Behavioural and Pharmacological Interactions between Drug and Nutritional Reinforcers

By

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ABSTRACT

BEHAVIOURAL AND PHARMACOLOGICAL INTERACTIONS BETWEEN DRUG AND NUTRITIONAL REINFORCERS

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The repeated consumption of highly palatable food shares several behavioural and neurobiological similarities with drugs of abuse. This has led to the “food addiction” hypothesis which predicts that highly palatable food can promote “addictive” behaviours. This hypothesis predicts that sugar and drug reinforcers should interact to influence responses to each other, and that the behaviours engendered by these reinforcers should be comparable in situations where their availability is similar. The studies in this dissertation were designed to test these predictions in male Sprague-Dawley rats using high fructose corn syrup (HFCS) and oxycodone (OXY). HFCS was selected because it is a widely used added sugar in our diets and OXY was selected because it is a widely used and abused prescription opioid.

Study 1 explored the effect of a HFCS-rich diet on behavioural and neurochemical responses to OXY. It was found that HFCS decreased OXY-induced locomotion and OXY-induced dopamine release in the nucleus accumbens but did not alter OXY conditioned place preference. Study 2 investigated interactions between HFCS and OXY on taking and seeking behaviors in the same subject using the innovative procedure of co-self-administration of intraoral (IO) HFCS and intravenous (IV) OXY. It was found that OXY and HFCS differentially controlled seeking expressed by the same subjects: OXY engenders more persistent behaviour than HFCS, both in the presence and absence of stimuli predicting their availability. Study 3 investigated how a pharmacotherapy used to treat obesity and drug craving could affect HFCS and OXY seeking in animals trained to self-administer both. It was found that acute bupropion
increased the magnitude of cue reinstatement for both OXY and HFCS-paired levers, and that the magnitude of the increase was larger for OXY.

Collectively, these studies demonstrate that opioid and sugar reinforcers act on similar systems of learning and reinforcement, although there are differences in the extent to which these systems appear engaged. This said, these reinforcers can affect responses to each other, and this suggests that nutrition can play a significant role in the development and maintenance of drug addiction.
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<tbody>
<tr>
<td>AgRP</td>
<td>Agouti-related protein</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>BED</td>
<td>Binge Eating Disorder</td>
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<tr>
<td>BLA</td>
<td>Basolateral amygdala</td>
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<td>BUP</td>
<td>Bupropion</td>
</tr>
<tr>
<td>CART</td>
<td>Cocaine- and amphetamine-regulated transcript</td>
</tr>
<tr>
<td>cBLA</td>
<td>Caudal basolateral amygdala</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<tr>
<td>CON</td>
<td>Continuous</td>
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<tr>
<td>CR</td>
<td>Continuous reinforcement</td>
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<td>CS</td>
<td>Conditioned stimulus</td>
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<tr>
<td>D2R</td>
<td>Dopamine receptor D2</td>
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<tr>
<td>DA</td>
<td>Dopamine</td>
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<td>DOA</td>
<td>Drugs of abuse</td>
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<tr>
<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
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<tr>
<td>GHSR</td>
<td>Growth hormone secretagogue receptor</td>
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<td>GLUT</td>
<td>Glutamate</td>
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<td>GTP</td>
<td>Guanosine-5'-triphosphate</td>
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<td>HFCS</td>
<td>High Fructose Corn Syrup</td>
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<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
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<tr>
<td>ICSS</td>
<td>Intra-cranial self-stimulation</td>
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<td>INT</td>
<td>Intermittent</td>
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<td>IO</td>
<td>Intra-oral</td>
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<td>IV</td>
<td>Intravenous</td>
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<td>LH</td>
<td>Lateral hypothalamus</td>
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<td>MOR</td>
<td>Mu-opioid receptor</td>
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<td>NAc</td>
<td>Nucleus Accumbens</td>
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<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
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<tr>
<td>NPY</td>
<td>Neuropeptide Y</td>
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<tr>
<td>OXY</td>
<td>Oxycodone</td>
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<tr>
<td>PFC</td>
<td>Prefrontal cortex</td>
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<tr>
<td>POMC</td>
<td>Pro-opiomelanocortin</td>
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<tr>
<td>PR</td>
<td>Progressive Ratio</td>
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<tr>
<td>rBLA</td>
<td>Rostral basolateral amygdala</td>
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<tr>
<td>SA</td>
<td>Self-administration</td>
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<tr>
<td>SC</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
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<tr>
<td>US</td>
<td>Unconditioned stimulus</td>
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<tr>
<td>VTA</td>
<td>Ventral Tegmental Area</td>
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<tr>
<td>YFAS</td>
<td>Yale Food Addiction Scale</td>
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Statement of Research Problem

