Sucrose Bingeing Reduces the Reward Value of Sucrose

By

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Abstract

Binge eating occurs primarily on highly palatable food (PF) suggesting that the reward value of food has an important role in this behaviour. Bingeing also leads to reward dysfunction in rats and humans. The rewarding effect of binge eating may involve opioid mechanisms as opioid antagonists reduce PF consumption in animals that binge eat and binge eating produces neuroadaptations of opioid receptors in rodents. We tested this hypothesis by using the conditioned place preference (CPP) paradigm. First we established a sucrose CPP in male and female Long-Evans rats (n=8 for each group) using 1%, 5%, 15%, or 30% sucrose solution. Next, rats underwent the sucrose bingeing model in which separate groups of rats (n=8 for each group) received 12hr and 24hr access to 10% sucrose solution and chow, 12hr access to 0.1% saccharin solution and chow, or 12hr access to chow only every day for 28 days. Immediately following these sessions, rats were conditioned and tested in the CPP paradigm using a 15% sucrose solution. Finally, we examined whether the sucrose bingeing model altered morphine reward in female rats. Rats (n=8 for each group) received 12hr and 24hr access to 10% sucrose solution and chow every day for 28 days. Immediately following this access period, rats were conditioned to morphine (6mL/kg) or saline solution in the CPP paradigm and tested for a CPP. In all experiments, rats drank more sucrose solution than water during conditioning sessions. Male rats did not develop a CPP to any concentration of sucrose solution and females developed a CPP to 15% sucrose solution only. Following the sucrose bingeing protocol, sucrose CPP was attenuated in male rats that binged on sucrose and in all female rats. Sucrose bingeing in females did not affect the development of a CPP to morphine. These results suggest that sucrose consumption and
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sucrose CPP are measures of different psychological components of reward. Furthermore, sucrose bingeing reduces the rewarding effect of sucrose, but not morphine, suggesting that opioid reward is still intact.
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<tr>
<td>American Psychiatric Association</td>
<td>APA</td>
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<tr>
<td>Anorexia Nervosa</td>
<td>AN</td>
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<tr>
<td>Anorexia Nervosa- restrictive</td>
<td>AN-r</td>
</tr>
<tr>
<td>Anorexia Nervosa- binge/purge</td>
<td>AN-b/p</td>
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<td>Binge Eating Disorder</td>
<td>BED</td>
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<td>Brain stimulation reward</td>
<td>BSR</td>
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<td>Bulimia Nervosa</td>
<td>BN</td>
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<tr>
<td>Body Mass Index</td>
<td>BMI</td>
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<td>Clinical impairment assessment</td>
<td>CIA</td>
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<td>Conditioned place preference</td>
<td>CPP</td>
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<tr>
<td>Dopamine</td>
<td>DA</td>
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<tr>
<td>Food restricted</td>
<td>FR</td>
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<td>Free fed</td>
<td>FF</td>
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<tr>
<td>Health related quality of life</td>
<td>HRQL</td>
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<tr>
<td>Intraperitoneal</td>
<td>IP</td>
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<tr>
<td>Lateral hypothalamic</td>
<td>LH</td>
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<tr>
<td>Medical Outcomes Study Short-Form Health Survey</td>
<td>SF-36</td>
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<tr>
<td>Nucleus Accumbens</td>
<td>NAc</td>
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<tr>
<td>Other Specified Feeding or Eating Disorder</td>
<td>OSFED</td>
</tr>
<tr>
<td>Palatable food</td>
<td>PF</td>
</tr>
<tr>
<td>Unspecified Feeding or Eating Disorder</td>
<td>UFED</td>
</tr>
<tr>
<td>United States</td>
<td>U.S.</td>
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</table>
Binge eating is common in patients with eating disorders and in obese populations (Hudson, Hiripi, Pope, & Kessler, 2007). It is the central component of Binge Eating Disorder (BED) (American Psychiatric Association, 2013), but is also prevalent in other eating disorder subtypes (Castellini et al., 2011). Binge eating is also associated with obesity (Hudson et al., 2007), although this relationship is somewhat complex since not all binge eaters are obese and not all individuals who are obese binge eat. This relationship is paralleled in animal models in which binge eating and weight gain do not always co-occur. For example, rats that binge on sucrose or have intermittent or limited access to highly palatable food (PF) do not gain excessive weight (Avena, Bocarsly, Rada, Kim, & Hoebel, 2008; Johnson & Kenny, 2010; Wojnicki, Johnson, & Corwin, 2008), whereas high sucrose-fat bingeing rats and rats that have extended access to a highly palatable diet gain a significant amount of weight (Berner, Avena, & Hoebel, 2008; Johnson & Kenny, 2010). Thus, macronutrient type, access to PF, and binge eating are all factors that likely influence weight gain.

Binge eating is associated with high relapse rates to disordered eating (Carter et al., 2012; Castellini et al., 2011), a characteristic common among drug abusers since drug relapse is common in this population (Brandon, Vidrine, & Litvin, 2007). Binge eating in animals occurs almost exclusively on PF that is high in fat and sugar (Hagan et al., 2002) and in humans (Drewnowski, 1997; Yanovski et al., 1992), which suggests that the behaviour is hedonically, rather than metabolically, driven although energy states (i.e., satiety vs. hunger) do affect the rewarding properties of food. Similar neurochemical changes occur during the development of binge eating and substance abuse, including
alterations in the mesolimbic dopamine (DA) reward pathway and neurocircuitry involved in compulsion. Examination of the alterations in these neurochemical systems are important in determining how binge eating develops and is maintained despite its negative impact in humans.

1.1 Eating Disorders and Obesity

Eating disorders fall into a number of diagnostic categories, including Anorexia Nervosa (AN), for which there are two subtypes, restrictive (AN-r) and binge/purge (AN-b/p), Bulimia Nervosa (BN), BED, Other Specified Feeding or Eating Disorder (OSFED), and Unspecified Feeding or Eating Disorder (UFED) (APA, 2013). Individuals who do not meet criteria for AN, BN, or BED but have the same types of symptoms are generally classified as OSFED, whereas individuals that do not show characteristics typical of AN, BN, or BED are diagnosed with UFED.

At any given time in Canada, between 600,000 and 999,000 individuals will meet diagnostic criteria for an eating disorder, primarily AN, BN, or BED and approximately 80% of these individuals are women (Report of the Standing Committee of the Status of Women, 2014). The lifetime prevalence of DSM-IV AN, BN, and BED in the United States (U.S.) is 0.9%, 1.5%, and 3.5% among women and 0.3%, 0.5%, and 2.0% among men (Hudson et al., 2007). The prevalence of eating disorders in Canada is similar to that of the U.S., with AN and BN affecting approximately 0.5% and 1% of the population (Report of the Standing Committee of the Status of Women, 2014). A more recent study in the U.S., using DSM-V diagnostic criteria, but only including women, reported that the lifetime prevalence of eating disorders by age 20 was 13.1%, with 0.8%, 2.6%, and 3.0% of the women meeting criteria for AN, BN, and BED respectively (Stice, Marti & Rhode,
Eating disorders tend to be very difficult to treat: only 50% of individuals ever make a full recovery (Report of the Standing Committee of the Status of Women, 2014).

Obesity, which is defined as a Body Mass Index (BMI) ≥30, can be broken down into three classes: class I obesity (BMI of 30 to 34.9), class II obesity (BMI of 35 to 39.9), and class III obesity (BMI ≥40). The prevalence of obesity across all three categories has risen drastically in the past thirty years. In Canada, the prevalence of obesity in adults rose from approximately 14% in 1980 to 26% in 2009 (Public Health Agency of Canada the Canadian Institute for Health Information, 2011). The proportion of adults that fall within class I obesity (BMI 30 to 34.9) is the greatest, followed by class II obesity (BMI 35 to 39.9), and then class III obesity (BMI ≥40). Although the greatest proportion of obese adults are categorized as class I obesity (15.1%), the proportion of adults that fall within class II (5.1%) and class III (2.7%) obesity is rising drastically. For example, from 1979 to 2009, class II and class III obesity increased by 122% and 200% respectively, whereas class I obesity increased by 45% (Public Health Agency of Canada the Canadian Institute for Health Information, 2011).

Obesity is associated with a number of health problems including cancer (kidney, colorectal, prostate, ovarian, oesophageal, pancreatic and post-menopausal), type II diabetes, heart disease (hypertension, coronary artery disease, congestive heart failure, pulmonary embolism and stroke), gallbladder disease, osteoarthritis, asthma, and back pain (Anis et al., 2010). The growing prevalence of obesity places a heavy burden on the health care system. In 2006, the direct cost of overweightness and obesity in Canada was approximately $6.0 billion, and made up 4.1% of the total health expenditures. A greater
cost was attributed to obesity ($3.9 billion) compared to overweightness ($2.0 billion) (Anis et al., 2010).

Health care costs for eating disorders are also quite substantial, although estimates vary greatly due to differences in inclusion versus exclusion of factors such as direct or indirect costs, cost of drugs, and outpatient or inpatient care. In a systemic review of cost-of-illness for eating disorders, Stuhldreher et al. (2012) found that annual costs per patient ranged from 1288 to 8042 U.S$-purchasing power parity. Ultimately, both direct costs, which include medical and non-medical costs (e.g., treatment, medication, transportation) and indirect costs, which is the monetary value associated with a loss of production (Stuhldreher et al., 2012), contribute to the annual cost of an individual with an eating disorder.

All cause mortality is the annual number of deaths for any reason, in a given population. In obese individuals, class II and III obesity (described as severe obesity), are associated with a significantly higher all-cause mortality, relative to normal weight controls (Flegal, Kit, Orpana, and Graubard, 2013). In terms of the number of deaths related to obesity, in 2000, 9.3% of all deaths in Canada could be attributed to obesity, a number that grew from 5.1% in 1985 (Public Health Agency of Canada the Canadian Institute for Health Information, 2011). Eating disorders are also associated with a higher risk of mortality. Compared to all other psychiatric disorders, AN has the highest mortality rate (10% to 15%) and BN has a mortality rate of approximately 5%. Together, eating disorders are the cause of mortality in approximately 1,000 to 1,500 Canadians per year (Report of the Standing Committee of the Status of Women, 2014).
Eating disorders and obesity are associated with a lower subjective health-related quality of life (HRQL). HRQL is measured using the Medical Outcomes Study Short-Form Health Survey (SF-36), which assesses eight domains including physical functioning, role limitations as a result of physical problems, bodily pain, general health perception, vitality, social functioning, role limitations as a result of emotional problems, and mental health (Winkler et al., 2014). Patients with eating disorders report a significantly lower HQRL, with lower scores compared to the general population on all domains of the SF-36, except for physical functioning (Winkler et al., 2014). Similarly, obese individuals report a lower HRQL, and score significantly lower on all eight domains of the SF-36 compared to a general population (Fontaine & Barofsky, 2001).

Compared to their non-eating disordered counterparts, individuals with eating disorders report having significantly greater functional impairment, defined as impairment in family, peer, group, romantic, and schools spheres; BN and BED patients report significantly greater emotional distress (Stice et al., 2013) and BED is strongly associated with a history of depression (Spitzer et al., 1993). Eating disorder patients are five times more likely to attempt suicide compared to the general population (Suokas et al., 2014), lending further evidence for the emotional distress associated with these disorders. Eating disorders are also associated with a high lifetime prevalence of comorbid psychiatric disorders leading to further psychosocial impairment. For example, the prevalence of eating disorders is positively correlated with mood, anxiety, substance use, and impulse control disorders. Using DSM-IV diagnostic criteria, Hudson et al. (2007) found that 56% of AN, 94.5% of BN, 78.9% of BED, and 63.6% of subthreshold BED patients met criteria for one of these disorders.
1.2 Binge Eating

Binge eating is a common feature of eating disorders, most notably BED, BN, and AN-b/p; it is also associated with obesity. Binge eating is defined as eating an objectively large amount of food within a discrete period of time (i.e., 2 hours), and feeling a sense of lack of control over eating (APA, 2013). In patients with an eating disorder, binge eating episodes are accompanied by marked distress, eating until uncomfortably full, eating rapidly, eating alone, and feeling disgusted or guilty with oneself after binge eating (APA, 2013). BED is an eating disorder characterized by objective binge eating episodes that occur at least once weekly, for a minimum of three months. BED differs from BN in that patients do not engage in any compensatory behaviours (e.g., purging, excessive exercise, fasting, laxative use, etc). AN-p/b is characterized by restriction of food intake leading to significantly lower body weight, intense fear of gaining weight, and disturbances in experienced weight and shape, in addition to recurrent binge/purge episodes.

Binge eating is present across all eating disorder subtypes, and is also reported by individuals who do not meet full criteria for an eating disorder (Stice et al., 2013). The lifetime prevalence of subthreshold BED, and any binge eating among women and men combined is 1.2% and 4.5% respectively (Hudson et al., 2007). The prevalence of BED and BN is much higher than AN in both women and men, and a large portion of AN-r patients develop binge eating (Eddy et al., 2002; Eddy et al., 2008). In fact, there is a large degree of diagnostic crossover between eating disorder subtypes, with the majority of eating disorder patients transitioning from non-binge eating to binge eating. For example, in a 6-year follow up study, Castellini et al. (2011) found that there was
crossover between all eating disorders subtypes: AN to BN (23.4%), BN to AN (8.4%),
BN to BED (8.4%), and BED to BN (7.1%). In addition to the 23.4% of patients that
transitioned from AN to BN, 46% of the AN-r patients developed binge eating, and thus
met criteria for AN-p/b, whereas no patients transitioned from binge eating to restrictive
eating only. Similarly, Eddy et al. (2002) found that a large proportion of patients
transitioned from AN-r to AN-b/p, with only 12% of AN patients never reporting
binge/purge episodes.

Binge eating is associated with a number of negative outcomes including obesity,
psychosocial impairment, and persistence of eating disorders (i.e., longer duration)
(Hudson et al., 2007). Binge eating and BED are strongly associated with obesity, in
particular class II and III obesity, which are the most rapidly escalating classes of obesity
and those that are associated with the greatest health risks and mortality. BED patients
are significantly more likely to have a BMI≥40 compared to the general population
(Hudson et al., 2007). The prevalence of BED is much greater (17%) in class III obese
populations seeking gastric bypass surgery compared to the general population (3%) (de
Zwaan et al., 2002), lending further evidence for the association between BED and
obesity.

