are known for their pain-relieving and euphoric properties. Opioid receptors come in a variety of subtypes, termed mu, kappa, delta, zetta, and the nociception receptor. In humans, ingestion of the mu-opioid receptor antagonist nalmefene reduces palatability ratings of food, and reduces consumption of a varied palatable "cafeteria" style diet, with the decrease in consumption of specific foods being correlated to their palatability (Yeomans & Wright, 1991). These findings provide support for the role of opioid receptors in processing food-reward palatability. The opioid system's role in manipulating these components of motivation is not very clean cut; opioid receptor stimulation's effects are dependent upon where in the brain, including at which receptor subtype, the stimulation takes place. Since opioid receptors became implicated in the evaluation of the hedonic qualities of food, further research has attempted to hone in more precisely on where this occurs in the brain.

When localized to the NAc, mu-opioid stimulation results in a sensitization of hedonic evaluative motivation, leading to an increase in consumption of palatable substances such as sucrose and a high-fat diet in rats (Pecina & Berridge, 2005; Skelly et al., 2010; Zhang & Kelley, 2000). However, some of the same research demonstrating these findings has also shown that more widespread, systemic opioid stimulation instead increases general consummatory motivation independent of perceived palatability, and has little effect on appetitive motivation (Pecina & Berridge, 2005). Similarly, when mu-

opioid receptors are systemically blocked in rats by the antagonist naloxone, consummatory motivation to feed on freely available, palatable sweetened-condensed milk was reduced (Schneider et al., 2010). Mu-opioid stimulation in the ventral tegmental area (VTA) in rats leads to an increase in feeding on rat chow (Mucha & Iverson, 1986). This increase in feeding on a food substrate of low palatability suggests that mu-opioid receptor stimulation in the VTA may bolster consummatory motivation. Taken together, these findings further illustrate how these various motivational components are differentially represented and impacted by an assortment of neurotransmitter systems.

Just as the aforementioned results appear to contain clear and distinct instances of specific components being controlled to a degree by neurobiology, there also exists many instances of studies examining the role of various neurotransmitter systems on food-related motivation where individual neurotransmitter systems clearly impact more than one aspect of motivation. Ambiguity of this variety can be seen in the serotonergic system (involving the neurotransmitter serotonin), which is best known for its regulatory role with respect to sleep, feeding, and mood. This particular neurotransmitter system is complex, with seven different receptor subtypes, some of which contain multiple subtypes. Thus it almost comes as no surprise that serotonin's role in motivated feeding behavior is not always easily deciphered.

Specifically, when stimulating serotonin (5-HT) 1 and 7 receptors of the nucleus accumbens in rats, food intake is decreased (Pratt et al., 2009). This reduction in feeding is the same for both food-deprived rats given access to unlimited, low palatability rat chow for 2 hours, and non-deprived rats given access to a palatable diet for 2 hours. The same research has also demonstrated that stimulation of 5-HT 6 receptors leads to an

increase in feeding for both food deprived and non-deprived rats, and that antagonism of those same receptors, in addition to the 5-HT 2C receptor, has no effect on food intake (Pratt et al. 2009). These findings suggest that several serotonin receptor subtypes exert different influences on consummatory motivation. Later research has demonstrated that serotonin receptors play an important role in influencing appetitive motivation as well. Rats not experiencing food deprivation were trained to lever press for a sucrose reward on a progressive-ratio schedule of reinforcement, meaning that following delivery of a sugar reward, more lever presses were required to receive the subsequent sugar reward (Pratt et al. 2012). Thus, this methodology provides a concrete way to examine changes in appetitive motivation in the rat. Serotonin receptor subtypes 1, 7, 6, and 2C in the nucleus accumbens were stimulated, and it was demonstrated that 5-HT 6 stimulation resulted in increased appetitive motivation, stimulation of 5-HT 2C had no effect, and stimulation of 5-$HT_{1/7}$ receptors decreased appetitive motivation at the highest dose of agonist 5-CT, and increased it at the lowest dose (Pratt et al. 2012). These results demonstrate that serotonin receptors play a role in the regulation of appetitive motivation as well, and again that this regulation depends upon receptor subtype. To date, there is little to no research examining what role these transmitters may play in the regulation of the hedonic evaluative aspects of motivation.

In summary, food-related motivation can be divided into distinct elements or components, of which hedonic evaluation is one. All of these components of motivation, hedonics included, can be studied empirically, and each possesses distinct neural processes that allow these components to manifest in motivated behavior. Dopaminergic projections into the NAc are essential for intact appetitive motivation, while dopamine

D1 and D2 receptors in general are necessary for proper hedonic evaluation. The opioid system affects hedonic evaluation and consummatory motivation in a neuroanatomically specific fashion, and serotonin's role in food-related motivation is comparably more complex. However, the ways in which caffeine interacts with these motivational attributes must be addressed as well, and remain relatively unexplored. The current state of the literature regarding these interactions will be examined next.

Current Findings Regarding Caffeine and its Influence on Food-Related Motivation

A small number of studies have examined the effect that adding caffeine has on both consumption and "liking" in human participants. As described previously, the term "liking" refers to the hedonic impact of a reward as it is consciously experienced by the individual (Berridge, 2001). Measuring changes in "liking" allows for the observation of changes in an individual's hedonic evaluation of a solution. Studies in humans have demonstrated that caffeine added to sweetened beverages may cause a positive hedonic shift in the evaluation of the palatability of those beverages, as measured by self-reported "liking" (Keast et al., 2015; Temple et al., 2012). In a study involving a large (n = 99) sample of adolescents, subjects were given novel soft drinks that were either paired with caffeine or placebo (Temple et al., 2012). In a subjective assessment measure, participants provided information related to their perceived palatability (i.e., "liking") of the soft drink following daily consumption for four consecutive days. Participants in the higher caffeine dose group (2 mg/kg) reported greater perceived palatability, and less perceived bitterness of the novel soft drink throughout the course of the test period, whereas the low dose (1 mg/kg) and no caffeine groups reported no change (Temple et al., 2012). A similar enhancement of "liking" over time has also been demonstrated in

participants consuming novel-flavored yogurts with added caffeine (Panek et al. 2013). These findings suggest that, in humans, ingestion of caffeine paired with a palatable food causes a positive shift in the hedonic evaluation of that food.

The animal literature regarding caffeine and feeding behavior is scarce, and the few studies that have examined caffeine and the consumption of palatable sugar solutions have only tested the impact of the drug on appetitive motivation or overall consumption of those solutions. These studies have not at all examined the role caffeine may play in the hedonic evaluation of sugar solutions, which is also a critical component to understanding caffeine's role in food-related motivation. Recall that appetitive motivation is the variable "searching" and pursuit behaviors that lead to later consumption of the reward, while consummatory motivation refers to the stereotyped behaviors that represent "consumption" of the pursued reward (Ball & Balthazart, 2008). Consumption of a food substrate is accompanied by an affective reaction to the food reward/goal, indicative of the hedonic value of that reward (Berridge 2004).

In rats, studies have shown that systemic administration of caffeine increases how hard they are willing to work for sucrose, as demonstrated by performance in progressive-ratio tasks (Brianna Sheppard et al., 2012). Studies such as these provide evidence to suggest that caffeine increases the appetitive motivation to pursue a food reward. Animal research with caffeine has also demonstrated that rats will consume more of a sweetened solution when it is paired with caffeine (Swithers et al., 2010). In addition, systemic caffeine administration increases fixed-ratio lever pressing for sucrose in rats (Retzbach et al., 2014). The same study also showed that acute, but not chronic, caffeine administration led to increased neuronal activity in the nucleus accumbens as

evidenced by *Fos* expression. Taken together, these findings demonstrate an effect of caffeine on both appetitive and consummatory motivation, but leave hedonic evaluation unassessed.