Frequent overconsumption of foods, particularly those classified as highly palatable (i.e., high in fat, sugar, and/or salt) leads to behavioral and neurobiological adaptations similar to those observed following repeated use of drugs of abuse (DOA; Volkow et al., 2012; Tomasi et al., 2015). Increased availability and consumption of palatable foods is proposed to be one environmental factor contributing to the obesity epidemic (Warner et al., 2006; Linderbeg et al., 2011). Research in humans and rodents indicates that palatable food can reinforce behaviour and precipitate craving that is comparable in magnitude to DOA (Goldstein et al., 2010; Lenoir et al., 2007; Ahmed, Guillem, & Vandaele, 2013; Madsen & Ahmed, 2015; Volkow and Wise, 2005). Hence, the notion of “food addiction” was put forward to explain, in part, the development of “addictive” like behaviour motivated by highly palatable foods. This would suggest a shared vulnerability, perhaps the result of similar underlying behavioural and neural processes recruited by both palatable foods and DOA. It is not surprising then, that substance related abuse disorders frequently present co-morbidly with eating disorders (Hudson, Hiripi, Pope, Kessler, 2007; Herzog et al., 2006). While there is considerable support for the food addiction construct (Volkow et al., 2012; Gearhardt et al., 2011), there is also intense debate about its validity (Ziauddeen & Fletcher, 2013; Benton, 2013).

The aim of this dissertation was to further characterize commonalities and distinctions in behavioural and neurobiological processes that underlie reinforcement of highly palatable food and DOA. Currently, there are a lack of direct comparisons of sugar and drug-reinforced behaviours in the same animal. To achieve these ends, we employed several animal models to characterize the behavioural and neurobiological similarities and differences between sugars and DOA, as well as interactions between them in the same animal. In the literature to date,
behavioural and neural responses to repeated administration of stimulant drugs is often compared to that of frequent consumption of palatable foods (Kelley et al., 2002). However, both drug and food reward/reinforcement can also recruit the opioid system (Di Chiara & North, 1992). Therefore, to further understand drug-sugar interactions, it is important to compare behavioural and neural responses elicited by repeated opioid and palatable food exposure.

Furthermore, there is evidence that different saccharide combinations/ratios (i.e., sucrose vs high fructose corn syrup) can affect behavioural and neural markers in different ways (Levy et al., 2015); however, most research typically uses sucrose. Since, high fructose corn syrup (HFCS) is a widely added sweetener in drinks and processed foods, it is also important to investigate its effects. It is particularly important given that HFCS appears to produce neural alterations that are similar to those produced by DOA; alterations reported to be more pronounced relative to sucrose (Levy et al., 2015). Finally, strategies for reducing the likelihood of relapse using pharmacotherapies that have been used to treat obesity and drug craving (Stahl et al., 2004; Dwoskin et al., 2006; Holm & Spencer, 2000), were tested in this dissertation.