Binge eating severity, but not obesity, is associated with psychological distress
(depressive symptomatology, general psychiatric symptomatology, lower self-esteem,
and interpersonal distress) (Telch & Agras, 1994) and binge eating itself is associated
with impairment in role functioning, including home life, work life, social life, and/or
personal life (Hudson et al., 2007). Patients who engage in binge eating are more
susceptible to psychosocial impairment compared to non-binge eating patients. In eating
disorder patients, binge eating, is a unique contributor to psychosocial impairment, measured by the Clinical Impairment Assessment (CIA), whereas restrictive eating or purging are not (Hovrud & De Young, 2015). The CIA measures impairment associated with mood and self-perception, cognitive functioning, interpersonal functioning, and work performance, which are aspects typically affected by eating disorders. Relapse rates are high among eating disorder patients, ranging from 40% to 50% (McFarlane, Olmsted, & Trottier, 2008). Subjective binge eating frequency across all eating disorder subtypes (Castellini et al., 2011) and an AN-b/p versus AN-r diagnosis (Carter et al., 2012) is predictive of eating disorder relapse, suggesting that binge eating increases relapse rates in eating disorder patients.

1.3 Animal Models of Binge Eating

Presuming that animals do not develop eating disorders from a social perspective, animal models of binge eating are valuable in that they eliminate a number of psychosocial factors that contribute to binge eating in humans, including weight concern, shame, and overall eating psychopathology (Duarte, Pinto-Gouveia, Ferreira, 2015). Thus, animal models provide a useful tool to examine the neurological underpinnings and neurophysiological changes in response to binge eating. Presently, there is no concrete definition of binge eating in animals, however experimenters tend to define binge eating in a way that follows APA guidelines for binge eating in humans: eating an objectively large amount of food within a discrete amount of time (e.g., two hours). For example, most studies describe eating behaviour in rodents as binge eating if the animals eat significantly more food, compared to control groups, within one to four hours, with the
majority of studies examining consumption after one to two hours following food presentation (Perello, Valdivia, Romero, & Raingo, 2014).

The majority of animal models of binge eating consist of cycles of food restriction (FR) or food deprivation and access to a highly PF. Food deprivation is described as the complete absence of all food for a given period of time, so that during this time, rodents only have access to water. FR on the other hand, refers to limited access to food. For example, rodents would have access to 50% of the total amount of chow that they would otherwise consume in that period of time. The three primary types of animal models of binge eating include FR in combination with stress, the limited access model, and food deprivation and re-feeding (the sucrrose bingeing model).

FR in combination with stress induces binge eating of highly PF in female rats (Hagan et al., 2002). In this paradigm, rats undergo three cycles of FR and re-feeding, followed by an acute stressor (e.g., footshock) and then are provided with access to highly PF, such as chocolate chips. Compared to rats that are exposed to stress alone, FR alone, or neither, rats that undergo a combination of FR and acute stress eat significantly more PF within a discrete time period (2 hours) than either of the control groups. Importantly, a history of FR and stress only induces binge eating when PF is made available during the re-feeding and testing phases (Hagan et al., 2002). This paradigm is useful because it incorporates dieting and stress, two factors that, in combination, induce binge eating in humans (Woods, Racine, & Klump, 2010). On the other hand, the numerous components in this model (highly PF, FR, and stress) make it more difficult to examine how the specific behaviour of binge eating may cause changes in neurocircuitry that perpetuate the behaviour.
In the limited access model, rodents receive limited access to PF and may or may not be FR. For example, Wojnicki et al. (2008) used a limited access model without FR to induce binge eating in male rats. The animals received sporadic (three days a week) and time limited (2 hours) access to a highly PF with unlimited access to chow. When compared to control groups that had daily, but time limited (2 hour) access to PF, rats with sporadic and limited access ate significantly more PF in a one-hour time period. Another example of the limited access model is a 12-hour PF-free period followed by a 12-hour period when PF is available. Numerous types of PF have been used to induce binge eating in the limited access model, including fat-only, sucrose-only, and sucrose-fat combinations (Berner, Bocarsly, Hoebel, & Avena, 2009). The limited access model is useful because it produces feeding behaviour similar to that of BED patients. In these patients, dietary restraint tends to not be as severe as in those with BN or AN-b/p (Elran-Barak et al., 2015), but bingeing on PF still occurs.

The food deprivation re-feeding model consists of cycles of food deprivation and re-feeding and produces binge eating in both male and female rats. Rats are subject to a 12-hour food deprivation period, followed by a 12-hour re-feeding period, at which time animals have access to PF and chow. This cycle is repeated for three to four weeks, at the end of which, rats exhibit binge eating, defined by eating significantly more PF within one hour following presentation, compared to control groups. The sucrose bingeing model is based on this paradigm: rats receive 12 hours of access to chow and sucrose followed by a 12-hour food deprivation period, at which time only water is available.

The sucrose bingeing model is beneficial for a number of reasons. First, there are likely differences in sucrose- and fat- bingeing, evidenced by behavioural and
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neurochemical differences following discrete macronutrient bingeing (Avena, Rada, & Hoebel, 2009). Binge eating is produced by intermittent access to PF that is high in fat, sugar or combination of both. Rats maintain their weight during sucrose- and fat-bingeing, by reducing their chow intake to compensate for the high caloric intake of PF (Avena et al., 2008; Berner et al., 2009), whereas fat-sucrose bingeing rats exhibit significant weight gain (Berner et al., 2008; Johnson & Kenny, 2010). Naloxone-precipitated withdrawal is not seen in fat- or sucrose-fat bingeing rats (Bocarsly, Berner, Hoebel, & Avena, 2011), but is observed in sucrose bingeing rats (Avena et al., 2008; Colantuoni et al., 2002). Furthermore, there are distinct differences in the effects of opioid and DA antagonists on PF intake following a history of binge eating that are dependent on the macronutrient type. For example, GABA, DA and opioid antagonists have differential effects on sucrose- versus fat- bingeing rats (Wong, Wojnicki, & Corwin, 2009; Avena et al., 2008). Thus, the sucrose bingeing model provides a number of advantages over other animal models of binge eating, particularly in studies focusing on specific neurobiological substrates that contribute to this behaviour.

1.4 Binge Eating and Reward Mechanisms

PF is a natural reward that activates the mesolimbic DA system. Like many substances of abuse, (Koob & Volkow, 2010; Prus, James & Roscrans, 2009), rats show a conditioned place preference (CPP) to PF and sucrose solutions (Duarte, Lefebvre, Chaperon, Hamon & Thiébot, 2003; Figlewicz, Higgins, Ng-Evans & Havel, 2001; Jarosz, Sekhon & Coscina, 2006; Larson, 2006). Rats also readily consume and self-administer PF and sucrose solutions (Dumont, Mark, Mader, & Williams, 2005; Nair, Golden, & Shaham, 2008; Zhang, Balmadrid, & Kelley, 2003).
DA and opioid signalling within the mesolimbic reward pathway are important mediators of PF reward. Opioid antagonists reduce PF, but not chow consumption in free fed (FF), FR (Levine, Weldon, Grace, Cleary, & Billington, 1995), and binge eating rats (Boggiano et al., 2005; Czyzyk, Sahr, & Statnick, 2010), whereas µ-opioid agonists increase consumption of PF in binge eating rodents (Boggiano et al., 2005). Specific regions that are important in mediating PF consumption through opioid signalling are the nucleus accumbens (NAc), hypothalamus, and amygdala, as µ-opioid receptor agonists injected into these regions increase high fat and high sugar consumption in rodents (Berthoud, 2002; Peciña & Berridge, 2005). Overall, this suggests that µ-opioid receptor agonism increases, whereas µ-opioid receptor antagonism selectively reduces, PF consumption in rodents.

DA and opioid manipulation also alters behaviour in paradigms that are used to measure reward. For example, DA and opioid agonists in the NAc increase the breaking point (a measure of motivation) and lever presses for sucrose pellets (Zhang et al., 2003), and rats that are trained in a CPP paradigm spend significantly more time in the PF-associated compared to the non food-associated side (Duarte et al., 2003; Figlewicz et al., 2001; Jarosz et al., 2006; Larson, 2006); this preference can be blocked by opioid (Ågmo, Galvan, & Talamantes, 1995; Jarosz et al., 2006) or DA (Ågmo et al., 1995) antagonists. DA antagonists also attenuate responding for lateral hypothalamic (LH) brain stimulation reward (BSR), amphetamine, cocaine, and PF (Wise, 2004). Together, this evidence indicates that PF reward, is at least in part mediated by opioid and DA signalling within the mesolimbic DA reward pathway.
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Although DA and opioid systems work in tandem, according to the incentive salience theory, they each have a particularly important role in two psychological components of reward: the hedonic impact of rewards (liking), and its attributed incentive salience (wanting). DA in the mesolimbic DA reward pathway mediates wanting. DA antagonism reduces lever pressing for food, but does not reduce food intake when it is readily available, indicating that within the NAc, DA mediates the response to incentive salience but does not affect the pleasure derived from sucrose (Blackburn, Phillips, & Fibiger, 1987). DA antagonists inhibit sucrose-induced CPP in FR rats (Figlewicz et al., 2001), but do not affect hedonic reactions to sucrose (Berridge & Robinson, 1998), and low doses of intraperitoneal (IP) DA antagonists attenuate CPP but do not affect sucrose consumption during conditioning, demonstrating that DA does not mediate liking sucrose at the time of consumption but does affect wanting sucrose (Ågmo et al., 1995). DA signalling within the NAc increases in anticipation of a PF reward and decreases during consumption, which is indicative of DA’s role in predicting and wanting a reward (Richardson & Gratton, 1996).

Liking is mediated through opioid signalling in certain regions of the mesolimbic DA pathway. Rats show positive orofacial expressions during the consumption of PF that indicate liking (Pool, Sennwald, Delplanque, Brosch, & Sander, 2016). µ-opioid agonists injected into the NAc decrease aversive reactions to quinine and increase sucrose consumption and the number of positive taste reactions to sucrose (Avena et al., 2008; Peciña & Berridge, 2005), suggesting that the increase in consumption is attributed to liking rather than wanting. In contrast, opioid antagonists reduce consumption during CPP conditioning and attenuate a CPP to sucrose (Ågmo et al., 1995), which indicates
that the attenuation of a CPP is attributed to reduced liking. Taken together, this evidence suggests that DA signalling is particularly important in mediating wanting, whereas opioid signalling is important in mediating liking, each of which are major components of reward.

The availability of PF is an important contributor to the development of binge eating (Mathes, Brownley, Mo, & Bulik, 2009). Intermittent access to PF leads to the development of binge eating (Avena et al., 2008), rats selectively binge eat PF but not chow (Hagan et al., 2002), and binge eating occurs in the absence of hunger (Corwin, Avena, & Boggiano, 2011), suggesting that binge eating is hedonically, rather than metabolically, driven. PF reward, neurochemical changes in the mesolimbic DA reward pathway, and opioid and DA signalling are altered following extended access to PF, weight gain, obesity, and possibly the development of binge eating (Mathes et al., 2009). Following extended access to a highly palatable diet, rats show a significant increase in reward thresholds in the self-stimulation paradigm that is associated with a down-regulation of D2 receptors in the NAc (Johnson & Kenny, 2010). Other studies have also shown a reduction in both D2 receptors, and D2 receptor mRNA in the NAc following binge eating in rodents (Avena, 2012). Compared to controls, obese individuals show increased activation when viewing images of PF, and decreased activation during consumption of PF in regions related to reward, including the NAc and dorsal striatum (Volkow, Wang, & Baler, 2011). This is indicative of discrete changes in liking and wanting of PF in obese individuals when compared to controls. PF, like drugs of abuse, increase intrasynaptic levels of DA in the NAc in bingeing animals, whereas in non-addicted and non-bingeing animals, DA release decreases after repeated exposure to
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drugs and PF (Goodman, 2008). An increase in µ-opioid receptor binding in the limbic forebrain, NAc, cingulate cortex and hippocampus is observed in sucrose bingeing rats (Colantuoni et al., 2001), opioid antagonists injected intraperitoneally are more effective at reducing PF intake in binge eating, compared to non-bingeing rats (Boggiano et al., 2005), and opioid antagonists significantly decrease taste preference and consumption of high fat-sugar foods in binge eaters, but not obese or normal weight controls (Drewnowski, Krahn, Demitrack, Nairn, & Gosnell, 1992).

In sum, binge eating is highly prevalent among obese and eating disorder populations. BED, for which binge eating is the primary component, is the most highly prevalent eating disorder when compared to AN, BN, UFED and OSFED. Binge eating is related to higher eating disorder relapse rates, psychological impairment, and substance abuse (Holderness, Brooks-Gunn, & Warren, 1994). Both obesity and binge eating lead to alterations in the mesolimbic reward pathway that are similar to those changes displayed in drug-addicted animals. This may explain the high relapse rates for binge eating and the addictive-like quality of the behaviour.

PF produces positive orofacial reactions reflecting ‘liking’. PF is also readily consumed, self-administered, and produces a CPP in rats suggesting that it is rewarding. Furthermore, manipulation of opioid and DA signalling, two neurotransmitters that are important in mediating reward, alter consumption and the rewarding effects of PF. Rats selectively binge-eat PF, which indicates that the reward value of food is an important component of binge eating. In accordance with this theory, alterations in opioid and DA signalling within the mesolimbic DA reward system are observed following the
development of binge eating. Taken together, this evidence suggests that binge eating alters the brain reward system, particularly opioid and DA mechanisms.

1.5 Current Experiments

The current set of experiments was designed to test the hypothesis that binge eating results in a reward deficit and alters the neural substrates of reward, specifically the opioid system that mediates the liking or hedonic aspects of PF. To test this, rats will be maintained on the sucrose bingeing model for 28 days, at which point binge eating, defined as drinking a significantly greater amount of sucrose within a one-hour period compared to controls, should be evident. Because sucrose bingeing does not lead to obesity, the sucrose bingeing model is beneficial in that the reward value of food can be examined independently of weight gain. Recent studies have suggested that the development of binge eating and obesity leads to a blunted reward system (Johnson & Kenney, 2010), and obese individuals show reduced activation of the mesolimbic reward pathway when consuming PF. In these studies, binge eating and weight gain co-exist, making it difficult to examine the effects of binge eating on the reward system, in isolation from weight gain.