In spite of the apparent lack of an examination of caffeine's impact on the hedonic processing of palatable solutions in animals, hedonic evaluation in other contexts has been a staple technique for other contexts within the realm of animal research. In prior studies in rats, hedonic reactions to the taste of various substances have been examined by two methodologies. The first method involves the direct observation of rats' affective facial reactions when being given an oral infusion of a particular substance via a tube attached to a cannula in a rat's mouth (Pecina & Berridge, 2005). This method has the advantage of forcing the rat to taste the solution tested and directly assesses the facial reactions to those solutions, but it has the disadvantage of not allowing other components of motivation to be examined, such as the incentive to seek out the solution (appetitive), or the amount of solution that the rats would drink if given free access (consummatory). The second method for analyzing the hedonic evaluation of substances, and other attributes of food substrates, is through the analysis of licking microstructure. Specifically, this method involves a detailed examination of rats' licking behavior when freely ingesting solutions of varying palatability. These observations allow inferences to be drawn regarding food-related motivation from specific patterns of licking attributes, such as the rate of licking and number of licks in a burst (Davis, 1989; Dwyer 2012).

Licking microstructure data is generally collected in two different ways. The first involves the placement of rats in operant chambers equipped with a licking spout attached to a bottle of solution to be ingested. The licking spout is equipped with a

photobeam that is broken by a rat's tongue when it licks from the spout to obtain the solution (i.e., Taha et al., 2009). This breakage of the beam is a solitary "lick", and the time at which occurs is recorded on a computer. The second technique involves a similar setup with a licking spout in the operant chamber, only here the licking spout is connected to an amplifier that allowed a current to pass through the rat whenever its tongue made contact with the spout (i.e., Davis and Perez, 1993). Each time the current passes through and the electrical circuit is completed, a "lick" is recorded by a computer, as well as the time at which it occurs.

Once all of the licks in a free-feeding session have been recorded, several parameters and attributes related to patterns of licking can be analyzed to yield valuable information regarding different components of food-related motivation, including perceived palatability. Though terminology and interpretation (such as what length of time constitutes a "pause" in licking) often vary from lab to lab and study to study, all of the procedures look at similar features of the microstructure. All of the licks in the session can be parsed into bursts of rapid licking separated by pauses (inter-burst interval) with the pauses in between individual licks in a burst being referred to as an inter-lick interval (Davis & Perez, 1993; Dwyer 2012). In addition, groups of licking bursts occurring within a pre-specified time period are referred to collectively as a meal. Measuring the initial lick rate and the size of licking bursts provides an interpretable measure of the hedonic evaluation of the solution (Dwyer 2012; Spector et al. 1998; Taha et al., 2009). These measures have been shown to increase as the concentration of sucrose solutions go up. These measures have also conferred ample useful information regarding the role that certain neurotransmitter systems play in hedonic processing. For example,

endogenous cannabinoid (CB1) receptor agonist administration leads to an increase in burst size/duration in rats licking for sucrose solutions, while CB1 antagonist administration has the opposite effect (Higgs et al., 2003). These findings demonstrate that the cannabinoid system plays a role in modifying the perceived palatability of substances.

Additionally, the analysis of licking microstructure possesses the advantage of being able to simultaneously address other motivational components alongside hedonics. For instance, by examining the latency to begin a meal, number of licking bursts, and meal duration, meal size, and changes in the rate of decline of consumption throughout a meal, one can ascertain information regarding changes in the appetitive and consummatory phases of motivated feeding behavior (Spector et al., 1998; Taha et al., 2009). Upon injection of the neuropeptide orexin into the fourth ventricle, researchers demonstrated that sucrose solution meal size and duration increased but initial lick rate and burst size remained unaffected (Baird et al., 2009). These findings provide an example of a neurotransmitter system that appears to affect motivational processes unrelated to hedonic processing. Thus, analysis of the microstructure of licking patterns provides a comprehensive array of information related to the motivational workings of feeding behavior, including (but not limited to) hedonics. For the purposes of this thesis, hedonic processing was assessed via an examination of burst length, while latency to initiate the first meal was used to assess appetitive motivation and total consumption was used for consummatory motivation. Number of bursts was reported as well, and was used to assess changes in both appetitive and consummatory processes. In this thesis, I examined changes in these dependent variables in both rats given caffeine in their

drinking solution (experiment 1), and in rats given access to sucrose solutions following intraperitoneal injections of caffeine (experiment 2).

METHODS

General Methods

Subjects and housing. Male Sprague-Dawley rats (Harlan, Madison, WI) were housed in pairs in clear plastic cages fitted with a water bottle, food access, and regularly changed bedding in a colony room maintained at ~21 °C with a 12-hr light-dark cycle (lights on/off at 7 A.M./ 7 P.M.). During the first week of acclimation to the lab, animals were handled in multiple sessions of five minutes per rat to minimize stress and facilitate testing via conveying familiarity with the experimenters. In addition, all rats' body weights were monitored daily throughout the experiment. All behavioral testing was conducted during the light phase. Rats were maintained on standard laboratory rat chow and tap water *ad libitum*. All animal care and experimental procedures were run in strict compliance with NIH and WFU IACUC guidelines for use of animals in research.

Testing apparatus. Consumption of solutions took place in specialized feeding chambers. Eight of these chambers (Med-Associates, St. Albans, VT, USA) were utilized for both experiments, and all resided within the same room with one 15-Watt house light in a corner, and a speaker for generating white noise at 65 decibels to block out potentially interfering background noise. Each individual chamber was equipped with a licking spout connected to a bottle with the sucrose solution. Each licking spout was outfitted with a photobeam, which was broken when a rat's tongue made contact with the licking spout. This is how each lick was individually recorded. Additional infrared beams lined the walls within each chamber, allowing for recording and observation of rats' rearing, movement, and time spent in particular parts of the chamber.

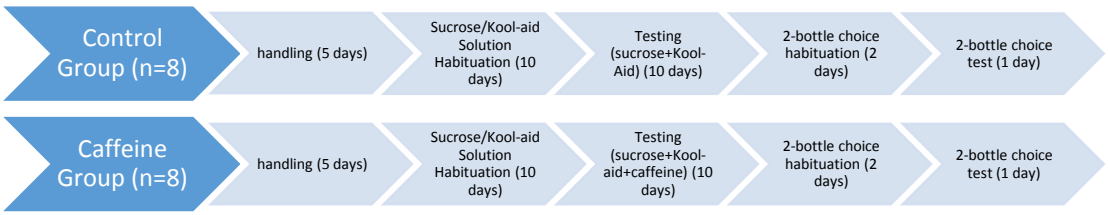
Behavioral/Pharmacological Methods

Validation experiment. Following arrival in the laboratory and sufficient handling, eight rats were exposed to increasing molarities of sucrose solutions (.03M, .1M, .3M, 1.0M), allowing for the determination of how licking behavior varies across a range of palatability. In addition, this preliminary experiment was conducted in order to determine if the microstructural attribute of "burst length" (i.e., duration of the burst in seconds) could be substituted for the more frequently used "burst size" (i.e., the number of licks in a burst) when examining changes in hedonic evaluation. In preliminary studies, we discovered that the lickometer equipment in our laboratory cannot collect burst size data as reliably as others have previously reported, but does accurately convey burst length. Thus, if this preliminary experiment could show that burst length linearly increases with increasing sucrose concentrations just as burst size does, we could confidently use this microstructural attribute for assessments of changes in hedonic evaluation.

Rats were allowed to freely consume the sucrose solutions for 90 minutes over the course of four days for each concentration, with the fourth day serving as the "test day" for purposes of analysis. Sucrose solutions were weighed prior to every session so that consumption could be measured for each session. The four test days were later compared to assess changes in licking microstructure and consumption across concentrations. The rats had constant access to the solution for the entirety of the session, and their licking behavior was simultaneously recorded and saved to a desktop computer equipped with MED-PC software. At the conclusion of each testing session, rats were returned to their home cages and the solutions were re-weighed to determine total consumption.