Testing of the “food addiction” hypothesis will enhance our understanding of how people eat, the development of compulsive like eating behaviour, and the extent to which the consumption of highly palatable food and DOA are comparable. Furthermore, many factors impact the abuse liability of opioids; it is believed that one of these factors may be diet. Therefore, it is important to further elucidate whether there is shared underlying processes between the two and the extent to which those contribute to a shared co-morbidity, as well whether treatments from the drug addiction field can be applied to the management of food consumption.
Main Research Objectives

Objective 1: Evidence suggests that sugars influence reward pathways in the brain in a manner similar to DOA. This leads to the hypothesis that sugars and DOA interact at both neural and behavioural levels. So, the first objective was to assess the effect of HFCS pre-exposure on:
(1) place conditioning (0, 0.16, or 2.5 mg/kg OXY; SC), (2) locomotion involving context-dependent treatment with OXY (0, 0.16, or 2.5 mg/kg; SC), or (3) in vivo microdialysis of DA concentrations in the nucleus accumbens (NAc) prior to, during, and following an injection of OXY (0 or 2.5 mg/kg; SC).

Objective 2: The food addiction hypothesis would postulate that sugar rewards can lead to “addictive” behaviours because they act on systems of learning and reinforcement that are targeted by DOA. This leads to the prediction that “taking” and “seeking” behaviours reinforced by sugar rewards and DOA should be comparable in a situation where their availability, or availability of their conditioned stimuli, is similar; as well they should interact at behavioral levels. Therefore, the second objective was to characterize how HFCS and OXY interact and whether there are differences in the persistence of seeking behaviour. To do this, animals were trained to self-administer HFCS (8%, 25%, or 50%) or OXY (0.05, 0.1, or 0.2 mg/kg) at different concentrations and doses, respectively (Single SA). Another subset of animals was trained to co-self-administer HFCS (50%) and OXY (0.2 mg/kg) (Dual SA). In both Single and Dual SA, responses were assessed during acquisition of self-administration, extinction, and during cue and prime induced reinstatement.

Objective 3: Bupropion (BUP) has been used as a pharmacotherapy strategy to treat drug dependence and obesity. Therefore, the objective of this study was to investigate the effect of BUP pre-treatment on cue-induced reinstatement for HFCS and OXY. To do this, animals were
trained to co-self-administer HFCS (50%) and OXY (0.2 mg/kg). Prior to a test of cue-induced reinstatement rats were pre-treated with BUP (0, 10, and 30 mg/kg SC), following which responses to HFCS- and OXY cues were measured.
Chapter 1

General Introduction
1. General Introduction

1.1. Food Addiction

Can food be addictive? The food industry has developed food in such a way that it has led to food with increasingly higher “rewarding properties.” Highly palatable food, processed to contain high concentrations of sugars, fats, and/or salts may be “addictive” because its consumption produces greater reward (Schulte et al., 2015). These foods are reported to induce patterns of excessive overeating that sometimes leads to obesity (Ludwig et al., 2014).

There has been an increase in the use of the term “food addiction” in the literature. In an attempt to operationally define food addiction, Gearhardt et al (2009) developed the Yale Food Addiction Scale (YFAS), which is an adaptation of the diagnostic and statistical manual V (DSM V; American Psychological Association, 2015). This scale characterizes food addiction as a loss of control over eating behaviour, continued food consumption despite negative consequences, consuming larger amounts of food than intended, etc. over the period of a year (Meule & Gearhardt, 2014). Individuals receive a diagnosis of food addiction if they present with three or more of the diagnostic criteria and clinically significant distress or impairment (Gearhardt, Corbin, & Brownell, 2009). The most common symptom observed with the YFAS scale is a persistent desire or unsuccessful effort to cut down or control eating (Flint et al., 2014; Gearhardt et al., 2014).