The effects of sucrose bingeing on the reward system will then be examined using the CPP paradigm. Rats will have access to sucrose in one CPP compartment and water in the other following sucrose bingeing. I predict that rats who have undergone sucrose bingeing will show a reduced CPP to the sucrose-paired compartment, compared to rats who have not gone through sucrose bingeing. I will then test the effect of sucrose bingeing on the opioid reward system by testing whether sucrose bingeing reduces a CPP to morphine.
Chapter 2: General Methods

2.1 Subjects

Female Long-Evans rats (Charles River Laboratories, Montreal QC), weighing 225-250g at the start of experiments were singly housed in polycarbonate cages (45.5 X 24 X 21 cm) within a climate-controlled room (21°C) and on a reverse 12:12h light cycle (lights off at 4:00h). All animals had free access to water and standard chow in their home cages, unless otherwise stated. All experiments were conducted in accordance with the guidelines for the ethical use of animals outlined by the Canadian Council on Animal Care and all experiments were approved by the Queen’s University Animal Care Committee.

2.2 Apparatus

Weight and food consumption (g) were recorded with a standard scale. Liquid solutions were presented to rats in 100 ml graduated glass drinking bottles fitted with rubber stoppers containing ball-tipped sipper tubes (Ancare Inc., Montreal, QUE). Sucrose and saccharin were mixed in tap water, measured as g/L. Solution concentrations for the sucrose consumption tests were 10% sucrose and 0.1% saccharin (>99% purity, Sigma-Aldrich, Oakville, ON). Solution concentrations during conditioning sessions for the CPP paradigm were 1%, 5%, 15%, and 30% sucrose.

The CPP apparatus (Figure 1) was made of plexiglas and consisted of two large compartments (46cm X 46cm X 30cm) connected by a tunnel (19cm X 38 cm X 30 cm). The two large compartments differed in terms of wall colour (black and white stripes or solid white) and floor texture (striated or bumpy). The tunnel was clear, since the plexiglass was not covered and the floor of the tunnel was made of sheet metal, spray
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Painted with a mat white finish. Guillotine doors, that could be raised or lowered, separated the tunnel from each compartment. The boxes were set on tables, elevated 33cm above the floor. Cameras were mounted directly above each set of boxes so that the rats’ movement throughout the entire compartment could be monitored and recorded. Movement was tracked using the video tracking software EthoVisonXT (Noldus Information Technology b.v. Wageningen, The Netherlands), which detects and extracts the size and position of the subjects from the digital image captured by the camera. This information is then transformed into a series of dependent variables quantifying the behaviour of the subject.

Figure 1. Conditioned place preference (CPP) apparatus. The photo shows two CPP boxes set side by side on a raised platform. The tunnels connecting the two large compartments in each apparatus are on the outer side of each apparatus.

2.3 Behavioural Procedures

2.3.1 Sucrose Consumption

All animals in Experiments 2 and 3 underwent a 28-day sucrose consumption phase. Each rat was randomly assigned to one of four groups: 12hr sucrose, 24hr sucrose, 12hr saccharin, or 12hr food only. For the 12hr sucrose, 12hr saccharin, and 12hr food
only groups, chow was removed from cages at 8:00pm on the night prior to the first day of the 28-day sucrose consumption phase. On the following morning, at 8:00am, 4hrs after onset of the active cycle, rats were provided with standard chow and sucrose solution, standard chow and saccharin solution, or standard chow only. Solution (mL) and chow intake (g) were measured at 1 and 12 hr following presentation. At 8:00 pm, 12hr following presentation of solution and chow, rats were weighed and chow and solution were removed. The 24hr sucrose group was provided with standard chow and sucrose 4hrs after onset of the active cycle (8:00am) on day 1 of the 28-day self-administration phase, and solution (mL) and chow (g) intake was measured 1hr, 12hr, and 24hr (when solution was refreshed) following presentation. All rats had *ad libitum* access to water throughout these sessions.

### 2.3.2 Sucrose Conditioned Place Preference

The CPP paradigm consisted of a habituation phase, conditioning phase, and testing phase. Rats were either FF or FR throughout the habituation, conditioning, and testing phases. FR rats received access to chow for two hours per day, following the afternoon conditioning session (3:00pm to 5:00pm). FR began the evening before the first habituation session. The habituation phase consisted of two, 30-minute session, separated by approximately 6 hours. During habituation, rats were placed in the tunnel and had access to all three compartments over the entire session. Over the next five days, rats underwent two, 30-minute conditioning sessions per day. In each session, rats were confined to one compartment, where they had access to a water bottle containing either tap water or sucrose solution. The assignment of sucrose-paired compartment was counterbalanced within groups. The amount of fluid consumed during each conditioning
A single, 30-minute test occurred on the day after the final conditioning session. Each rat was placed in the tunnel and allowed to roam freely throughout all three compartments for 30 minutes. Neither sucrose solution nor water was available in any of the compartments. The time spent in each compartment was recorded across the session.

2.3.3. *Morphine Conditioned Place Preference*

Morphine was dissolved in sterile saline. All rats were conditioned to morphine (6mL/kg) in one compartment and saline (6mL/kg) in the alternative compartment. IP injections of morphine and saline were administered on alternating days, immediately prior to the conditioning sessions. The CPP paradigm consisted of a habituation, conditioning, test day and state-dependent test day. Rats received two consecutive days of habituation sessions, 10 consecutive days of 30-minute conditioning sessions, one 30-minute, drug free test session, and finally one, 30-minute state-dependent test day. Rats did not receive an IP injection on test day but on state-dependent test day, both groups were administered morphine IP immediately prior to the test session.

2.4 Statistical Analysis

2.4.1 *Sucrose Consumption*

Group differences in body weight across the 28-day sucrose consumption sessions were analyzed using a two-way analysis of variance (ANOVA) with group as a between-subjects factor and session as a within-subjects factor. All consumption data are presented as a percent of body weight (g/g for food and ml/g for solution). For missing data from solution spills, the average solution intake of the remaining rats for the given
time period was calculated and used. Sucrose consumption data were analysed using a two-way ANOVA with group as a between-subjects factor and session as a within-subjects factor. Separate analyses were conducted for intake at 1 hr and 24 hr (12 hr intake for the 12 hr sucrose and saccharin groups). In cases in which sphericity was violated (Mauchley’s $p < .001$), the Greenhouse – Geisser correction was used (df rounded to the nearest whole number). Significant interactions were followed up with simple main effects analyses and multiple pairwise comparisons using a bonferroni correction. To analyse group differences, simple main effects analyses were conducted comparing data on the first and last four days of sucrose consumption sessions.

2.4.2 Conditioned Place Preference

Consumption of solution (sucrose and water) during the five conditioning sessions and time spent in the water-paired and sucrose-paired compartment during the 30-minute test session were analyzed using planned orthogonal comparisons. Planned orthogonal comparisons are *a priori* tests that are used to analyze a limited number of predicted hypotheses. Orthogonal planned comparisons are those that are independent from one another, such that the same term is never presented in more than one comparison. Each comparison is treated independently, so that no correction is made for using multiple tests. In planned orthogonal comparisons, the mean square for the contrast is divided by the mean square error for the ANOVA. The primary advantage of conducting planned orthogonal contrasts is that these minimize the number of comparisons to those of interest based on specific hypotheses (e.g., rats will spend more time in sucrose- than water-paired compartment). As such, a 5% risk of type I error is accepted for each comparison, and no power is lost in order to maintain control over type I error inflation. Importantly,
because each comparison tests a unique hypothesis, it can be carried out regardless of the outcome for the overall ANOVA.

Prior to testing, it was hypothesized that rats would consume more sucrose than water during conditioning, and would spend more time in the sucrose-paired than the water-paired compartment during testing. Thus, the dependent measures were solution consumed (ml/g) and time (s) spent in the water- or sucrose-paired compartment. The between subject factor was sucrose concentration (1%, 5%, 15%, and 30%) in Experiment 1 and food group (12hr sucrose, 24hr sucrose, 12hr saccharin, and 12hr food only) in Experiment 2, 3, and 4. The Levene’s F test was used to examine the assumption of homogeneity of variance. If Levene’s test was significant ($p<.05$), the results associated with the “does not assume equal variance” were used, which adjusts for the standard error of the estimate and degrees of freedom.

Chapter 3: Experiment 1

Effects of Food Restriction on Sucrose Conditioned Place Preference in Females

3.1 Introduction

The primary goal of this preliminary experiment was to establish a sucrose CPP in female rats in our lab and to determine which concentration of sucrose should be used in subsequent experiments. Other labs have shown a CPP to sucrose in male rats using either sucrose pellets (Figlewicz et al., 2001) or sucrose solution (Delamater, Sclafani, & Bodnar, 2000). We used sucrose solution in our experiments because it allowed us to measure consumption during conditioning sessions and to vary the concentration of
sucrose provided to different groups. We also tested animals in both FR and FF conditions, assuming that the magnitude of a CPP would be larger when animals were hungry during conditioning and testing, since other labs have shown that FR potentiates the rewarding effects of LH BSR, PF, and addictive drugs (Volkow & Wise, 2005). In a separate group of animals, we added a tactile cue (floor covering) to enhance the discrimination between the two large compartments. This allowed us to measure whether the size of the CPP would increase under these conditions.

During CPP testing, animals have no exposure to the reinforcer (i.e., sucrose) so the test assesses the ability of animals to remember an association between a rewarding stimulus and environmental cues (Prus, James & Rosecrans, 2009). In the absence of the reinforcer, the response may extinguish such that animals spend less time in the sucrose-paired compartment over testing. That is, the magnitude of the CPP could be greater earlier in the session. For this reason, we also examined the time course of the CPP across testing in order to establish the most appropriate timeframe for CPP testing.

Following the final CPP test, we administered a state-dependent test in which animals had access to sucrose prior to CPP testing. The state-dependent test assesses whether the ability to retrieve memories of a reward-environment association depend on the physiological state during conditioning. For example, if animals show a CPP during state-dependent, but not reinforcer-free testing, it would infer that they have formed a reward-environment association but can only retrieve this memory when they are in a particular physiological state. State-dependent tests, therefore, are a powerful way to assess whether disruptions in memory retrieval affect the expression of a CPP.
Methods

3.2.1 Subjects

Sixty-four female, Long-Evans rats were assigned to either a FF or FR condition (n=32 each). The FF rats had *ad libitum* access to chow and water throughout habituation, conditioning, and testing. For the FR rats, chow was removed the afternoon before the first habituation session and, for the remainder of the experiment, these animals received access to chow for two hours per day in their home cage. Chow was provided following the afternoon conditioning session, two to four hours following the end of conditioning. All rats had *ad libitum* access to water in their home cage.

3.2.2 Behavioural Procedures

Rats in each food condition (n=8) were then assigned to one of four concentration groups: 1%, 5%, 15%, or 30% sucrose solution. All rats underwent CPP habituation, conditioning, and testing as described earlier. The day after CPP testing, all rats underwent a single state-dependent test session (30 min). The state-dependent test day was identical to test day, but immediately prior to the state-dependent test, rats received access to sucrose solution (1%, 5%, 15%, or 30%) for 30 min.

In order to verify that 15% sucrose produces a robust CPP, a separate group of female Long-Evans rats (N=8) was conditioned and tested in the CPP paradigm. In this experiment, a tactile cue (floor type) was added to the CPP apparatus. Plastic drawer liners that were either striated or bumpy were glued onto plasticized cardboard and placed in one or the other large compartments. These covered the entire floor of the two large compartments. Liner was not added to the tunnel floor, so it remained smooth. The floor type and visual cues (striped versus solid) were mixed such that one apparatus had
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striated/striped versus bumpy/solid compartments and the other had bumpy/striped versus
striated/solid. These rats were FR and underwent the same habituation, conditioning, and
testing phases as in the previous experiments.

3.3 Results

3.3.1 Habituation

To test whether the rats had a preference for the striped or solid compartment, the
time spent in each of the large compartments during the habituation sessions for FF and
FR rats was analyzed. An independent *t*-test revealed no significant difference between
the time spent in the striped and solid compartments for FF [*t*(62)=−1.7, *p*>.05] or FR
[*t*(62)=−1.5, *p*>.05] rats (see Figure 2).

\[\text{Figure 2}. \text{Time (s) spent in each compartment (striped, solid, and tunnel) of the} \]
\[\text{conditioned place preference (CPP) apparatus during habituation sessions for A) free fed} \]
\[\text{(FF) and B) food restricted (FR) rats. There were two, 30-min habituation sessions, one} \]
\[\text{in the morning and one in the afternoon. Each rat underwent the first habituation session} \]
\[\text{in the morning and the second in the afternoon. Data are presented as the mean (+SEM)} \]
\[\text{time spent in each compartment over the two habituation sessions.} \]

3.3.2 Conditioned Place Preference: Conditioning

As shown in Figure 3, FF rats drank significantly more sucrose than water when
they had access to 15% [*t*(8)=2.6, *p*<.05] and 30% [*t*(13)=3.4, *p*<.05] sucrose during
conditioning. In contrast, consumption of sucrose and tap water were not significantly
different when FF rats had access to 1%, $[t(12) = 1.2, p > .05]$ or 5% $[t(9) = 1.8, p > .05]$ sucrose during conditioning. Rats that were FR drank significantly more sucrose in the 5% $[t(8) = 5.8, p < .001]$, 15% $[t(8) = 8.6, p < .001]$, and 30%, $[t(8) = 5.6, p < .001]$ conditions, but not in the 1% $[t(14) = 1.5, p > .05]$ condition. Levene’s test indicated unequal variances for solution consumption in FF $[F(7, 56) = 5.0, p < .001]$ and FR $[F(7, 56) = 4.1, p < .001]$ rats.

![Figure 3](image)

**Figure 3.** Solution consumption during conditioned place preference (CPP) conditioning sessions for rats that were A) free fed (FF) or B) food restricted (FR). Data are presented as the mean (+SEM) solution consumed (per body weight) over five 30-min sessions. Separate groups of rats had access to 1%, 5%, 15%, or 30% sucrose solution in one compartment and tap water in the other compartment.

### 3.3.3 Conditioned Place Preference: Testing

During CPP testing, FF rats did not spend significantly more time in the sucrose-paired, compared to the water-paired, compartment following conditioning with 1% $[t(56) = -0.07, p > .05]$, 5% $[t(56) = 0.756, p > .05]$, 15% $[t(56) = 1.10, p > .05]$, or 30% $[t(56) = 1.24, p > .05]$ sucrose. Similarly, there was no significant difference in time spent in the sucrose- and water-paired compartments for FR rats following conditioning with
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1% \([t(52)= 1.02, p>.05]\), 5% \([t(52)= .909, p>.05]\), or 30% \([t(52)= 1.37, p>.05]\) sucrose. However, FR rats that had access to 15% sucrose during conditioning spent significantly more time in the sucrose-paired compartment during testing \([t(52)= 2.15, p<.05]\) (see Figure 4). Two FR rats, each from the 30% sucrose condition, were eliminated from analysis because one of the compartment doors was left closed during the testing session.