Experiment 1. Following arrival in the laboratory and sufficient handling, sixteen rats were tested for consumption and licking microstructure analysis of two different solutions. Regular sucrose solutions were prepared by mixing 100 grams of granulated sugar with 1 liter of tap water to create a 10% (~0.29M) sucrose solution. The caffeinated solution consisted of the same 10% sucrose solution, but with .125mg/g of caffeine added. This caffeine dosage has been used by Swithers and colleagues (2010), and reflects the amount of caffeine present in regularly consumed sweetened beverages. As adapted from Swithers et al. (2010), all solutions contained .1% cherry or grape Kool-Aid to mask the taste of the caffeine. Each day following preparation of solutions, they were weighed and then attached to the lickometers in the feeding chambers.

Figure 1: Time course for experiment 1.



For a period of 10 days, rats habituated to a 10% sucrose plus Kool-Aid solution (see Figure 1). To control for the potential that one Kool-Aid flavor may be objectively more desirable than another, the flavors were counterbalanced across rats such that half of them received cherry and the other half receive grape. Each day, rats were placed in the feeding chambers for 90 minutes to freely consume the solution. Following the

habituation period, rats were randomly assigned to one of two groups (n=8 per group). One group continued to receive sucrose solutions, and the other had caffeine added to their sucrose solutions. For all rats, the flavor of Kool-Aid added to all solutions was switched to the one they didn't have during habituation. This flavor switch served the purpose of ensuring that for each rat, the pharmacological effects of caffeine were only associated with one flavor, so that flavor preference could later be assessed independently. This assessment allowed for interpretation of the role that caffeine's reinforcing properties has in a potential enhancement of hedonic evaluation. Rats were tested for consumption and licking microstructure of either a sucrose solution or a caffeinated sucrose solution 90 minutes daily for 10 days. On each day, rats were removed from their home cages and placed into the feeding chambers for the duration of the 90-minute testing session. The rats had constant access to the solution for the entirety of the session, and their licking behavior was simultaneously recorded and saved to a desktop computer equipped with MED-PC software. At the conclusion of each testing session, rats were returned to their home cages and the solutions were re-weighed to determine total consumption.

In order to help assess the impact of an association between flavor and the arousal-inducing effects of caffeine, rats participated in a two bottle choice task. Both bottles contained sucrose solutions, however each contained a different kool-aid flavoring: one that had previously been paired with caffeine, and the other that wasn't. Preference was based solely on the amount of solution from each bottle that was consumed; microstructural data was not collected. Rats underwent two days of habituation immediately following the ten days of testing described previously. During

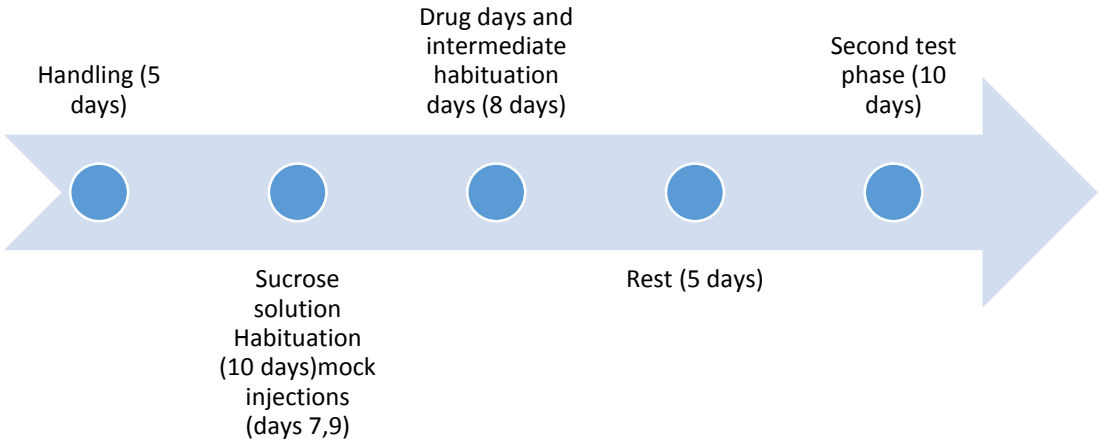
habituation, each rat was placed in a standard plastic rat cage of the same variety used to house them, but with no bedding or food access. Rats had access to both bottles for 30 minutes. During this habituation phase, each bottle contained unflavored solutions of 10% sucrose. On the day following this habituation period, rats underwent a single 30 minute test period identical to habituation, with kool-aid flavoring now added. Both bottles were weighed before and after to determine if the rats had developed a preference.

Experiment 2. Eight rats were tested for consumption and licking microanalysis of a sucrose solution following systemic injections of caffeine or saline. Sucrose/Kool-aid solutions were prepared and weighed daily just as in experiment 1. Rats were given ten days to habituate to the sucrose solutions in the feeding chambers (see Figure 2). During habituation days, rats were moved from their home cages to the feeding chambers for 90-minute licking sessions of sucrose. On the seventh and ninth days of habituation, rats received mock injections. On each mock injection day, each rat received a systemic (via intraperitoneal) injection of saline (1cc/kg) 30 minutes prior to being placed in the feeding chambers. The purpose of these mock injections was to allow the rats time to habituate to the injection procedure, so that any effects that being injected had on licking behavior were minimized or eliminated for the testing phase.

Following the habituation day after the final mock injection day (i.e. day 11), rats underwent testing. 10% sucrose solutions were prepared, weighed and placed in the feeding chambers as before. The following procedure for testing is adapted from Retzbach et al. (2014). There were four testing days where each rat received an intraperitoneal injection of either saline, 1mg/kg, 5mg/kg, 20mg/kg of caffeine (one for each day, in a random order for each rat, 1cc/kg) 30 minutes prior to being placed in the

feeding chambers. These specific caffeine doses are taken from Retzbach et al. (2014) and represent low, moderate, and high doses that are comparable to amounts regularly consumed by humans. In addition, the moderate dose was shown to effectively increase sucrose self-administration compared to low and high doses (Retzbach et al., 2014). In between each injection day, there was one day with no injection where rats were still placed in the feeding chambers with access to sucrose as before. Following the last testing session, rats had 5 days off before one final testing phase. This phase was identical to the previously described test days, except that all rats received the moderate (5mg/kg) injection of caffeine, and that this phase took place across ten days of sucrose exposure. This testing period at the moderate dose allowed for comparison to experiment 1, where rats instead consumed a moderately caffeinated solution across ten days of access to the sweetened solution. At the conclusion of all the testing sessions, rats were returned to their home cages and the solutions were re-weighed to determine total consumption.

Figure 2: Time-course for experiment 2.



Data Analysis

Following conclusion of the experiments, licking microstructure relevant to hedonic evaluation and appetitive/consummatory motivation, as well as total consumption, was compared. Bursts of licking were defined as groups of lickometer beam breaks, where the inter-beam break interval did not exceed one second. A burst of licks terminated once an inter-beam break interval had exceeded one second. These time-course standards and dependent measure parameters were adapted from Taha et al. (2009). The dependent variables examined were total consumption, mean burst length, number of bursts and latency to initiate the first meal. Total consumption reflects the amount of solution the rats consumed during the session (measured in grams), and mean burst length reflects the average amount of time any given rat spent licking during one burst (in seconds). Number of bursts reflects how many total bursts of licking occurred during the session, and latency refers to how much time elapsed before a given rat began licking for the solution (in seconds).