The construct of food addiction has been used in some instances to understand obesity and binge eating disorder. In fact, high rates of “food addiction” are observed in individuals with a body mass index (BMI) classified as overweight or obese. For instance, Eichen et al (2013) found that 15% of overweight and obese individuals seeking treatment met the criteria for “food addiction;” a number even higher in obese individuals seeking out bariatric surgery, where
approximately 40% met the food addiction diagnosis (Meule et al., 2014). These numbers are higher than those observed in the general adult population, where the diagnosis tends to be 0.0-8.7% in the general adult population (Long et al., 2015). Additionally, the reward and learning deficits that are identified in those with substance use disorders are similar to those observed in obesity (Coppin et al., 2014; Geiger et al., 2009), which may indicate a shared co-morbidity. Because of this, a considerable amount of food addiction research makes comparisons between substance related abuse disorders and obesity. It is important to note that not all individuals who meet the criteria of food addiction are obese, nor do all obese individuals meet the criteria for food addiction (Meule et al., 2014). This would then suggest that BMI is not an accurate marker of compulsive overeating (Gadalla & Piran, 2007). Instead, the criteria used by the YFAS to diagnose FA closely resemble the behavioural profile of binge-eating disorder (BED; Davis et al., 2011; Hatsukami, Mitchell, Eckert, & Pyle, 1986; Krahn, Kurth, Demitrack, & Drewnowski, 1992). Conditions like BED are characterized by recurrent episodes of uncontrolled eating that become compulsive (Mathes, Brownley, Mo & Bulik, 2009). Approximately 57–72% of those diagnosed with BED meet the criteria for food addiction (Gearhardt et al., 2011) and show 36% co-morbidity with obesity (Gadalla & Piran, 2007). Even in those obese individuals meeting the diagnosis for food addiction using the YFAS, only 50% also met the criteria for BED (Davis et al., 2011). So, while there is some overlap, BED, FA, and obesity are separate conditions. In fact, obesity is a heterogeneous disorder and so it is more likely that the overconsumption of palatable food is one of many factors that could contribute to its development. Consequently, food addiction research in animals and humans needs to go beyond observations in obese populations. Additionally, not all individuals who are diagnosed with BED meet the criteria of food addiction
(Gearhardt et al., 2011). This suggests that although there are clinical similarities, BED, obesity, and FA are not the same.

1.2. Animal Models of Food Addiction

The notion that certain foods may be “addictive” prompted Hoebel and colleagues to develop an animal model that is widely used in studies of food addiction today. This model centres on providing animals with intermittent access to sucrose, which typically involves a cyclic diet of 12-h deprivation followed by 12-h access to sucrose and chow pellets over the course of three weeks (Avena and Hoebel, 2003a, 2003b; Avena et al., 2004, 2005; Avena, Rada, & Hoebel, 2008). Experiments using this paradigm have revealed that: (1) rats consume more sucrose solution over time, and develop patterns of consumption where there are increases in intake at the onset of access, often referred to as “bingeing”; (2) following a period of sucrose abstinence, there are increases in responses for sucrose an effect often termed as “incubation of craving”; (3) opioid antagonists can precipitate opiate-like withdrawal symptoms (i.e., teeth chattering, forepaw tremors, and headshakes) in animals that consume highly palatable foods; and (4) bingeing on sucrose is accompanied by increases in phasic dopamine (DA) release and increased mu-opioid receptor binding in the nucleus accumbens (NAc) (Avena & Hoebel, 2003; Avena, 2010; Rada, Avena, & Hoebel, 2005; Avena et al., 2006; Vucetic et al., 2010).

The food addiction hypothesis has been evaluated using this highly structured intermittent access paradigm in the home cage. While this model reveals important information about binge-like consumption that is central to the development of compulsive/addictive-like behaviour (Koob & Volkow, 2010), there are important confounds that perhaps limit its use. One potential confound in the paradigm is food restriction/deprivation. It would be difficult to determine if behaviour was motivated by the reinforcing properties of the food or deficits in
energy. Furthermore, food restriction enhances sensitivity to reinforcers, as evidenced by increases in intake, lower thresholds for the reinforcing properties of DOA, and increases in motivation to obtain DOA (Carr, 2007; Holsen et al., 2012). These consequences limit the interpretation of the findings. And so, while these paradigms offer a means to study some features of food addiction, they likely capture neural mechanisms and anatomical substrates involved in the reinforcing value of highly palatable food during very specific situations. Consequently, these findings may not generalize to populations that experience food addiction in the absence of binge-like behaviour or food restriction. Hence, it is necessary to explore paradigms that study additional features of food addiction to encapsulate the entire spectrum of palatable food consumption.