Figure 4. Conditioned place preference (CPP) to sucrose in A) free fed (FF) and B) food restricted (FR) rats. Data are presented as the mean (+SEM) time spent in sucrose- and water-paired compartments over a 30-min test session. Separate groups of rats had access to 1%, 5%, 15%, or 30% sucrose solution in one compartment and tap water in the other compartment during conditioning sessions. Rats did not have access to water or sucrose solution during testing.

3.3.4 Conditioned Place Preference: State-Dependent Testing

Figure 5 shows the results of the state-dependent test for FF and FR rats. There were no significant differences in time spent in the sucrose-paired and water-paired compartments during the 30-min state-dependent test session for FF rats in the 1% \([t(56)=-.30, p>.05]\), 5% \([t(56)=-.80, p>.05]\), 15% \([t(56)=-.52, p>.05]\), or 30% \([t(56)=-1.0, p>.05]\) concentration conditions. Nor were there any significant differences between time spent in the sucrose- and water-paired compartments during the state-dependent test for
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FR rats in the 1% [t(56)=-.606, p>.05], 5% [t(56)=.270, p>.05], 15% [t(56)=.499, p>.05],
or 30% [t(56)=-.715, p>.05] concentration conditions.

Figure 5. State-dependent conditioned place preference (CPP) to sucrose in A) free fed (FF) and B) food restricted (FR) rats. Data are presented as the mean (+SEM) time spent in sucrose- and water-paired compartments over a 30-min state-dependent test session. Separate groups of rats had access to 1%, 5%, 15%, or 30% sucrose solution in one compartment and tap water in the other compartment during conditioning sessions. Rats had a 30-minute access period to 1%, 5%, 15%, or 30% sucrose solution immediately before the 30-minute test session, but did not have access to water or sucrose solution during testing.

3.3.5 Conditioned Place Preference: Time Course

The time course of CPP testing for FF and FR rats is shown in Figures 6 and 7 respectively. During the 30-min test, the mean difference in time spent in the sucrose- and water-paired compartments was greatest at 30 min for all concentrations of sucrose in both FF and FR rats. For this reason, all of the following test sessions were run for 30 min.
Figure 6. Conditioned place preference (CPP) to sucrose across time in free fed (FF) rats for the A) 1%, B) 5%, C) 15%, and D) 30% sucrose concentration. Data are presented as the mean (+SEM) time spent in sucrose- and water-paired compartments at five-min bins over a 30-min test session. Separate groups of rats had access to different concentrations of sucrose solution in one compartment and tap water in the other compartment during conditioning sessions.
Figure 7. Conditioned place preference (CPP) to sucrose across time in food restricted (FR) rats for the A) 1%, B) 5%, C) 15%, and D) 30% sucrose concentration. Data are presented as the mean (+SEM) time spent in sucrose- and water-paired compartments at five-min bins over a 30-min test session. Separate groups of rats had access to different concentrations of sucrose solution in one compartment and tap water in the other compartment during conditioning sessions.

3.3.6 Tactile Cues

During the two habituation sessions, rats did not differ in time spent between the solid and striped wall types, \([F(1, 14)=3.9, p>.05]\). Rats did spend significantly more time in the striated compared to the bumpy floor type \([F(1, 14)=4.9, p<.05]\) (see Figure 8). When the wall and floor type were combined, there was no significant difference in time spent between the water- and sucrose-paired compartment during habituation \([F(1, 14)=0.006, p>.05]\) (see Figure 9).
Figure 8. Time (s) spent in each A) wall type B) floor texture and C) sucrose- versus water- paired side of the CPP apparatus during habituation sessions for food restricted (FR) rats. There were two, 30-min habituation sessions, one in the morning and one in the afternoon. Each rat underwent the first habituation session in the morning and the second in the afternoon. Data are presented as the mean (+SEM) time spent in each compartment over the two habituation sessions.

As in the previous experiment, rats drank significantly more sucrose than water during conditioning sessions in an experiment with modified tactile cues \[t(7)=20, p<.0001\] (see Figure 9).
Rats also spent significantly more time in the sucrose-paired, compared to the water-paired, compartment during testing \([t(14)=-3.18, p<.01]\) (see Figure 10). A comparison of the size of the CPP to 15% sucrose in FR rats across experiments showed that the addition of the tactile cue did not significantly increase the size of the CPP: mean difference scores (sucrose-paired versus water-paired compartments) with no tactile cue \((M=243.1, \text{SEM}=178)\) and with tactile cues \((M=171, \text{SEM}=65.6)\) \([t(9)>.05]\). Levene’s test indicated unequal variances for mean difference scores \([F(7)=.38, p<.05]\).
3.4 Discussion

This experiment verified that rats exhibit a CPP to a sucrose solution (Delamater et al., 2000) and that 15% sucrose was the most effective concentration that we tested. We also confirmed our hypothesis that sucrose is more rewarding when animals are FR. Indeed, none of the FF groups exhibited a CPP suggesting that the solution is not rewarding when animals are sated. The problem with this interpretation is that the FF group drank significantly more sucrose than water during conditioning, with the exception of the 1% and 5% sucrose solutions, while the FR group drank significantly more sucrose than water with the exception of the 1% sucrose solution. Rats can discriminate between sucrose and water at a concentration of .45% when satiated, and .15% when food deprived and food deprived animals find lower sucrose concentrations more reinforcing than satiated animals (Campbell & Campbell, 1958). This experiment confirms that animals can discriminate water from sucrose and that they prefer the latter, at least at these higher concentrations.

Although both FF and FR groups drank more sucrose than water, the greatest contrast between sucrose consumption and water consumption was in the FR, 15% sucrose group, which was the only group that developed a CPP. Perhaps the 15% sucrose concentration was rewarding enough to produce a CPP whereas the other concentrations weren’t, even though they were still preferred over water. Another possibility is that sucrose consumption and CPP are measures of different components of reward. Discrepancies between solution consumption and CPP, and self-administration and CPP magnitude, have been observed in other studies. For example, rats drink as much
saccharin as sucrose during conditioning, but fail to develop a CPP to saccharin (White & Carr, 1985), low doses of opioid antagonists decrease sucrose consumption but do not attenuate sucrose CPP (Ågmo et al., 1995), and the magnitude of amphetamine- and cocaine-induced CPP are not correlated with self administration of these substances (Bardo & Bevins, 2000). Neurobiological evidence for the discrimination between self-administration and CPP exists as well. D2 antagonists injected systemically and into the NAc do not alter cocaine-induced CPP but do prevent cocaine self-administration (Bardo & Bevins, 2000). Incentive salience (wanting), which is one of the processes that mediate a CPP (Huston, Silva, Topic, & Müller, 2013), is enhanced by food deprivation, whereas food deprivation does not affect hedonic pleasure derived from food (Epstein, Truesdale, Wojcik, Paluch, & Raynor, 2003). The consumption of sucrose may therefore rely on hedonic pleasure (liking), or some other process whereas a CPP is mediated, at least partially, by incentive salience (wanting).

By analyzing the time course of the CPP, we observed that animals spent increasing amounts of time in the sucrose-paired compartment over testing. Rather than extinguishing, the CPP grew across testing. Although we did not test the possibility, the size of the CPP may continue to increase with a longer testing period. Given that 20-30 minutes are standard test lengths in drug CPP studies (Bardo & Bevins, 2000), and because the majority of studies examining sucrose-induced CPP used short test sessions (Delamater et al., 2000; Jarosz, Sekhon, & Coscina, 2006), we terminated the test after 30 minutes.

None of the groups exhibited a CPP during state-dependent testing, suggesting that the lack of a CPP during the first test does not reflect deficits in memory retrieval.
The state-dependent test was conducted after the reinforcer-free test so the lack of a CPP in this test could reflect extinction (i.e., the sucrose-paired cues loses the ability to elicit a preference). This seems unlikely because the size of the CPP was still large at the end of the first test. Another interpretation for state-dependency is that the relative novelty of each compartment is dependent on the state of the animal (Bardo & Bevins, 2000). For example, when the animal is under the influence of a drug, the compartment that was previously paired with saline would be novel, whereas when the animal is not under the influence of the drug, the compartment that was previously paired with the drug is more novel. Rats spend more time in novel environments (Bardo & Bevins, 2000), so this might have an effect on the time spent in each compartment. Since rats did not spend significantly more time in the water-paired compartment during state-dependent testing, it is unlikely that relative novelty related to the animal’s state affected the results of the first CPP test.

Chapter 4: Experiment 2
Effects of Sucrose Bingeing on Conditioned Place Preference to Sucrose in Females

4.1 Introduction

Under conditions of restricted access to highly PF, rats develop binge eating, a behaviour that is prevalent among individuals suffering from eating disorders and obesity. In humans and animals, binge eating is defined as consuming a large amount of food within a discrete period of time (e.g., 1-2 hours). The prevalence of BED among females and males is 3.5% and 2% respectively (Hudson et al., 2007). Because the
prevalence of binge eating is higher among females, female rats were used in this experiment. Sucrose bingeing models do not lead to weight gain in animal models (Avena, Rada, & Hoebel, 2008), whereas fat- and sucrose/fat- bingeing models often do (Avena et al., 2009; Johnson & Kenny, 2010). Thus, binge eating can be explored separately from weight gain in sucrose-bingeing models. This is important when the reward value of food is examined since the development of obesity is also associated with behavioural and neurobiological changes in this system (Volkow et al., 2011).

Similar to substance abuse, relapse rates are high among eating disorder patients and a history of binge eating increases this risk even further (Brandon, Vidrine, & Litvin, 2007; Carter et al., 2012). Behavioural and neurobiological studies indicate that sucrose-bingeing resembles substance abuse, at least in animal models. For example, rats escalate their daily sucrose intake over time, display somatic signs of withdrawal following the administration of an opiate antagonist, exhibit behavioural signs of craving and increased motivation for sucrose following abstinence, as well as sensitization in the DA reward system (Avena, 2010).

Obesity is associated with differences in perceived and actual rewarding value of food. For example, obese individuals ascribe a higher reward value and show greater activation within the mesolimbic DA reward system when presented with images of food compared to controls, but also show reduced activation during consumption of these foods (Volkow et al., 2011). Similarly, following extended access to a highly PF diet and the development of binge eating, rats show an increased reward threshold in the brain stimulation paradigm. This is an indication of a blunted reward system that is associated with the development of obesity, extended access to PF, or both (Johnson & Kenny,
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2010). Rats also show a CPP to PF under conditions of FR (Jarosz et al., 2006), a finding that was replicated in Experiment 1. The current experiment aims to examine the changes in the rewarding value of sucrose following sucrose bingeing. In the sucrose bingeing paradigm, rats develop binge eating but do not gain weight, so that changes in the reward value of sucrose can be examined independently of weight gain. Furthermore, the 24hr sucrose control group has extended access to sucrose so that the effect of binge eating and extended access to PF on the rewarding effect of sucrose can also be evaluated independently.

4.2 Materials and Methods

Thirty-two female Long-Evans rats (n=8 per group) were assigned to one of four groups: 12hr sucrose, 24hr sucrose, 12hr saccharin, or 12hr food only. In contrast to predictions, the 12r sucrose group did not develop bingeing behaviour over the 28-day sucrose consumption phase. Thus, a second group of rats (n=8) were tested under the same conditions (i.e., 12hr access to sucrose over 28 days). The first, non-bingeing 12hr sucrose group will be referred to as 12hr- no binge (12hr- NB) sucrose and the second 12hr sucrose group, which did develop binge intake of sucrose, will be referred to as 12hr-binge (12hr-B) sucrose. The data from these two groups were analyzed separately. All rats underwent a 28-day sucrose consumption phase followed by CPP conditioning, as described in the General Methods. Based on the previous experiment, a 15% concentration of sucrose was used in the CPP conditioning phase.

4.3 Results

4.3.1. Body Weight

Analysis of the data across the sucrose consumption phase revealed that body
weights changed across the 28 days of these sessions in the 12hr-NB sucrose ($p<.05$),
24hr sucrose ($p<.05$), 12hr saccharin ($p<.05$), and 12hr food only ($p<.05$) groups, but not
the 12hr-B sucrose ($p>.05$) group. Pairwise comparisons revealed that body weight did
not differ significantly between groups on day 28 ($p<.05$) (see Figure 11).

![Figure 11. Body weight across sucrose consumptions sessions for rats given different
access to sucrose, saccharin, or food only. Data are presented as mean (+SEM) body
weight (g) for each group across the 28-day cycle. Body weight was measured 12 hours
following the presentation of sucrose, saccharin, or food on each day; measurements were
taken at the same time point for rats given 24 hour access to sucrose. B= binge; NB= no
binge.]

4.3.2. Sucrose Consumption

Figure 12 shows sucrose consumption of each group during the first hour
following presentation of sucrose across 28 days. ANOVA revealed that solution intake
at 1hr changed significantly across days [$F(7, 201)=2.6, p<.05$] with significant group
differences in this measure [$F(3, 28)= 43, p<.05$] and a significant group by day
interaction [$F(22, 201)=1.8, p=.05$]. Solution intake increased over time for all groups,
with the exception of the 12hr saccharin group. Pairwise comparisons between days 1, 2,
3, 4, 25, 26, 27, and 28 revealed that solution consumption increased between days 1 and
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28 for all groups, except the 12hr saccharin group. Rats in the 12hr-NB, and 12hr-B sucrose groups consumed significantly more solution on days 25, 26, 27, and 28 compared to day 1 ($p<.05$) and the 24hr sucrose group consumed significantly more solution on days 25, 26, and 28 compared to day 1 ($p<.05$). Unlike the sucrose groups, there was no significant difference in solution consumption for the saccharin groups between days 25, 26, 27, or 28 and day 1 ($p>.05$).