For the validation experiment, each of these dependent variables was assessed via a one way repeated measures ANOVA with sucrose concentration as the independent variable. Significant pairwise differences between concentrations were assessed with Tukey's post-hoc analysis as appropriate. For experiment 1, the dependent variables were assessed via a 2x3 mixed ANOVA with testing day (habituation day 10, testing days 1 and 10) and caffeine exposure group as the independent variables. For the two-bottle portion of the experiment, differences in consumption between the choices were assessed via paired samples t-tests. In the second experiment, the dependent variables were examined with one way repeated measures ANOVAs with dose as the independent

variable. Significant pairwise differences between doses were assessed with Tukey's post-hoc analysis as appropriate. For the 10-day administration portion of the experiment, differences in the dependent variables between the vehicle injection day and 10^{th} day of moderate caffeine administration were assessed via paired samples t-tests.

RESULTS

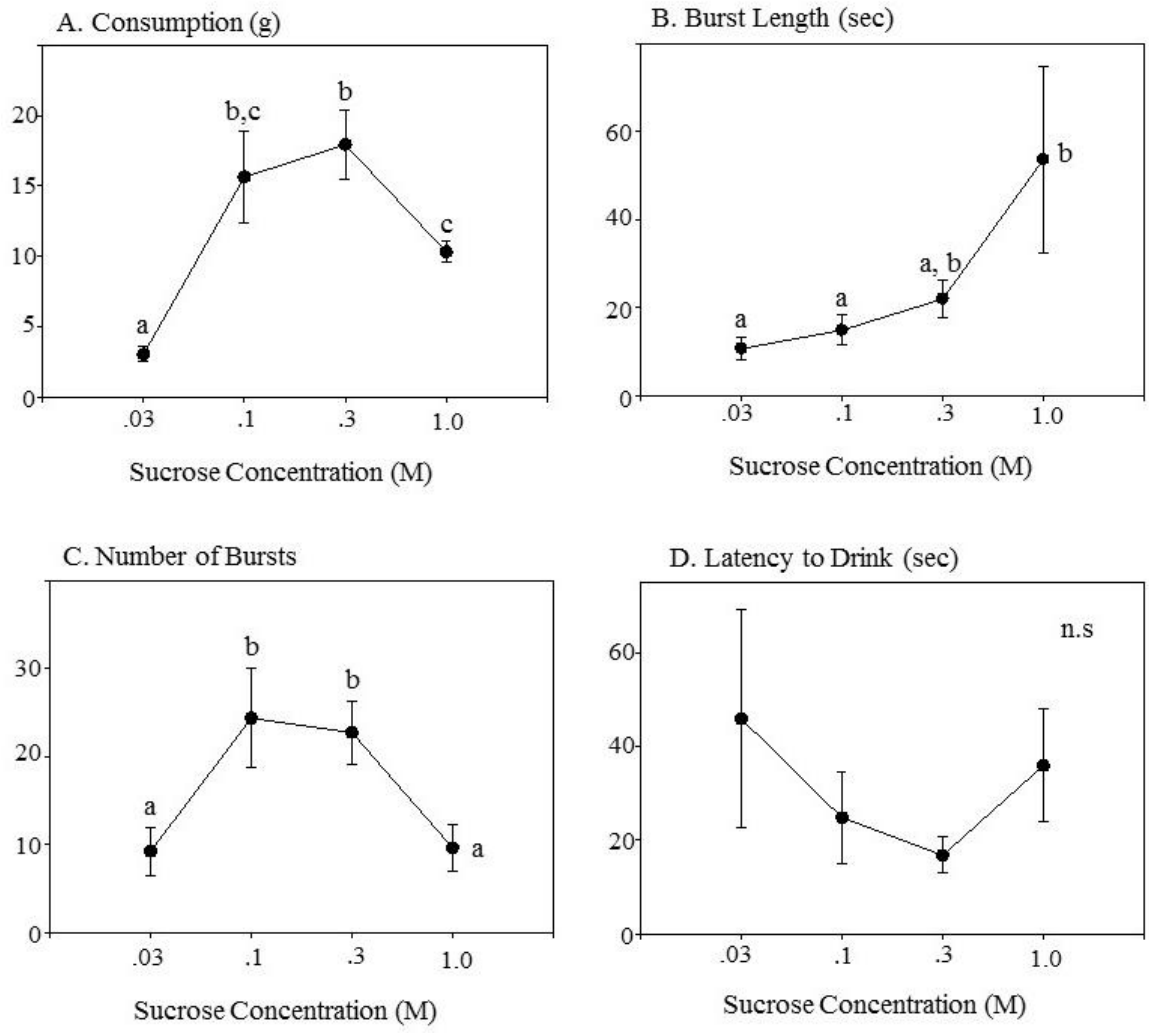
Validation Experiment: Effects of Increasing Sucrose Concentration on Consumption and Licking Microstructure

The effects that increasing the concentration of sucrose had on the parameters of interest varied for each parameter. Initial analysis via one-way repeated measures ANOVA revealed a significant within-subjects effect of sucrose concentration on consumption, $F(3, 21) = 13.589$, $p < .001$ (Figure 3A). Follow up analyses with Tukey's HSD showed that rats consumed more as sucrose concentration increased from .03M to .1M, and consumed less as concentration increased from .3M to 1.0M. Additionally, one-way repeated measures ANOVA revealed a significant within-subjects effect of sucrose concentration on burst length, $F(3, 21) = 4.441$, $p = .014$ (Figure 3B), demonstrating that the length of rats' licking bursts depended upon the sucrose concentration. Specifically, analysis via Tukey's HSD revealed that burst length increased as sucrose concentration was increased from .1M to 1.0M.

With respect to the number of bursts, analysis via one-way repeated measures ANOVA demonstrated a significant within-subjects effect of sucrose concentration, $F(3, 21) = 6.908$, $p = .002$ (Figure 3C). Thus, the number of licking bursts varied as a function of sucrose concentration. Follow up analyses via Tukey's HSD demonstrated that the number of bursts increased as sucrose concentration was increased from .03M to .1M, and decreased as concentration increased from .3M to 1.0M. Increasing the concentration of sucrose had no effect on the latency to begin licking, $F(3, 21) = 1.184$, $p = .34$ (Figure 3D).

The purpose of the validation experiment was to demonstrate that we collect get meaningful, interpretable data using our setup. A complication that arose during the data collection for this portion of the experiment was that we could not capture individual licks with the lickometers we have. Instead, our lickometers tell us for how long the

Figure 3: Effects of increasing sucrose concentration on consumption and licking microstructure. Significant differences between concentrations are represented by differing letters between those concentrations.



infrared beam that the rat's tongue comes into contact with is broken. Thus, our bursts do not consist of individual licks, but rather of segments of time that are each comprised of a variable number of licks. In order to assess changes in hedonic evaluation however, we needed to examine the length of each burst, and compare the trends with that dependent variable to trends from past papers who use burst size to assess hedonic evaluation instead. The trend exhibited by our measure of changes in burst length over increasing sucrose concentrations is comparable to what has been shown in past research regarding changes in burst size (as measured by individual licks) over the same concentrations (Spector et al., 1998). Thus, we felt confident in our usage of burst length as a substitute dependent variable for representing hedonic evaluation. In addition, the overall patterns our methods demonstrated for the other dependent variables mirror findings from previous microstructural research (Davis & Perez, 1993; Spector et al., 1998). These similarities informed us that we could confidently produce meaningful and interpretable data with our setup.