There were no significant differences in 1hr solution consumption between groups on day 1, except that the 12hr-B sucrose group consumed significantly more solution compared to the 12hr saccharin group ($p<.05$). On day 28, all of the sucrose groups (12hr-B, 12hr- NB, and 24hr) consumed significantly more solution compared to the 12hr saccharin group ($p<.01$), and the 12hr-B sucrose group consumed significantly more solution compared to all of the other groups (12hr-NB sucrose, 24hr sucrose, and 12hr saccharin) ($p<.01$). The 12hr-B sucrose group drank significantly more solution compared to the 12hr saccharin group on all the days analyzed, including days 1, 2, 3, 4, 25, 26, 27, and 28.
Figure 12. Solution consumption during the first hour of access across sucrose consumption sessions for rats given different access to sucrose, saccharin, or food only. Graphs represent A) 12hr-B sucrose, B) 12hr-NB sucrose, C) 24hr sucrose, and D) 12hr saccharin groups. Graph E shows the comparison between all groups for 1 hour solution consumption. Data points represent mean (+SEM) solution consumed (mL/per g body weight) on each day of the 28-day cycle. B= binge; NB= no binge.

The 24- hour solution consumption for each group across the 28-day cycle is shown in Figure 13. As with the 1 hour intake measure, solution consumption over 24 hours increased across sessions $[F(10, 280)=5.0, p<.001]$, with a significant difference
between groups \( [F(3, 28)=52, p<.001] \). There was also a significant day by group interaction \( [F(30, 280)=3.0, p<.001] \) due to the fact that both 12hr-B and 12hr-NB sucrose groups increased solution consumption over the 28 days (pairwise comparison). Both groups consumed significantly more solution on days 3, 4, 25, 26, 27, and 28 compared to day 1 \((p<.05)\), and the 12hr-B sucrose group also consumed significantly more solution on day 2 compared to day 1 \((p<.01)\). This in contrast to the 12hr-NB sucrose group, whose solution intake was much more variable in the days following the initial increase in consumption (from day 1 to 2). Neither the 24hr sucrose nor the 12hr saccharin group consumed significantly more solution on day 28 compared to day 1 \((p>.05)\).

Pairwise comparisons of groups on day 1 revealed that the 24hr sucrose group consumed significantly more solution compared to all of the other groups (12hr-B, 12hr-NB, and 12hr saccharin) \((p<.05)\) and the two 12hr sucrose groups (12hr-B and 12hr-NB) consumed significantly more solution compared to the 12hr saccharin group \((p<.05)\). The 12hr-B and 12hr-NB sucrose groups did not differ significantly from one another on this measure \((p>.05)\). On day 28, all groups consumed significantly more solution compared to the 12hr saccharin group \((p<.01)\). The 12hr-B and 24hr sucrose groups also consumed significantly more solution compared to the 12hr-NB sucrose group \((p<.05)\), and the 12hr-B and 24hr sucrose groups did not differ significantly from one another \((p>.05)\).
Figure 13. Solution consumption in each 24-hour period across sucrose consumption sessions for rats given different access to sucrose, saccharin, or food only. Graphs represent A) 12hr-B sucrose, B) 12hr-NB sucrose, C) 24hr sucrose, and D) 12hr saccharin groups. Graph E shows the comparison between all groups for 24hr solution consumption. Data points represent mean (+SEM) solution consumed (mL/per g body weight) on each day of the 28-day cycle. B= binge; NB= no binge.

There was a significant difference in regular chow intake, measured at 24hr intervals, across the 28 day binge cycle \([F(10, 339) = 11.5, p < .001]\), with significant
group differences on this measure \( F(4, 35)= 74, p<.001 \) and a significant day by group interaction \( F(39, 339)=1.7, p<.01 \). Pairwise comparisons revealed no significant difference between day 1 and day 28 in 24hr chow consumption in the 24hr sucrose group \( (p>.05) \). In contrast, the 12hr-NB sucrose group consumed more chow at the beginning of the binge cycle compared to the end, with rats consuming significantly more chow on each of the first four days (1, 2, 3, and 4) compared to each of the last four days (25, 26, 27, and 28) of the 28-day cycle \( (p<.05) \). In the 12hr-B group, rats consumed significantly more chow on days 2, 3, and 4, compared to days 27 and 28 \( (p<.05) \), also demonstrating that they consumed more chow at the beginning of the 28-day cycle compared to the end. There was no significant difference in chow consumption between days 1 and 28 \( (p>.05) \) in the 12hr saccharin group. In the 12hr food only group, rats consumed significantly more chow on day 1 compared to all other days (2, 3, 4, 25, 26, 27, and 28) \( (p<.05) \).

On day 1, there was no significant difference in chow consumption between any of the sucrose groups (12hr-B, 12hr-NB, 24hr) \( (p>.05) \). The 12hr food only group consumed significantly more chow compared to all of the sucrose groups (12hr-B, 12hr-NB, 24hr) \( (p<.05) \) and the 12hr saccharin group consumed significantly more chow compared to the 12hr-B and 24hr sucrose groups \( (p<.01) \). The 12hr saccharin and 12hr food only groups consistently consumed significantly more chow compared to the 12hr-B sucrose group on all of the days analyzed (1, 2, 3, 4, 25, 26, 27, and 28) \( (p<.05) \). On day 28, the 12hr saccharin and 12hr food only groups consumed significantly more chow compared to all of the sucrose groups (12hr-B, 12hr- NB, 24hr) \( (p<.001) \), and did not differ significantly from one another \( (p>.05) \). The sucrose groups (12hr-B, 12hr- NB,
24hr) did not differ significantly from one another on any of the days (\(p>.05\)), except for days 4 and 27, where the 12hr-NB sucrose group consumed significantly more chow compared to the 12hr-B sucrose group (\(p<.05\)) (see Figure 14).

\[\text{Figure 14. Food intake in each 24-hour period across sucrose consumption sessions for rats given different access to sucrose, saccharin, or food only. Data points represent mean (+SEM) chow consumed (g/g body weight) on each day of the 28-day cycle. B= binge; NB= no binge.}\]

4.3.3 Conditioned Place Preference

As shown in Figure 15, all rats, including those in the 12hr-B sucrose \([t(9)=15, p<.001]\), 12hr-NB sucrose \([t(8)=9, p<.001]\), 24hr sucrose \([t(8)=7 p<.001]\), 12hr saccharin, \([t(7)=6, p<.001]\), and 12hr food only \([t(7)=7, p<.001]\) groups consumed significantly more sucrose compared to water over conditioning sessions. Levene’s test indicated unequal variances for solution consumption \([F(9, 70)= 8.1, p<.001]\).
Figure 15. Solution consumption during conditioned place preference (CPP) conditioning sessions for rats given different access to sucrose, saccharin, or food only. All rats were food restricted (FR) following the 28-day sucrose consumption phase. Rats had access to 15% sucrose solution in one compartment and tap water in the other during conditioning. Data are presented as the mean (+SEM) solution consumed (per body weight) over five 30-min sessions. B= binge; NB= no binge.

Planned comparisons revealed that there was no significant difference in time spent between the sucrose-paired and water-paired compartments during the 30-minute test session for the 12hr-B sucrose [t(14)=.04, p>.05], 12hr-NB sucrose [t(13)=1, p>.05], 24hr sucrose [t(12)=−1, p>.05], 12hr saccharin [t(14)=.8, p>.05], or the 12hr food only [t(10)=.08, p>.05] groups (see Figure 16). Levene’s test indicated unequal variances for solution consumption [F(9,70)= 2.6, p<.05].
Figure 16. Conditioned place preference (CPP) to sucrose for rats given different access to sucrose, saccharin, or food only during sucrose consumptions sessions. Data are presented as the mean (+SEM) time spent in sucrose- and water-paired compartments over a 30-min test session. All rats were food restricted (FR) following the 28-day sucrose consumption phase. Rats had access to 15% sucrose solution in one compartment and tap water in the other during conditioning, with no solution available during testing. B= binge; NB= no binge.

4.4 Discussion

Surprisingly, the first group of rats given 12-hour access to sucrose (12hr-NB sucrose) did not develop binge intake over the 28-day sessions. This contrasts with previous findings from our lab and from other labs. A possible reason for this discrepancy is that the lights were being turned on during the rats active cycle, and rats were being disturbed during the first hour of intake. Because the sucrose bingeing model is well validated, the same procedure was used on a second group of rats (12hr-B sucrose) with a modified time schedule (lights were not turned on during the active cycle) and these rats did develop binge eating. The 12hr-B sucrose group drank significantly more sucrose in the first hour of access on day 28, compared to all of the other groups, including the 12hr-NB sucrose, 24hr sucrose, and 12hr saccharin groups, which is
indicative of binge eating.

Despite developing binge eating, rats given 12-hour access to sucrose did not display a CPP to sucrose when tested at a later time. As with the first experiment, rats consumed more sucrose during conditioning sessions, but this was not associated with a CPP to 15% sucrose. Interestingly, the 24hr sucrose group spent more time in the water-paired than the sucrose-paired compartment, whereas all of the other groups spent either more time in the sucrose-paired compartment or approximately equal amounts of time in the sucrose-paired and water-paired compartments. Previous research has shown that extended access to a diet high in fat and sucrose, which leads to the development of binge eating and obesity, results in increased LH reward thresholds, a measure of reward sensitivity and evidence of a deficit in reward processing (Johnson & Kenny, 2010). It is possible that extended access to sucrose in our experiment led to a deficit in reward processing. This would explain why the sucrose groups (12- and 24-hour access) did not develop a CPP, although this would not account for the lack of CPP in the food only or saccharin groups.

An alternative explanation for the lack of CPP effect in the 24hr and 12hr sucrose groups is that rats developed an association between sucrose intake and environmental cues in their home cages during the 28-day consumption phase. These groups may then have had difficulty associating cues in the CPP compartment with sucrose consumption, even if they found the solution rewarding. Again, this does not explain why the food only group or the 12hr saccharin group did not show a CPP to sucrose.

A third reason that animals may not have developed a CPP to sucrose is that the sucrose consumption sessions produced stress that interfered with reward systems. In all
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groups except the 24-hour sucrose group, food and sucrose solution were removed every 12 hours. This procedure induces stress in animals, which may alter functioning of the mesolimbic DA system. If so, it may alter the ability to form a CPP to sucrose. This could explain why none of the 12hr access groups showed a CPP to sucrose, although it doesn’t explain why animals given consistent access to food and sucrose solution (24hr sucrose) did not show a CPP to sucrose.

Finally, a major limitation to this study is that the rats began to chew the texturized floors during CPP conditioning and testing days. It is possible that part of the time spent in the compartments is attributable to the time they spent chewing as opposed to the rewarding value of sucrose. In other words, the chewing behaviour may have disrupted the expression of a CPP during testing.

Chapter 5: Experiment 3

Effects of Sucrose Bingeing on Morphine Conditioned Place Preference in Females

5.1 Introduction

Similar neurochemical mechanisms, including opioid and DA transmission within the mesolimbic DA reward pathway, mediate reward for PF and drugs of abuse (Smith & Robbins, 2013). Novel food increases extracellular DA in the dorsal striatum and NAc, but this effect diminishes upon subsequent exposure to the same food (Avena, Rada, et al., 2008; Volkow et al., 2011). In contrast, repeated exposure to drugs of abuse continually increase extracellular DA in this region. Following the development of binge eating, PF continually increases extracellular DA (Avena et al., 2009), providing evidence for neuroadaptations in DA transmission within the mesolimbic DA reward
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pathway in response to extended access to PF. Decreased D2 receptor availability is also observed following the development of obesity and substance abuse, however it is unclear whether this is a risk factor for obesity and substance abuse, or an effect of extended access to a drug or PF (Berridge, 2009; Smith & Robbins, 2013).

Opioid signalling is also an important mediator of food reward acting in a number of neural ‘hotspots’ of reward, including the NAc shell, ventral pallidum, and lateral amygdala (Volkow et al., 2011). Opioid agonists increase, whereas opioid antagonists decrease, PF consumption by altering the hedonic pleasure (liking) derived form PF (Olszewski & Levine, 2007). As with DA, neuroadaptations of opioid receptors is evident following extended access to sucrose, including an upregulation of µ-opioid receptors in several brain regions, one of which is the NAc (Colantuoni et al., 2001; Gosnell & Levine, 2009). In addition, extended access to sucrose alters opiate-mediated decreases in pain thresholds (Roane & Martin, 1990) and includes opiate-like withdrawal symptoms (Avena, Rada, et al., 2008), providing further evidence for the role of opioids in food reward.

It is important to note that the effects of PF on neurochemical systems are smaller in magnitude than those induced by drugs of abuse (Avena, Rada, et al., 2008), yet these similarities suggest that substance abuse and binge eating share similar underlying mechanisms. Cross-sensitization between drugs of abuse is one way to examine the similar effects of different drugs on a neurochemical system. Cross-sensitization between extended access to PF and amphetamine was shown when animals fed a high fat diet failed to show a CPP to amphetamine (Davis et al., 2008). Similar to PF, the reinforcing effects of morphine are at least partially mediated through the activation of µ-opioid
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receptors (Blumenthal & Gold, 2010). For this reason, the effect of sucrose bingeing on morphine will be examined in the following experiment.

In previous experiments, female rats that binged on sucrose failed to develop a CPP to sucrose, and this could be explained by a dysregulation in the reward system caused by sucrose bingeing. An alternative explanation is that access to sucrose in the home cage (i.e., during the sucrose consumption phase) disrupted the ability to form sucrose-environmental cue associations during CPP conditioning. This experiment was designed to distinguish between these two possibilities. I tested whether sucrose bingeing alters the development of a CPP to morphine. If sucrose bingeing produces a dysregulation in reward systems, rats should not show a CPP to morphine, but if it disrupts the formation of sucrose-cue associations, rats should show a CPP to morphine. I used a dose of morphine (6 mg/kg) that produced a CPP in preliminary studies. I also limited the study to animals given 12 or 24 hours access to sucrose because the other two groups (12 hour saccharin and food only) would both be expected to show a CPP to morphine.

5.2 Materials and Methods

Sixteen rats were assigned to one of two groups (n=8 per group): 12hr sucrose or 24hr sucrose. All rats underwent the 28-day sucrose consumption phase followed by CPP conditioning to morphine, testing, and state- dependent testing. Rats underwent one, 30-minute conditioning session per day for 10 consecutive days. Rats received IP injections of either morphine (6mg/kg) or saline (6ml/kg) on alternating days, so that each rat was conditioned to morphine and saline five times. IP injections were administered immediately prior to the conditioning sessions and state-dependent test.
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The assignment of conditioning compartment and order of drug injection were counterbalanced within groups.

5.3 Results

5.3.1 Body Weight

Analysis of the data across the sucrose consumption phase revealed that body weights changed across the 28 days of these sessions \( F(2, 28)=81, p<.001 \). There was also a significant group effect \( F(1, 14)=14, p<.01 \), but no significant day by group interaction \( F(2, 28)=1.3, p>.05 \) (see Figure 17).

![Figure 17. Body weight across sucrose consumptions sessions for rats given different access to sucrose. Data are presented as mean (+SEM) body weight (g) for each group across the 28-day sucrose consumption phase. Body weight was measured 12 hours following the presentation of sucrose for the 12 hour group; measurements were taken at the same time point for rats given 24 hour access to sucrose. B= binge; NB= no binge.](image)

5.3.2 Sucrose Consumption

Analysis of solution consumption during the first hour of intake revealed increased intake across the 28 days \( F(3, 44)=4.4, p<.01 \), with a significant difference between groups \( F(1, 14)=20, p<.01 \), but no significant interaction \( F(3, 44)=1.2, p>.05 \). Pairwise comparisons between days 1, 2, 3, 4, 25, 26, 27, and 28 revealed that the 12hr sucrose group drank significantly more solution in the first hour than the 24hr sucrose group on days 1, 25, and 28 \( p<.05 \). In addition, the 24hr sucrose group drank
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significantly more solution during the first hour of intake on days 2, 3, 4, and 28 compared to day 1 \(p<.05\), and the 12hr sucrose group drank significantly more solution in the first hour of intake on day 2 compared to day 1 \(p<.05\), and on day 28 compared to days 1, 2, 3, 4, 25, and 27 \(p<.05\) (see Figure 18).

Analysis of 24hr solution consumption revealed a significant difference across 28-days \([F(7, 97)=5.5, p<.001]\), no significant group effect \([F(1, 14)= 3.5, p>.05]\), and a significant day by group interaction \([F(7, 97)=2.3, p<.05]\). Pairwise comparisons between days 1, 2, 3, 4, 25, 26, 27, and 28 revealed that the 24hr sucrose group drank significantly more solution within 24hrs on days 2, 3, 4, and 28 compared to the 12hr sucrose group \(p<.05\). The 12hr and 24hr sucrose groups drank a comparable amount of solution on all other days analyzed. The 24hr sucrose group drank significantly more solution on all of the days (days 2, 3, 4, 25, 26, 27, and 28) compared to day 1 \(p<.05\). The 12hr sucrose group also drank significantly more solution on days 4, 25, 26, 27, and 28 compared to day 1 \(p<.05\) (see Figure 19).
Figure 19. Solution consumption in each 24-hour period across sucrose consumption sessions for rats given 12 or 24 hours access to sucrose across 28 days. Data points represent mean (+SEM) solution consumed (mL/per g body weight) on each day of the 28-day sucrose consumption phase.

Analysis of food intake revealed that 24hr chow intake changed across 28-days \([F(5, 67)=4.6, p<.01]\). There was no significant difference between groups \([F(1, 14)=2.8, p>.05]\) on this measure, although there was a significant day by group interaction \([F(5, 67)=2.9, p<.05]\). Pairwise comparisons between days 1, 2, 3, 4, 25, 26, 27, and 28 revealed that the chow intake did not differ significantly between the 24hr sucrose and 12hr sucrose groups on any days except on day 1, when the 24hr sucrose group consumed significantly more chow compared to the 12hr sucrose group \((p<.05)\), and on day 4, when the 12hr sucrose group consumed significantly more chow compared to the 24hr sucrose group \((p<.05)\). Chow consumption was significantly greater on days 1, 2, and 4 compared to days 25, 26, 27, and 28 \((p<.05)\). Similarly, the 12hr sucrose group consumed significantly more chow on day 2 compared to day 26 \((p<.05)\), and 27 \((p<.05)\), on day 3 compared to 26 \((p<.05)\) and on day 4 compared to days 26, 27, and 28 \((p<.05)\) (see Figure 20).
Figure 20. Food intake in each 24-hour period across sucrose consumption sessions for rats given 12 or 24 hours access to sucrose across 28 days. Data points represent mean (+SEM) chow consumed (g/kg body weight) on each day of the 28-day sucrose consumption phase.

5.3.3 Conditioned Place Preference

Planned comparisons revealed that both the 12hr sucrose \([t(26)= 5.9, p<.001]\) and the 24hr sucrose \([t(26)= 3.2, p<.01]\) groups spent significantly more time in the morphine-paired compartment than in the saline-paired compartment on test day (see Figure 21). There was no significant difference in the magnitude of the CPP in the 12hr and 24hr sucrose groups \([t(13)= 1.4, p>.05]\) (see Figure 22).

Figure 21. Conditioned place preference (CPP) to morphine for rats given 12 or 24 hours access to sucrose across 28 days. Data are presented as the mean (+SEM) time spent in saline- and morphine-paired compartments over a 30-min test session. All rats were food restricted (FR) following the 28-day sucrose consumption phase. Rats were administered
an intraperitoneal (IP) injection of morphine (6mg/kg) before being placed in one compartment and saline (6mL/kg) before being placed in the other during conditioning. All rats were drug-free during testing.

Figure 22. Magnitude of the Conditioned Place Preference (CPP) to morphine for rats given 12 or 24 hours access to sucrose across 28 days. Data are presented as the difference (+SEM) between times spent in the morphine- and saline- paired sides for the 12hr and 24hr sucrose groups over a 30-minute test session. All rats were food restricted (FR) following the 28-day sucrose consumption phase. Rats were administered an intraperitoneal (IP) injection of morphine (6mg/kg) before being placed in one compartment and saline (6mL/kg) before being placed in the other during conditioning. All rats were drug-free during testing.

Planned comparisons revealed that, on state-dependent test day, the 12hr sucrose group spent significantly more time in the morphine- than the saline-paired compartment \([t(26)=4.9, p<.001]\) but there was no significant difference between time spent in the morphine- and saline-paired compartments for the 24hr sucrose group \([t(26)=.57, p>.05]\) (see Figure 23).
Figure 23. State-dependent conditioned place preference (CPP) to morphine for rats given 12 or 24 hours access to sucrose across 28 days. Data are presented as the mean (+SEM) time spent in saline- and morphine-paired compartments over a 30-min test session. All rats were food restricted (FR) following the 28-day sucrose consumption phase. Rats were administered an intraperitoneal (IP) injection of morphine (6mg/kg) before being placed in one compartment and saline (6mL/kg) before being placed in the other during conditioning. All rats were administered an IP injection of morphine (6mg/kg) prior to testing.

5.4. Discussion

It is unclear whether sucrose bingeing was established in the 12hr sucrose group because these animals did not consistently consume significantly more solution during the first hour of access on the last few days of the consumption phase. Although animals in this group drank a comparable amount of solution in 12 hours as the 24hr sucrose group, sucrose bingeing is usually defined as increased intake within the first one to two hours of access. Notably, both the 12hr and 24hr sucrose groups showed a decrease in consumption of chow across the 28-day sucrose consumption phase, a sign of caloric compensation that is common in sucrose bingeing rats (Avena et al., 2009).

Based on the findings of the previous experiment, I hypothesized that sucrose bingeing and extended access to sucrose would result in a reward deficit, evidenced by a lack of CPP to morphine. Contrary to this prediction, both sucrose groups (12hr and 24hr) showed a CPP to morphine following the 28-day sucrose consumption phase. This
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suggests that neither extended access nor sucrose bingeing reduces the reinforcing effects of morphine. Extended access to sucrose and/or sucrose bingeing could result in the sensitization of the reward system, although there was no non-sucrose control group in this experiment, so it is difficult to know if this is the case. This would lend further evidence to the similarities of sucrose bingeing and opiate reward since extended access to morphine lowers the threshold for LH BSR (Esposito & Kornetsky, 1977) and increases the reinforcing efficacy of morphine (Bie et al., 2012; Gaiardi et al., 1991).

There was no significant difference in the magnitude of the CPP to morphine between the 12hr and 24hr sucrose but the 12hr sucrose group continued to show a CPP to morphine on state-dependent test day, whereas the 24hr sucrose group did not. This may reflect the fact that the CPP to morphine was extinguished in the 24hr sucrose, but not the 12hr sucrose, group since both groups received a 30-minute test session in a drug free state the day before state-dependent testing. Extinction of CPP occurs when animals experience the environmental cues in the absence of the reinforcer (i.e., drug-free or sucrose-free testing). For example, repeated CPP testing weakens and eventually extinguishes a CPP to morphine (Wang, Luo, Zhang, & Han, 2000). These results indicate that the morphine CPP in the 12hr sucrose group was not as easily extinguished as the morphine CPP expressed by the 24hr sucrose group on the first test session.
Chapter 6: Experiment 4

Effects of Sucrose Bingeing on Conditioned Place Preference to Sucrose in Males

6.1 Introduction

The purpose of this experiment was to examine the effects of sucrose bingeing on sucrose reward in male rats to determine whether the unexpected results using female rats could be replicated. Sucrose bingeing has been established in male and female rats with no differences in consumption (Avena, Rada, & Hoebel, 2006) but differences in sucrose taste thresholds and sucrose preferences have been observed, indicating that sucrose reward differs between males and females (Koh & Teitelbaum, 1961). The purpose of the first part of the experiment was to establish a CPP to sucrose in male rats and to determine which concentration of sucrose should be used in subsequent experiments. Since female rats showed a CPP to sucrose under FR, but not FF conditions, and FR potentiates the reward value of PF and addictive drugs (Volkow & Wise, 2005), all rats were FR in this experiment. The second part of this experiment was used to examine the effects of sucrose bingeing on sucrose reward in male rats using the CPP paradigm. Neuroadaptations in the DA reward pathway, as well as alterations in LH BSR are observed in binge eating rats (Johnson & Kenny, 2010). This evidence suggests that binge eating leads to changes in the reward pathway and might result in a change in the reward value of PF.

6.2 Methods

6.2.1 Subjects

Forty-eight male, Long-Evans rats were assigned to either a sucrose dose
response curve (n=32), or sucrose bingeing (n=32) group. The sucrose dose response curve group was further separated into one of four groups (n=8): 1%, 5%, 15%, or 30% sucrose, and the sucrose bingeing group was separated into one of four groups (n=8): 12hr sucrose, 24hr sucrose, 12hr saccharin, or 12hr food only.

6.2.2 Behavioural Procedures

All rats in the sucrose dose response curve group were FR. Chow was removed in the afternoon before the first habituation session and, for the remainder of the experiment, these animals received access to chow for two hours per day in their home cage. Chow was provided in the afternoon, two to four hours following the end of the afternoon conditioning session. All rats had ad libitum access to water in their home cage. The sucrose dose response curve group underwent CPP habituation, conditioning, and testing as described earlier. The day after CPP testing, all rats underwent a single state-dependent test session (30 min). The state-dependent test day was identical to test day, but immediately prior to the state-dependent test, rats received access to sucrose solution (1%, 5%, 15%, or 30%) for 30 min. The sucrose bingeing group underwent a 28-day sucrose consumption phase followed by CPP conditioning, as described in the General Methods. A 15% concentration of sucrose was used for the CPP conditioning phase.

6.3 Results: Sucrose Dose Response Curve

6.3.1 Habituation

Over the two habituation sessions, rats spent significantly more time in the striped wall compared to the solid wall compartment, $[F(1, 62) = 7.0, p < .01]$ and in the striated compared to the bumpy floor compartment, $[F(1, 62) = 34, p < .001]$. However, when
taken together, there was no significant difference between time spent in the water-paired compared to the sucrose-paired compartment during habituation [\(F(1, 62)=.99, p>.05\)] (see Figure 24).

Figure 24. Time (s) spent in each A) wall type, B) floor texture, and C) sucrose versus water-paired side of the CPP apparatus during habituation sessions for male, food restricted (FR) rats. There were two, 30-min habituation sessions, one in the morning and one in the afternoon. Each rat underwent the first habituation session in the morning and the second in the afternoon. Data are presented as the mean (+SEM) time spent in each compartment over the two habituation sessions.

6.3.2 Conditioned Place Preference: Conditioning

Rats consumed significantly more sucrose than water in the 5% [\(t(7)=4.06, p<.005\)], 15% [\(t(8)=7.92, p<.001\)], and 30% [\(t(7)=11.1, p<.001\)] sucrose groups, but not
the 1% \( t(10)=1.16, p>.05 \) sucrose group (See Figure 25). Levene’s test indicated unequal variances for solution consumption \( [F(7,56)=11, p<.001] \)

Figure 25. Solution consumption during conditioned place preference (CPP) conditioning sessions for male rats that were food restricted (FR). Data are presented as the mean (+SEM) solution consumed (per body weight) over five 30-min sessions. Eight rats had access to 1%, 5%, 15%, or 30% sucrose solution in one compartment and tap water in the other compartment.

6.3.3 Conditioned Place Preference: Testing

As shown in Figure 26 male rats did not develop a CPP to sucrose following conditioning with 1% \( t(13)=.34, p>.05 \), 5% \( t(14)=.23, p>.05 \), 15% \( t(11)=1.22, p>.05 \), or 30% \( t(14)=-.52, p>.05 \) sucrose solution. Levene’s test indicated unequal variances for solution consumption \( [F(7,56)=2.2, p<.05] \).
Figure 26. Conditioned place preference (CPP) to sucrose in male, food restricted (FR) rats. Data are presented as the mean (+SEM) time spent in sucrose- and water-paired compartments over a 30-min test session. Separate groups of rats had access to 1%, 5%, 15%, or 30% sucrose solution in one compartment and tap water in the other compartment during conditioning sessions. No solution was available during testing.

6.3.4 Conditioned Place Preference: State-Dependent Testing

Figure 27 shows the results for the state-dependent CPP. There was no significant difference in the time spent between the sucrose-paired and the water-paired compartments for the 1% [t(56)= .2, p>.05], 15% [t(56)= .02, p>.05], and 30% [t(56)= .02, p>.05] sucrose concentrations, but there was a significant difference between time spent in the water- and sucrose-paired compartment in the 5% sucrose group [t(56)= 2, p<.05].
Figure 27. State-dependent conditioned place preference (CPP) to sucrose in male, food restricted (FR) rats. Data are presented as the mean (+SEM) time spent in sucrose- and water-paired compartments over a 30-min state-dependent test session. Separate groups of rats had access to 1%, 5%, 15%, or 30% sucrose solution in one compartment and tap water in the other compartment during conditioning sessions. Rats had a 30-minute access period to 1%, 5%, 15%, or 30% sucrose solution immediately before the 30-minute test session. Rats did not have access to water or sucrose solution during state-dependent testing.