Experiment 1: Consumption and Microstructural Differences Between Rats Licking for Caffeinated and Caffeine-Free Sucrose Solutions

For the purposes of this thesis, data analysis was restricted to the final day of habituation, the first day of caffeine exposure with the novel flavor, and on the final (10^{th}) day of sucrose/caffeine presentation. For rats tested for the consumption of both caffeinated and non-caffeinated sucrose solutions, initial analysis via mixed 2x3 ANOVA yielded no effect of group, $F(1, 14) = .021$, $p = .887$ (Figure 4A) and no effect of day, $F(2, 28) = 2.167$, $p = .133$ (Figure 4A). However, these analyses did show a significant interaction effect between group and day, $F(2, 28) = 5.184$, $p = .012$ (Figure 4A). In order

to ascertain more information regarding the nature of this interaction effect, one-way repeated measures ANOVAs were conducted for each group (control, caffeine). For the control group, no significant differences in consumption across the days of interest were demonstrated, $F(2, 14) = .390$, $p = .684$ (Figure 4A). However, a significant within-subjects effect of day on sucrose consumption was present for the rats consuming caffeinated solutions, $F(2, 14) = 6.803$, $p = .009$ (Figure 4A) and a post-hoc analysis via Tukey's HSD showed that, for these rats, consumption of the caffeinated solution was greater following ten days of caffeine exposure compared to both the last day of habituation to a non-caffeinated sucrose solution, and the first day of exposure to a caffeinated solution.

With respect to burst length, analysis via mixed 2x3 ANOVA yielded no significant effects of group, $F(1, 14) = .701$, $p = .416$ (Figure 4B) or day, $F(2, 28) = .150$, $p = .861$ (Figure 4B). No significant interaction effect was illustrated either, $F(2, 28) = 1.746$, $p = .193$ (Figure 4B). However, due to the presence of the simple main effect of day for the caffeine group that was present in the consumption data, we decided to conduct follow up analyses on the caffeine group for this dependent variable as well. The simple effect of day present for consumption within the caffeine group piqued our interest with regards to whether or not it may have been related to changes in burst length and/or number of bursts. The follow up analyses were conducted via a one-way repeated measures ANOVA and revealed a trend towards increased burst length following ten days of caffeine exposure that failed to reach significance, $F(2, 14) = 3.358$, $p = .065$ (Figure 4B).

Caffeine in the sucrose solution had no effect on the number of rats' licking bursts, as demonstrated by the lack of significant effects of group, $F(1, 14) = .053$, $p = .821$ (Figure 4C) or day, $F(2, 28) = 1.07$, $p = .357$ (Figure 4C) and no significant interaction, $F(2, 28) = .03$, $p = .971$ (Figure 4C). Similarly to the analysis of burst size, we conducted follow up analyses on the caffeine group in order to find out if a simple main effect of day was present, as it could help to explain the simple main effect of day that was present for the caffeine group. No such effects were found, $F(2, 14) = 1.181$, $p = .336$ (Figure 4C). For the latency to begin licking, there was a significant effect of group, $F(1, 14) = 4.69$, $p = .048$ (Figure 4D) but no significant effect of day, $F(2, 28) = 2.005$, $p = .153$ (Figure 4D), nor was there a significant interaction, $F(2, 28) = 1.132$, $p = .337$ (Figure 4D). As seen in figure 4, this effect of group appears to be the result of a group difference that was in place before caffeine exposure.

To determine whether or not either Kool-Aid flavor was intrinsically more desirable than the other, a paired samples t-test was conducted to compare the difference in consumption of both flavors by the control group. The analysis revealed no differences in consumption between the grape or cherry flavors, $t(7) = .397$, $p = .703$ (Figure 5A). Analysis via paired samples t-test also failed to demonstrate any difference between consumption of caffeine-associated and non-caffeine associated flavors for the caffeine group of rats, $t(7) = -.913$, $p = .392$ (Figure 5B).

Figure 4: Consumption and microstructural differences between rats licking for caffeinated and caffeine-free sucrose solutions. Significant differences between concentrations for the caffeine group are represented by differing letters between those concentrations. Significant group by day interactions are denoted with a "†."

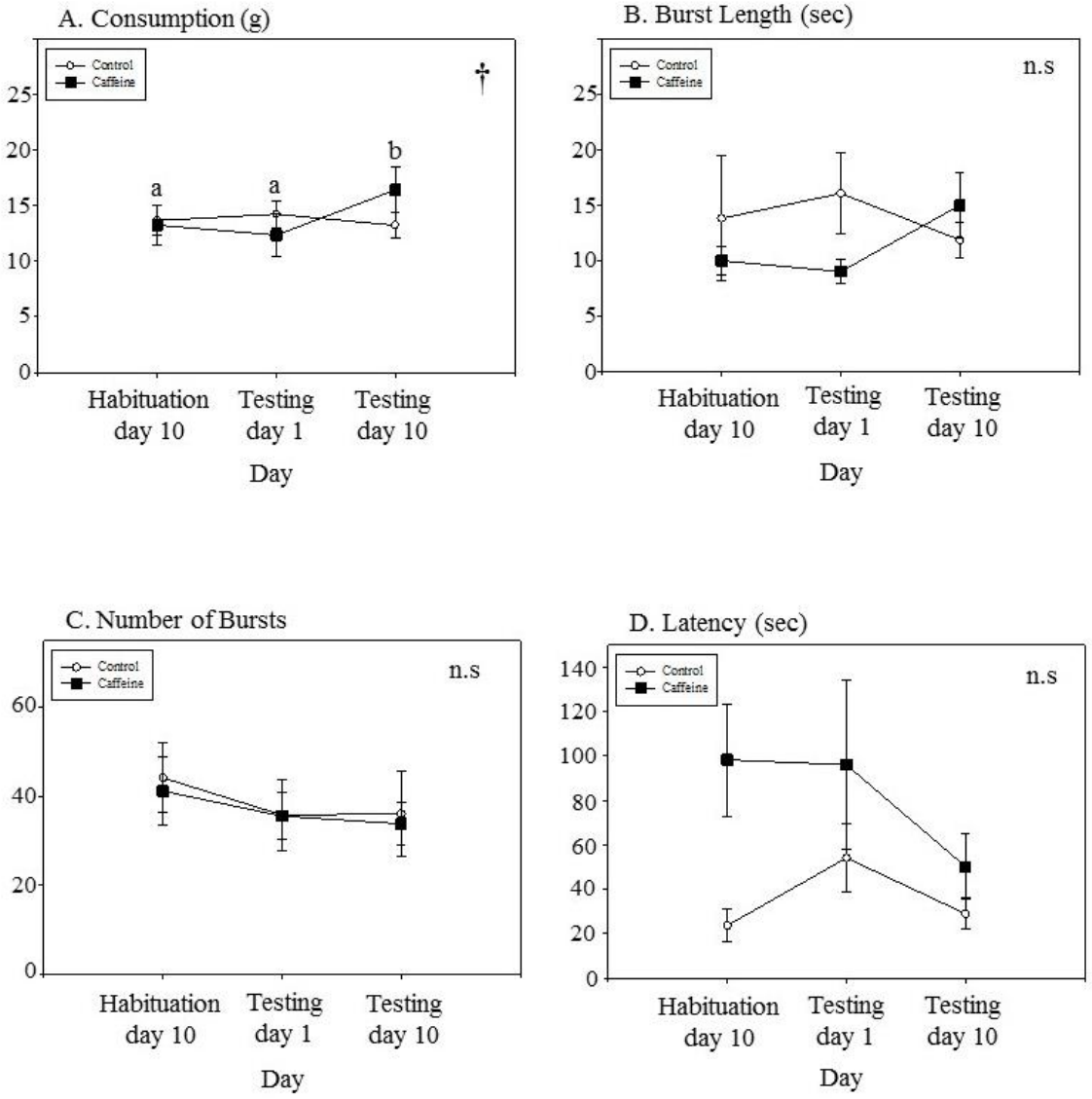
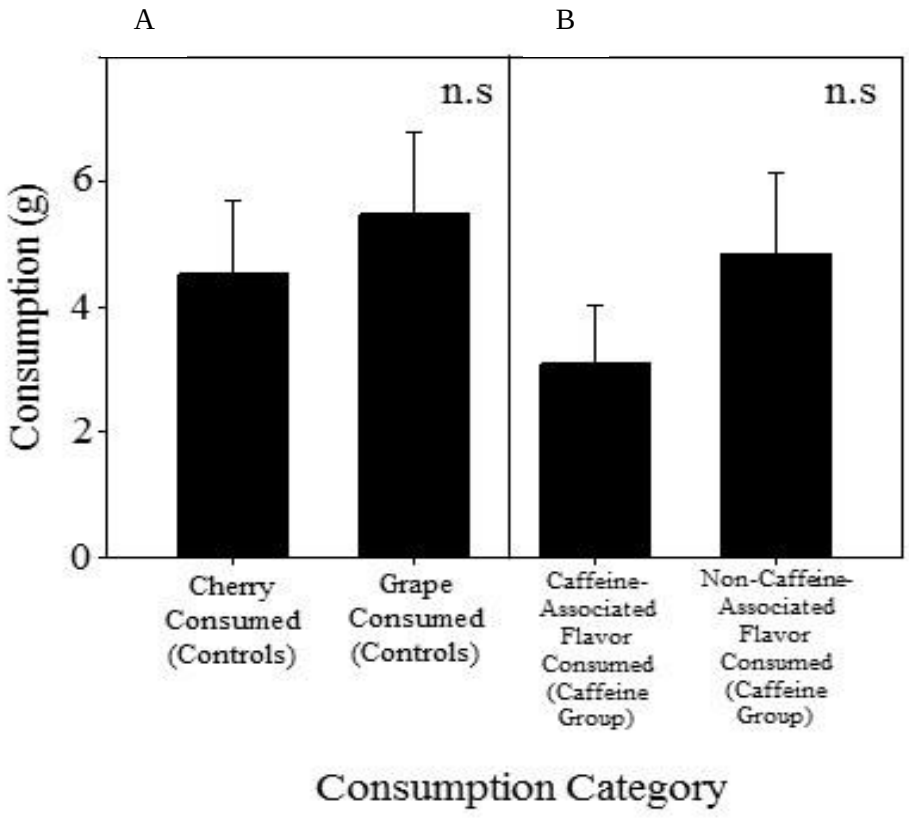