6.3.5 Conditioned Place Preference: Time Course

The time course of CPP testing in male FR rats is shown in Figure 28. The mean difference in time spent in the sucrose compared to the water-paired side was greatest within the first 25 minutes for all of the sucrose groups.
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Figure 28. Conditioned place preference (CPP) to sucrose across time in male, food restricted (FR) rats for the A) 1%, B) 5%, C) 15%, and D) 30% sucrose concentrations. Data are presented as the mean (+SEM) time spent in sucrose- and water-paired compartments at five-min bins over a 30-min test session. Separate groups of rats had access to different concentrations of sucrose solution in one compartment and tap water in the other compartment during conditioning sessions.

6.4 Results: Sucrose Bingeing

6.4.1 Body Weight

Analysis of the data across the sucrose consumption phase revealed that body weights changed across the 28 days of these sessions \( F(4, 111) = 316, p < .001 \), although there was no significant effect of group \( F(3, 28) = 1.4, p > .05 \). There was a significant group by day interaction \( F(12, 111) = 1.9, p < .05 \) (see Figure 28). Pairwise comparisons
revealed that body weight did not differ significantly between groups on day 1 ($p>.05$), and on day 28, the 24hr sucrose group weighed significantly more than the 12hr food only group ($p<.05$). There were no other significant differences in body weight between groups (see Figure 29).

Figure 29. Body weight across sucrose consumption sessions for rats given different access to sucrose, saccharin, or food only. Data are presented as mean (+SEM) body weight (g) for each group across the 28-day sucrose consumption phase. Body weight was measured 12 hours following the presentation of sucrose, saccharin, or food on each day; measurements were taken at the same time point for rats given 24- hour access to sucrose.

6.4.2. Conditioned Place Preference

Figure 30 shows sucrose consumption of each group during the first hour following presentation of sucrose across 28 days. ANOVA revealed that solution intake at 1hr changed significantly across days [$F(10, 214)= 24, p<.001$] with significant group differences in this measure [$F(2, 21)= 12, p<.001$] and a significant group by day interaction [$F(20, 214)=2.9, p<.001$]. Pairwise comparisons between days 1, 2, 3, 4, 25, 26, 27, and 28 revealed that on day 1, both sucrose groups (12hr and 24hr) consumed significantly more solution compared to the saccharin group ($p<.01$). On days 2, 3, and 4, the 12hr sucrose group continued to consume significantly more solution compared to the 12hr saccharin group ($p<.01$), whereas the 24hr sucrose group only drank significantly
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more solution on day 4 ($p<.01$). On the last four days of the binge cycle (days 25, 26, 27, and 28), the 12hr and 24hr sucrose groups consumed a comparable amount of sucrose solution within the first hour of intake ($p>.05$), except for day 25, when the 12hr sucrose group consumed significantly more than the 24hr sucrose group ($p<.05$). Both sucrose groups (12hr and 24hr) consumed significantly more solution during the first hour of intake compared to the saccharin group on days 25 and 26 ($p<.01$). On day 27, the 24hr sucrose group consumed significantly more solution compared to the saccharin group ($p<.05$), and on day 28, all three groups (12hr sucrose, 24hr sucrose, and 12hr saccharin) consumed comparable amounts of solution during the first hour of intake ($p>.05$).

The 12hr sucrose group consumed significantly more solution on day 25 compared to days 1, 3, and 4 ($p<.05$), but consumed significantly less solution on days 26, 27, and 28 compared to days 1, 2, 3, and 4.
Figure 30. Solution consumption during the first hour of access across sucrose consumption sessions for rats given different access to sucrose, saccharin, or food only. Graphs represent A) 12hr sucrose, B) 24hr sucrose, and C) 12hr saccharin groups. Graph D shows the comparison between all groups for 1-hour solution consumption. Data points represent mean (+SEM) solution consumed (mL/per g body weight) on each day of the 28-day sucrose consumption phase.

Figure 31 shows sucrose consumption of each group during 24 hours across 28 days. ANOVA revealed that solution intake at 24hrs changed significantly across days [$F(6, 121)=4.5, p<.001$] with significant group differences in this measure [$F(2, 21)=24, p<.001$] and a significant group by day interaction [$F(12, 121)=1.9, p<.05$]. Pairwise
comparisons between days 1, 2, 3, 4, 25, 26, 27, and 28 revealed that the 24hr sucrose
group consumed significantly more solution than the 12hr sucrose ($p<.05$) and 12hr
saccharin ($p<.05$) groups on all of the days analyzed, including days 1, 2, 3, 4, 25, 26, 27,
and 28. Twenty-four hour solution consumption in the 12hr sucrose group did not differ
significantly from the 12hr saccharin group on days 1, 2, 3, and 4, but on days 25, 26, and
27, rats in the 12hr sucrose group consumed significantly more solution compared to
those in the 12hr saccharin group ($p<.05$). Rats did not drink significantly more solution
on any of the last four days of the binge cycle, including days 25, 26, 27, and 28
compared to days 1, 2, 3, and 4 of the binge cycle in the 12hr sucrose ($p>.05$), 24hr
sucrose ($p>.05$), and 12hr saccharin ($p>.05$) groups. One exception to this is that the 12hr
sucrose group consumed significantly more solution on day 25 compared to all of the
days analyzed, including days 1, 2, 3, 4, 26, 27, and 28 ($p<.05$).
Figure 31. Solution consumption in each 24-hour period across sucrose consumption sessions for rats given different access to sucrose, saccharin, or food only. Graphs represent A) 12hr sucrose, B) 24hr sucrose, and C) 12hr saccharin groups. Graph D shows the comparison between all groups for 24hr solution consumption. Data points represent mean (+SEM) solution consumed (mL/per g body weight) on each day of the 28-day sucrose consumption phase.

Figure 32 shows the intake of regular chow, measured at 24hr intervals. Chow intake decreased across the 28 day sessions \([F(10, 276) = 31, p < .001]\), with significant group differences on this measure \([F(3, 28) = 36, p < .001]\) and a significant day by group
interaction \[F(30, 276)=1.9, p<.01\]. Pairwise comparisons revealed that the 12hr saccharin and 12hr food only groups consumed significantly more chow compared to the 12hr and 24hr sucrose groups on all of the days analyzed, including day 1, 2, 3, 4, 25, 26, 27 and 28 \((p<.05)\) of the 28-day sucrose consumption phase. There were no other significant differences between groups except that on days 25, 26, and 27, the 12hr sucrose group consumed significantly more chow compared to the 24hr sucrose group \((p<.05)\). In the 24hr and 12hr sucrose groups, rats consumed significantly more chow on days 2, 3, and 4 compared to days 25, 26, 27, and 28 \((p<.05)\), showing that in the sucrose groups, chow consumption decreased significantly over time.

**Figure 32.** Food intake in each 24-hour period across sucrose consumption sessions for rats given different access to sucrose, saccharin, or food only. Data points represent mean (+SEM) chow consumed (g/g body weight) on each day of the 28-day sucrose consumption phase.

Total solution consumption across the five conditioning days is shown in Figure 33. Rats drank significantly more sucrose than water in all of the food groups, including the 12hr sucrose \([t(8)=12, p<.001]\), 24hr sucrose \([t(7)=11, p<.001]\), 12hr saccharin\([t(8)=18, p<.001]\) and 12hr food only \([t(7)=7, p<.001]\) groups. Levene’s test indicated unequal variances \([F(7,56)=4.3, p<.01]\).
Figure 33. Solution consumption during conditioned place preference (CPP) conditioning sessions for rats given different access to sucrose, saccharin, or food only. All rats were food restricted (FR) following the 28-day sucrose consumption phase. Rats had access to 15% sucrose solution in one compartment and tap water in the other during conditioning. Data are presented as the mean (+SEM) solution consumed (per body weight) over five 30-min sessions.

Planned comparisons revealed that rats in the 24hr sucrose \[t(56)=-2.6, p<.05\], 12hr saccharin \[t(56)=-2.4, p<.05\] and 12hr food only groups \[t(56)=-2.2, p<.05\] spent significantly more time in the sucrose-paired compartment compared to the water-paired compartment, but there was no significant difference in time spent between the two sucrose-paired and water-paired compartment in the 12hr sucrose group \[t(56)=-1.6, p>.05\] during the 30-minuted CPP test session (see Figure 34).
Figure 34. Conditioned place preference (CPP) to sucrose for rats given different access to sucrose, saccharin, or food only during sucrose consumptions sessions. Data are presented as the mean (+SEM) time spent in sucrose- and water-paired compartments over a 30-min test session. All rats were food restricted (FR) following the 28-day sucrose consumption phase. Rats had access to 15% sucrose solution in one compartment and tap water in the other during conditioning, with no solution available during testing.

6.5 Discussion

During habituation, rats preferred the striated floor and striped walls. Floor and wall type were counterbalanced and when taken together, there was no significant difference between the time spent in the water- and sucrose-paired compartments.

The lack of a sucrose CPP in this study differs from previous studies, which show that male rats develop a CPP to 16% sucrose solution under FR conditions (Delamater et al., 2000). All rats were conditioned to water in the morning sessions and sucrose in the afternoon sessions, but the test sessions were counterbalanced so that half of the rats were tested in the morning and the other half were tested in the afternoon. The sucrose conditioning and test sessions were therefore matched (afternoon sucrose conditioning/afternoon testing) for half and non-matched (afternoon sucrose conditioning/morning testing) for the other half of each group. The time of day could be an important environmental cue for sucrose, so that when the conditioning and test sessions were non-
matched, a preference for sucrose was not shown. Other studies have found a time-state dependency for food-induced CPP as well. Rats show a CPP to food when the time of the conditioning and test sessions are matched but not when they are non-matched (Cain, Ko, Chalmers, & Ralph, 2004). When the test data was separated into matching and non-matching sucrose conditioning and test sessions, there was a greater difference between time spent in the sucrose and water paired compartments in the matched animals compared to the non-matched animals. This difference was most notable in the 5% and 15% sucrose concentration groups. Because there were very few animals in each of the matched and non-matched test sessions, this data was not analyzed for statistical significance.

The length of the test session could also explain the absence of a sucrose-CPP in male rats, since the test session in this study was longer than other studies. For example, Delamater et al. (2000) found that male rats showed a CPP to a 16% sucrose solution, but the test session was 15 minutes, as opposed to the 30-minute test session used in this study. The time course of the CPP revealed that the greatest difference between the sucrose and water-paired compartments was within the first 15 minutes for the 5% and 15% sucrose solution, and within the first 10 minutes for the 30% sucrose concentration groups. It is possible that the longer test duration did reduce the expression of a CPP to sucrose in male rats.

The 12hr sucrose group did not drink significantly more solution during the first hour of intake compared to the 24hr sucrose group, and did not show an escalation in 1hr solution consumption across days, indicating that the 12hr sucrose group was not sucrose bingeing by the end of the 28-day sucrose consumption phase. Even though the 12hr
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sucrose group was not sucrose bingeing, the results show that this type of access schedule (limited access in combination with food deprivation) does affect sucrose reward since the 12hr sucrose group was the only group that did not show a CPP to sucrose following the 28-day sucrose consumption phase. Other studies have shown that binge eating PF high in fat and sugar leads to a general desensitization of reward (Johnson & Kenny, 2010), so it is possible that limited access to sucrose reduces the reward value of sucrose but also reduces the rewarding effects of other substances. Interestingly, all of the groups, including the 12hr sucrose group, drank significantly more sucrose solution than water during conditioning sessions. This suggests that sucrose consumption and CPP to sucrose are measures of different components of reward, possibly liking and wanting.

Chapter 7: General Discussion

The purpose of these experiments was to examine the effect of sucrose bingeing on reward function, and to evaluate sex differences in this effect. The most consistent finding in these experiments is that rats consumed significantly more 15% sucrose solution than water during CPP conditioning sessions. This was true for both females and males under all feeding conditions, regardless of whether or not rats showed a CPP to sucrose. Interestingly, sucrose consumption and the development of a CPP to sucrose were unrelated, in that increased sucrose consumption during conditioning sessions did not produce a sucrose-induced CPP. There were also a number of differences between female and male rats, the most apparent being that sucrose CPP was attenuated in all female rats, whereas sucrose CPP was only attenuated in the 12hr sucrose group in males.
The attenuation of sucrose CPP in the male 12hr sucrose group indicates that intermittent access to sucrose, and sucrose bingeing results in decreased sucrose reward. Finally, both the 12hr and 24hr sucrose groups showed a CPP to morphine, indicating that sucrose bingeing had no effect on morphine reward in females.

7.1 Liking and Wanting

Although surprising, the finding that sucrose consumption and sucrose reward were not correlated fits with behavioural, pharmacological, and neurobiological evidence that these two behaviours are dissociable. For example, stress attenuates sucrose CPP in rats but does not affect sucrose consumption during conditioning (Papp, Willner, Muscat, & Street, 1991), rats develop a CPP to sucrose and not saccharin despite consuming a comparable amount of both solutions during conditioning (White & Carr, 1985), and low doses of opioid antagonists reduce sucrose consumption but do not attenuate a sucrose-induced CPP (Ågmo et al., 1995). Similar discrepancies between self-administration and CPP are evident with drugs of abuse: D2 antagonism attenuates self-administration of cocaine, while having no effect on a CPP to cocaine (Bardo & Bevins, 2000) and the magnitude of a cocaine- and amphetamine-induced CPP is not correlated with self-administration of these substances (Bardo & Bevins, 2000). Furthermore, drugs that induce a CPP do not necessarily produce self-administration and vice versa (Bardo & Bevins, 2000). Together, this evidence indicates that consumption and CPP are measures of different components of reward.

According to the incentive salience theory, reward is comprised of two major components: the immediate pleasure derived from a rewarding stimulus (liking), and incentive salience attributed to a reward and its associative cues (wanting) (Berridge,
Wanting enhances the motivation for reward and its associative cues, consequently increasing approach and consummatory behaviour (Berridge, Robinson, & Aldridge, 2009). Incentive salience ‘wanting’ differs from cognitive ‘wanting’ in that incentive salience ‘wanting’ is implicit and unconscious, requires simpler mechanisms, and involves subcortical mesolimbic DA neurotransmission. In contrast, cognitive ‘wanting’ is conscious, requires more complex cognitive mechanisms, and involves cortical brain regions (Berridge, 2009). For the purpose of this discussion, wanting will refer to incentive salience attributed to reward and its associative cues. Other components of reward include anticipatory and consummatory pleasure, which are associated with wanting and liking, respectively (Sherdell, Waugh, & Gotlib, 2012). Liking is measured using affective orofacial expressions that are elicited during consumption, whereas wanting is generally measured using consumption tests, choice tests, place preference, and instrumental performance (Berridge & Robinson, 1998).

For the most part, rewards that are liked are also wanted, but certain manipulations are capable of disentangling these two components. DA agonism (Berridge & Robinson, 1998), stress (Pool et al., 2016), and \( \mu \)-opioid agonism in the central nucleus of the amygdala (Mahler & Berridge, 2012) increase wanting while either reducing or having no effect on liking reactions to sucrose. On the other hand, depletion of DA induces aphagia without affecting liking, suggesting that DA is necessary for wanting but not liking food reward (Berridge & Robinson, 1998). Moreover, specific brain regions mediate liking and wanting discretely: \( \mu \)-opioid receptor activation in certain hedonic “hotspots” located in the NAc shell and ventral pallidum mediate liking while opioid and DA transmission within larger areas of the mesolimbic DA system.
mediate wanting (Berridge, 2009). In humans, discrete brain regions implicated in liking and wanting have also been identified. For example, amygdalar activation is associated with the expectation (wanting) while the orbitofrontal cortex is active during consumption (liking) of a reward (Pool et al., 2016).

A number of studies have evaluated reward using consumption and CPP as measures of liking and wanting, but the results of these studies vary greatly. For example, stressed rats consume more sucrose, show fewer hedonic liking reactions at the time of consumption, and do not develop a CPP to sucrose when compared to control rats (Silveira et al., 2010), indicating that a CPP is hedonically driven but consumption is not. On the other hand, liking has been shown to mediate sucrose consumption but not a CPP. For example, DA antagonism in FR rats attenuates a CPP to sucrose, but does not influence hedonic liking reactions (Berridge & Robinson, 1998), and opioid agonists increase hedonic liking reactions and consumption concurrently (Peciña & Berridge, 2005), indicating that liking is a mediator of consumption. Because of this, consumption is used to measure wanting in some animal studies, and liking in others, and this is also true in human studies (Pool et al., 2016). It is possible that under certain conditions, consumption is mediated by liking, whereas in other cases, it is not. For example, Johnsons and Kenny (2010) proposed that following the development of binge eating and weight gain, the reward system is blunted, which leads to compulsive intake of palatable food. In conclusion, consumption and CPP are not always associated with one another in these experiments and this is likely due to changes in the reward value of sucrose through alterations in either liking, wanting, or both.

7.2 Conditioned Place Preference to Sucrose Following Sucrose Bingeing
In our experiments both female and male rats were given access to 12hr sucrose, 24hr sucrose, 12hr saccharin or 12hr food only. CPP to sucrose was attenuated in all female rats, and in the 12hr sucrose group for male rats, revealing that a history of intermittent access to sucrose results in decreased sucrose reward. Furthermore, the reward value of sucrose was not dependent on weight gain in males, since weight did not differ significantly between groups. These findings are somewhat consistent with previous studies, which show that access to a highly palatable diet results in decreased sensitivity to reward. Extended access to a high fat diet and weight gain results in reduced reward sensitivity and decreased striatal D2 receptors in rodents (Johnson & Kenny, 2010) and a reduction in striatal response to palatable food in women (Stice, Yokum, Blum, & Bohon, 2010). Reduced reward sensitivity to food is also evident in non-obese individuals that have a history of high consumption of PF or binge eating, and in animals maintained on a high fat diet that do not gain weight, indicating that reward dysfunction is not only associated with weight gain. For example, frequent consumption of a particular food leads to reduced activation in the striatum in response to that specific food (Burger & Stice, 2012), women with BN showed reduced activation in reward related areas in response to anticipation and consumption of palatable food (Bohon & Stice, 2011), and individuals who have recovered from BN show reduced activation in the right anterior cingulate cortex, an area involved in the anticipation of reward (Frank et al., 2006). Furthermore, rats that are maintained on an intermittent high fat diet to prevent weight gain, display lower breakpoints and fewer lever presses for sucrose (Davis et al., 2008). Extended access to PF, binge eating, and the development of obesity have also been shown to lead to dissociated wanting and liking. For example, obese females
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show decreased activation in reward related areas, including the striatum, in response to consumption, but not in anticipation of PF (Stice, Spoor, Bohon, Veldhuizen, & Small, 2008), obesity is associated with a marked increase in willingness to work for a reward with no change in the hedonic value of food (Saelens & Epstein, 1996), and obese binge eaters display a higher preference and lower wanting for sweet foods when fasted compared to when satiated (Dalton, Blundell, & Finlayson, 2013). In conclusion, our experiments suggest that, similar to extended access to highly PF and the development of obesity, intermittent access to sucrose, which is a feeding schedule that often leads to binge eating, results in decreased sucrose reward.

7.3 Sex Differences

The current results also suggest that male and female rats show different sensitivity to sucrose reward: FR male and female rats both consumed more sucrose solution but only female rats developed a CPP to sucrose. There are a few possible explanations for these results, based on previous literature. Internal physiological state (i.e., hunger/satiety) modulates the relevance of a reward, which affects both liking and wanting (Pool et al., 2016). This is likely the reason that the females developed a CPP to sucrose under FR, but not FF, conditions. Compared to females, males are less sensitive to hunger following food deprivation, which results in reduced reactivity to food related stimuli (Uher, Treasure, Heining, Brammer, & Campbell, 2006). This could explain the lack of CPP to sucrose in males, since FR might not have had the same impact on males in terms of increasing hunger and reward relevance as it did on females. Furthermore, compared to males, females develop CPP to drugs of abuse, including cocaine and morphine with fewer pairings and at lower doses, suggesting that they are more sensitive
to the rewarding effects of these substances (Nazarian, Russo, Festa, Kraish, & Quinones-Jenab, 2004). However, these findings do not explain the lack of CPP to sucrose in male rats because other studies have shown that males develop a CPP to sucrose under FR conditions (Delamater et al., 2000). Another possibility is that sucrose CPP is time-state-dependent, meaning that sucrose conditioning and testing must occur at the same time of day for a CPP to be expressed. In our study, half of the FR males underwent conditioning and testing at different times of the day, whereas this was not the case for females.

Males and females differed from one another in a number of other ways that may explain why we observed a CPP in one sex but not the other. The time course of the CPP test differed between males and females, and sucrose CPP was attenuated in females under all food conditions, whereas sucrose CPP was attenuated only in the male, 12hr sucrose group. Females could be more sensitive to changes in the reward value of sucrose following abnormal feeding conditions, including FR and extended or intermittent access to highly PF. For example, following 28 days of 2hr access to sucrose during adolescence, adult males showed reduced, whereas adult females showed increased, motivation for sucrose reward (Reichelt, Abbott, Westbrook, & Morris, 2016), and anhedonia produced by Interleukin-1-β-induced depression, is more prominent in females than males (Merali, Brennan, Brau, & Anisman, 2003). Females also become more stressed when individually housed, substantiated by higher levels of corticosterone (Brown & Grunberg, 1996); this type of stress, like conditioned stressors, results in reduced food intake (Brown & Grunberg, 1996). Animals were singly housed during the 28-day sucrose consumption phase, which may have produced differences in stress-
related responses of males and females. In addition to having an impact on food consumption, stress has also been shown to influence reward. For example, stress potentiates reward dysfunction during abstinence (Koob, 2006), and more specifically, chronic, mild stress decreases the reward value of food (Goodman, 2008). Females may also be particularly vulnerable to the stress effects on food reward since reward deficits are observed in female hamsters following social isolation, whereas this is not the case in males (Shannonhouse, Grater, York, Wellman, & Morgan, 2015). It is likely that the reward value of sucrose following FR, or intermittent and extended access to a palatable solution differed between females and males because of the food schedule, or as a result of the impact of social isolation on food reward.

7.4 Opioids and Sucrose Reward

The µ-opioid receptor is an important mediator of PF reinforcement. µ-opioid receptor agonists increase, while µ-opioid receptor antagonists decrease PF consumption (Glass, Billington, & Levine, 1999). More specifically, µ-opioid receptors are involved in the consumption of preferred foods (Gosnell & Levine, 2009), through their influence on liking and wanting (Giuliano, Robbins, Nathan, Bullmore, & Everitt, 2012). Animals that are pre-exposed to morphine or the µ-opioid receptor agonist fentanyl show enhanced CPP to morphine, an effect that is attenuated by administration of the µ-opioid receptor antagonist, naloxone, providing evidence for the involvement of alterations in µ-opioid receptors in mediating this effect (Shippenberg, Heidbreder, & Lefevour, 1996). Alterations in the opioid reward system are observed in individuals that meet criteria for BN and BED, suggesting that this is a result of binge eating. For example, opioid antagonists decrease liking and consumption of palatable food in binge- but not non
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binge-eaters (Adam Drewnowski, Krahn, Demitrack, Nairn, & Gosnell, 1995). Altered reward sensitivity in response to intermittent access to sucrose is likely specific to PF since females under the 12hr and 24hr sucrose access schedules showed a CPP to morphine, but not sucrose. There is some evidence to support these findings. Morphine-induced CPP is inhibited in D2 null mice, whereas sucrose CPP is not (Maldonado et al., 1997), indicating that there are clear differences between morphine and sucrose reward. Consumption of a specific PF results in decreased activation in reward related areas in response to that particular food (Burger & Stice, 2012), and there are no alterations in the genes encoding opioid peptides and receptors in the hypothalamus or brainstem following intermittent access to sucrose (Olszewski et al., 2009). Synaptic alterations produced by extended and intermittent access to drugs are unique to the particular drug used, which could explain these results. For example, alterations in the dendritic structure of regions, including the NAc, differ following extended access to opiates (e.g., morphine) and stimulants (e.g., amphetamine). These differences exist between drugs of the same class as well (e.g., morphine and amphetamine), albeit to a much lesser degree (Robinson & Kolb, 2004). Furthermore there are clear differences between drug- and sucrose-reward training on synaptic plasticity, since self-administration of amphetamine increases spine density in the NAc, whereas sucrose self-administration does not (Crombag, Gorny, Li, Kolb, & Robinson, 2005). These differences could explain the disparity between sucrose and morphine-CPP following intermittent access to sucrose and binge eating.

In contrast to these findings, extended access to a high fat diet results in altered reward sensitivity. For example, high fat diets result in reduced food reward, increased expression of tyrosine hydroxylase, decreased expression of FosB (Sharma, Fernandes, &
Sucrose bingeing alters sucrose reward

Fulton, 2013), and decreased reward sensitivity, measured by LH BSR (Johnson & Kenny, 2010). Similar findings are observed in humans: obese binge eaters display enhanced liking and a greater frequency of the gain of function “G” allele, which is associated with an increase in responsiveness to opiates and alcohol, and a susceptibility to addiction (Davis et al., 2009). Furthermore, access to a high fat diet without the development of obesity attenuates a CPP to amphetamine, decreases the number of lever presses made for sucrose, and results in reduced DA turnover in the NAc (Davis et al., 2008). In all of these studies, a high fat as opposed to a high sucrose diet was used, so it is likely that fat and sucrose differ in their effect on reward circuitry.

Sucrose bingeing may not have had an effect on morphine CPP because intermittent access to highly PF, and binge eating results in a dysregulation of reward that is mediated through long lasting alterations of the DA, as opposed to the opioid reward system. Intermittent access to glucose attenuates cocaine CPP (Rorabaugh, Stratford, & Zahniser, 2015), and extended access to nicotine enhances both cocaine and sucrose CPP, suggesting that cross-tolerance and cross-sensitization exists between sucrose and drugs of abuse which directly activate DA. In fact, there is a large body of evidence to suggest that binge eating, intermittent, and extended access to highly PF leads to alteration in DA transmission within the mesolimbic DA system. Rats that binge eat show reduced DA release in the NAc following food deprivation (Avena, Bocarsly, et al., 2008), obese and fat bingeing rats display decreased reward sensitivity and a downregulation of D2 receptors (Johnson & Kenny), and long-term alterations in the DA reward pathway are observed following intermittent access to a high fat, high sucrose diet. Interestingly, this high-fat, high-sucrose diet, which does not lead to weight gain results in a
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downregulation of D1 and µ-opioid receptors in the NAc, but only the downregulation of D1 receptors is evident following a withdrawal period (Alsiö et al., 2010).

7.5 Future Studies

These studies demonstrate that sucrose CPP is attenuated in male rats following intermittent access to sucrose in combination with food deprivation. This finding was not replicated in female rats since sucrose CPP was attenuated in all food groups, but this was likely due to a stress effect from social isolation and/or the sucrose bingeing cycle, itself. It would be interesting to examine the difference between the stress responses in females and males following the sucrose bingeing model to determine if, in fact, this was the reason that sucrose CPP was attenuated in all females. Future studies should aim to control for this by using control groups where female rats are double housed.

These studies also demonstrated that consumption and CPP are not measures of the same psychological component of reward, since rats drank a large amount of sucrose regardless of whether or not they developed a CPP to sucrose. The discrepancy between consumption, self-administration, and CPP is well documented, however there is still no consensus concerning these behavioural tests and the discrete component of reward that each measures. The most consistent behavioural tests used to measure wanting and liking are operant conditioning and orofacial expressions, respectively. Future studies should examine the effect of sucrose bingeing on wanting and liking separately by using these behavioural tests. Finally, although sucrose CPP was attenuated in all rats following the 28-day sucrose administration phase, both the 12hr and 24hr sucrose groups developed a CPP to morphine. Future studies should use more control groups, including a 12hr saccharin, 12hr food only, and standard chow group since it is possible that 12hr and 24hr
access to sucrose had a similar effect on morphine reward. Furthermore, it would be interesting to examine the effects of sucrose bingeing on a morphine CPP dose response curve to determine whether sucrose bingeing results in cross-sensitization or cross-tolerance to the rewarding effects of morphine at various doses. Since sucrose bingeing did not affect morphine reward in this study, future studies should also examine the effect of sucrose bingeing on neuroadaptations of DA in the mesolimbic reward pathway. Finally, future studies should examine the effect of sucrose bingeing on LH BSR, since this is a more direct measure of reward sensitivity.
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