Figure 5: Two-Bottle flavor choice task. Differences in consumption between Kool-Aid flavors and differences in consumption between caffeine-paired and unpaired flavors.

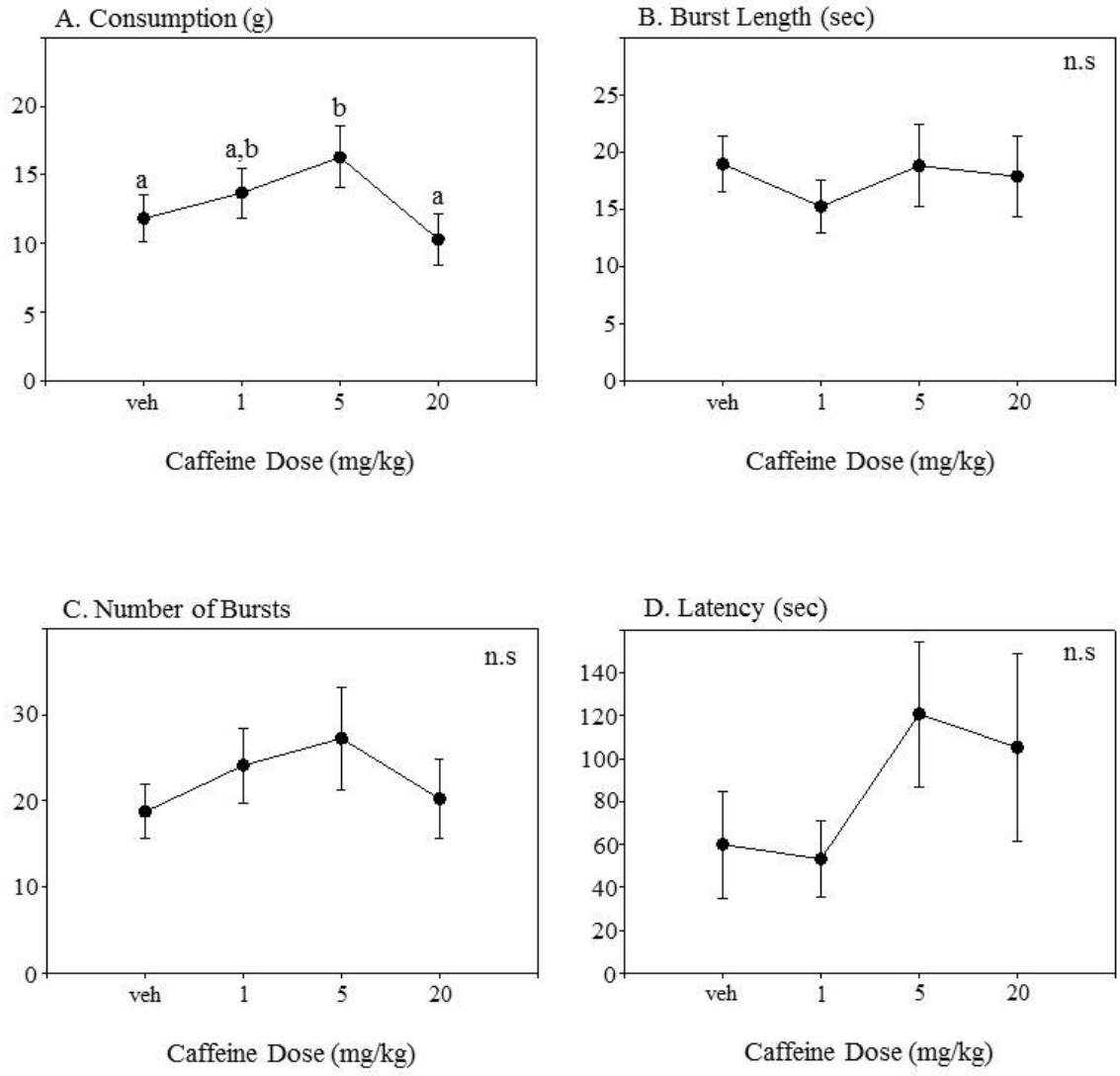


Experiment 2: Effects of Systemic Caffeine Administration on Licking Microstructure and Consumption of Sucrose Solutions

Analyses via one-way repeated measures ANOVAs were used to examine the effect of drug dose on the parameters of interest. For rats that were tested for sucrose consumption following systemic injections of caffeine, there was a significant within-subjects effect of drug dose on consumption, $F(3, 21) = 5.262$, $p = .007$ (Figure 6A)

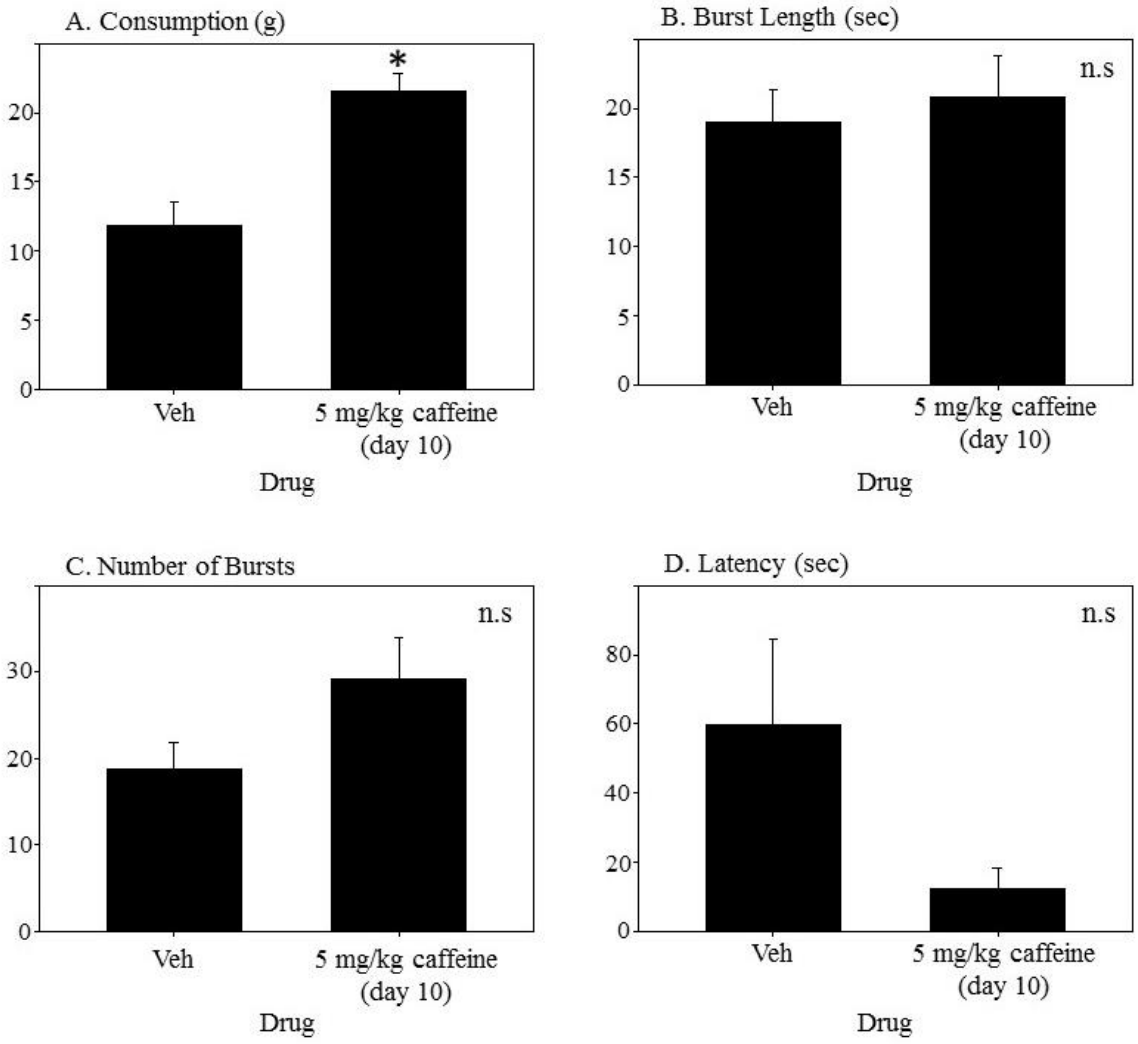
indicating changes in consumption that were dependent upon the dose of caffeine being administered. Follow-up analyses with Tukey's HSD revealed that rats being given the moderate 5mg/kg injection of caffeine consumed more sucrose solution than rats being given vehicle or the high 20mg/kg dose of caffeine. No significant effects were obtained for burst length, $F(3, 21) = .478$, $p = .701$ (Figure 6B), number of bursts, $F(3, 21) = 1.102$, $p = .370$ (Figure 6C), or latency, $F(3, 21) = 1.315$, $p = .296$ (Figure 6D).

Figure 6: Effects of systemic administration of various caffeine doses on consumption and licking microstructure. Significant differences between doses are represented by differing letters between those doses.



Paired samples t-tests were used to assess changes in consumption and licking microstructure following 10 days of chronic administration of the moderate 5mg/kg caffeine dose. Regarding consumption, rats consumed significantly more sucrose solution on their tenth day of moderate caffeine administration compared to when vehicle was administered, $t(7) = -5.657$, $p = .001$ (Figure 7A). However, rats' burst length did not differ between their vehicle injection day and their 10th day of moderate caffeine

Figure 7: Effects of 10 days of moderate systemic caffeine administration on consumption and licking microstructure of a sucrose solution compared to vehicle. Significant differences between days are represented by an asterisk.



administration, $t(7) = -.637$, $p = .544$ (Figure 7B). In terms of the number of bursts, analysis revealed a trend towards more bursts on the 10th moderate injection day, but this did not reach statistical significance, $t(7) = -1.856$, $p = .106$ (Figure 7C). Similarly, latency trended towards rats' being more quick to commence licking on the 10th day of moderate caffeine injections, but this did not reach significance, $t(7) = 2.353$, $p = .051$ (Figure 7D).

DISCUSSION

The purpose of the experiments comprising this thesis was to better characterize the role of caffeine in modulating food-related motivation, specifically as it applies to sweetened solutions. These goals were crafted in response to the real-world prevalence of caffeine in highly palatable, calorie-dense beverages, as well the overall lack of research covering the topic. Changes in consumption and microstructural attributes were examined in response to both manipulation of the presence of caffeine in sucrose solutions, as well as pharmacological administration of caffeine systemically. These changes can be related back to specific aspects of food-related motivation, and thus reveal valuable information regarding caffeine's role in promoting intake of palatable drinks.

During the validation study, we demonstrated that rats consumed more of a sucrose solution when the concentration of that solution was increased from .03M to .1M. These same rats also consumed less of the solution when the concentration was further increased from .3M to 1.0M. Similarly, we found that increasing the concentration from .03M to .1M also increased the number of licking bursts, and that these too decreased as the concentration was increased from .03M to 1.0M. These findings illustrate the fact that consummatory motivation related to a sucrose solution varies with regard to the concentration of that solution, and mirrors what has been shown by other researchers (Davis & Perez, 1993; Spector et al., 1998). Additionally, during the same experiment we found that increasing the sucrose concentration trended towards an increase in the length of licking bursts, which reached statistical significance when increasing the concentration from .3M to 1.0M. Prior studies have shown that burst size, a commonly used measure of hedonic evaluation, increases as sucrose concentration increases (Davis & Perez, 1993;

Spector et al., 1998). This suggests that burst length is an acceptable substitute for burst size in the examination of hedonic evaluation since our data exhibit the same trend. We observed no significant changes in latency to initiate licking as sucrose concentration was varied. These findings allowed us to demonstrate that our laboratory setup was suited for collecting meaningful and interpretable data regarding licking microstructure. Because of this, we were confident in our ability to assess and interpret the two experiments that followed.

The first caffeine experiment examined the differences in licking microstructure and consumption of sucrose solutions with or without caffeine added to the solution. While the amount of solution consumed did not vary for the control rats, follow-up analyses demonstrated that following 10 days of exposure to caffeinated sucrose solutions, rats consumed more than they did following 10 days of exposure to a non-caffeinated sucrose solution. They also consumed more following 10 days of caffeine exposure than they did on their first day of exposure. This is consistent with findings from Swithers et al., who also reported that rats consumed more of a sweetened solution over time when it was paired with caffeine (2010). However, unlike Swithers and colleagues, this thesis examined the microstructure of licking following caffeine-sucrose pairing. Analysis of the microstructural attributes revealed that the increase in consumption on the tenth day of exposure was paralleled by a trend illustrating increased burst length for the caffeine group on the tenth day of exposure. However, this trend failed to reach statistical significance. The number of bursts were unaffected throughout the experiment and did not differ between groups or across treatment days. The rats receiving caffeine were slower to begin drinking the solution compared to control rats,

but this difference was present during habituation. Since no caffeine was administered during habituation, these differences in latency are not due to the effects of caffeine and are likely due simply to individual differences between the two cohorts of rats. The two bottle test demonstrated no base preference for grape or cherry among the control rats. In addition, this test also showed that the rats who had been exposed to caffeine exhibited no preference or aversion for the caffeine-associated flavor. These findings are consistent with past work from Myers and Izbicki, which involved the exposure of rats to both non-caffeinated and caffeinated palatable solutions, and a post-conditioning two bottle choice test to test for flavor preference (2006). These rats did express a caffeine-associated flavor preference when the flavor was paired with a lower dose of caffeine (.07mg/ml) and a preference for a non-caffeine associated flavor when a higher caffeine dose (.25mg/ml) was used. However, they demonstrated no preference for either flavor when the moderate dose of caffeine (.125mg/ml) was used. Recall that this is the same dose we used for our experiment.

Taken together, the findings from this first experiment tell us several things. First, they suggest that caffeine's presence in a palatable solution increases the consumption of that solution over time, implying that caffeine may increase consummatory motivation. Additionally, the trend towards increases in burst length suggest that this increase in consummatory motivation may be due to a positive shift in the hedonic evaluation of the solution. This is supported by past studies in the human literature that paired caffeine with palatable food and drink items, and demonstrated that this pairing led to self-report increases in the hedonic evaluation of those items (Keast et al., 2015; Panek et al. 2013; Temple et al., 2012). In addition, a lack of a caffeine-associated flavor preference

demonstrates that the increase in consumption of caffeinated solutions during testing was likely not due to a positive association between the arousing effects of caffeine and the flavor it was paired with. Since many sweetened beverages that humans regularly consume contain caffeine levels comparable to what we used for the solutions in this experiment, these findings also have major implications for the obesity epidemic. Specifically, they suggest that the overconsumption of high-calorie, sweetened beverages may be due at least in part to the consummatory-enhancing effects of caffeine that we showed here.

In the second caffeine experiment, the effects of systemic caffeine administration on consumption and licking microstructure were examined. The effects of various doses of caffeine were examined, as well as the effects of ten days of administration of the moderate (5mg/kg) dose. This chronic phase of the experiment with a moderate dose of caffeine allowed us to compare the findings to the first caffeine study we conducted, which involved ten days of exposure to a moderate amount of caffeine, but in the solution. This comparison allows us to better isolate the pharmacological aspect of caffeine's effects, and examine how it differs from having caffeine in the solution itself. When testing rats with various doses of caffeine in single testing sessions per dose, we found that the moderate (5mg/kg) caffeine injection increased consumption of sucrose solutions, and that administration of the low (1mg/kg) and high (20 mg/kg) doses resulted in no difference in consumption compared to the vehicle injection. Injection of increasing doses of caffeine had no effect on burst length or latency. The low and moderate doses of caffeine appeared to produce an increasing trend in the number of licking bursts, but this was not statistically significant.

During the second phase of this experiment, we administered daily injections of the moderate dose (5mg/kg) of caffeine for ten days. We compared the tenth day of moderate caffeine administration to the vehicle injection day to examine the more long-term effects of caffeine administration prior to sucrose availability. Following the ten days, rats consumed substantially more sucrose solution than they had on their vehicle injection day. The tenth day of moderate caffeine administration yielded no change in the length of licking bursts, but did demonstrate non-significant trends towards an increase in the number of bursts and a decrease in latency. These findings suggest that a moderate amount of caffeine increases the consummatory motivation to drink an excess of a sweetened solution, and that this increase occurs as soon as the first day of exposure to the caffeine. In addition, these findings provide no evidence to suggest the heightened consummatory motivation is tied to changes in hedonic evaluation. The trends towards increased number of bursts and decreased latency following ten days of caffeine exposure however, suggest that moderate doses of caffeine may increase appetitive motivation as well. These findings are consistent with past animal work which has used lever-press paradigms to show that systemic caffeine administration increases the appetitive motivation to work for sugar pellet rewards (Brianna Sheppard et al., 2012; Retzbach et al., 2014). Thus, in isolating the pharmacological effects of caffeine, we discovered evidence to suggest that different aspects of motivation are engaged in the consumption of sweetened solutions depending on the route in which caffeine is administered. Important to note is that both experiments demonstrated an enhancement of consummatory motivation as demonstrated by increased consumption, but this increase occurred on the first day of exposure when caffeine was systemically injected.

Due to time constraints on the submission of this thesis, the data corresponding to testing days in between the first and tenth days of testing were not included in the final analyses. This applies both to the ten days of caffeine exposure in solution in experiment 1, and the portion of experiment 2 that involved ten days of systemic caffeine injections. For many of the dependent variables, we established meaningful trends that failed to reach statistical significance. Further analysis examining the remaining testing days in both experiments needs to be done in the future in order to determine if the trends we found are real. If they are, then a comparison of the dependent variables across time should yield significant findings for those instances where we demonstrated trends.

Should these trends turn out to be significant following further analysis, the next logical step would be to better characterize the neural instantiation of caffeine's effects on sweetened-solution consumption. For example, if the trend for increased burst length for caffeinated solutions is statistically significant, then caffeine's actions within the brain's reward circuitry may be the cause. There are many experimental directions this could take. Recall that caffeine exerts its effects in the brain via the non-selective antagonism of adenosine receptor subtypes, which are located throughout the brain. One way to test whether or not caffeine is changing hedonic evaluation via the reward circuitry would be to administer intracranial injections of caffeine or other more selective adenosine antagonists directly into the nucleus accumbens, a crucial neuroanatomical locus of the reward system. While consuming a non-caffeinated sucrose solution, if rats receiving these injections produce similar microstructural and consumption data compared to rats consuming caffeinated sucrose solutions, then that would provide support for the implication of caffeine in altering the function of the reward system.

Another way to test this would to stimulate adenosine receptors prior to allowing rats to consume a caffeinated sucrose solution. Pretreatment with a non-selective agonist for adenosine receptors, such as adenosine itself, to the nucleus accumbens via intracranial injections would activate adenosine receptors and block their binding sites before the caffeine from the solution would be able to. If caffeine is indeed acting on neural reward circuitry such as the nucleus accumbens, then agonism of this nature should reverse any hedonic evaluative enhancing effects caffeine would have. If this agonism has no effect on the consumption and microstructure related to a caffeinated sucrose solution, then it is likely that caffeine is having an effect elsewhere in the brain to enhance hedonic evaluation and consumption.

Another potential way to help characterize how caffeine's enhancement of consumption is instantiated in the brain would be through the utilization of c-Fos expression. This approach would be more exploratory and less confirmatory in nature when compared to the pharmacological manipulations described in the previous paragraphs. c-Fos is a protein that is upregulated in neurons that have recently been firing. Thus c-Fos serves as a marker for increases in neuronal activity. To utilize this protein, one group of rats would need to be given systemic injections of caffeine and another would need to be given vehicle. By administering intraperitoneal injections of caffeine, or intracranial injections of caffeine into the brain's ventricles, caffeine will be able to spread diffusely throughout the brain. Following consumption of non-caffeinated sucrose solutions, sacrificing the rats and histologically analyzing the brains of both groups for c-Fos expression could help reveal which clusters of neurons that caffeine is interacting with are more active during the consumption of a sweetened solution.

Though there are clearly many directions that future research in this area could take, the present findings from this thesis hold major implications for the obesity epidemic. Recall that a critical component in the etiology of the obesity epidemic is the overconsumption of highly palatable foods and drinks that contain a disproportionately large amount of calories compared to other foods. The findings in this thesis suggest that the moderate amount of caffeine that is present in high calorie, highly palatable beverages is sufficient to promote their overconsumption. Additionally the microstructural data suggest that this enhanced intake may be due to an increase in the length of licking bursts, suggesting that caffeine may promote overconsumption by enhancing the perceived palatability of sweetened beverages. These findings then suggest that people may drink more of these beverages in a sitting than they would have were the caffeine not present. Individuals who are prone to obesity should take special considerations into their dieting plans to reduce or eliminate the amount of caffeinated sweet beverages they allow themselves access to. The findings from this thesis suggest that the removal of caffeine may be an effective strategy for reducing caloric intake of palatable beverages.

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