In less utilized models of food addiction, those that avoid food restriction, results have been mixed. Shin et al (2011) showed that both diet-induced obesity and obesity-prone rats appear to prefer higher concentrations of sucrose than lean rats, while not showing an interest in the lower concentrations, suggesting tolerance to the rewarding effects of sucrose, possibly attributable to decreased reward sensitivity. However, Pothos (2001) reported that rats who became overweight due to a cafeteria style diet show greater levels of extracellular DA when administered systemic amphetamine compared to controls; suggesting increased rather than decreased reward sensitivity. In part, differences in the observed behavioural phenotype appear to be driven by differences in schedule of access to food. For example, comparisons between a group of rats given access to sugar three times a week for 2 hours a day, and another group with daily ad libitum access to the same sugar, revealed that animals given limited access to sucrose showed "bingeing" to low and moderate concentrations of sucrose compared to animals given daily ad libitum access to sucrose (Wojnicki, Roberts, & Corwin, 2006). These animals also
worked harder to obtain food, as measured on a progressive ratio schedule of reinforcement (Wojnicki, Roberts, & Corwin, 2006). It therefore appears that the consumption of highly palatable food can engender differences in behavioural and neurochemical outcomes depending on the type of food and paradigm/access model utilized to assess these effects.

1.3. Assessment of responses to drugs of abuse and palatable food

In addition to manipulations in the home cage, various models used to study responses to DOA can also be utilized to study food reinforcement. Addiction is characterized as a chronically relapsing disorder that involves compulsive drug seeking and use (Koob & Volkow, 2010). Various animal models have been used to characterize aspects of these addictive behaviours. The following behavioural models will be employed in this dissertation to study drug and sugar reinforcement and interactions between the two: conditioned place preference (CPP), self-administration (SA), and locomotor sensitization.

1.3.1. Conditioned place preference

CPP is based on the notion that an animal will display approach behaviour to contextual cues that have previously been associated with an appetitive stimulus (for review see Sanchis-Segura & Spanagel, 2006). In this model, the animal is given passive administration of a reinforcer (unconditioned stimulus; US) and confined in a neutral environment. Following multiple pairings, the neutral stimulus acquires the ability to act as a conditioned stimulus (CS), capable of eliciting an approach response (for review see Sanchis-Segura & Spanagel, 2006). There are many advantages of using the CPP model: (1) it allows the testing of animals in a drug-free state; (2) can assess both CPP and locomotor activity at the same time (Bardo & Bevins, 2000); and (3) it does not require surgery. Typically, a CPP apparatus consists of two distinctive environments that are differentiated from each other based on color and/or texture.
Despite having an apparatus that is unbiased, animals can nonetheless display a preference for either of the place cues. Therefore, to avoid potential confounds in the interpretation of the results, sometimes a preconditioning phase is employed to assess pretest preferences. If the drug has the potential to produce approach behaviour, it is paired with the nonpreferred environment. This avoids the interpretational issue that the place preference was a result of both the interceptive effects of the drug and an innate preference for the side paired with the drug (even before any context-drug pairings).

There are disadvantages of relying only on the CPP paradigm. First, it involves forced exposure to a drug; non-contingent models of drug administration can induce different behavioural and neurochemical outcomes than those observed with contingent administration (Markou, Arroyo, Everitt, 1999; Lecca et al., 2007). Second, non-contingent models of drug administration are not consistent with the human drug-taking experience. Third, novelty preference can skew data. For example, it is possible that pairing a drug with one context may retard or block familiarization to that context therefore making it more novel relative to the other context (Bardo & Bevins, 2000). Lastly, CPP paradigms are not always able to measure progressive changes in drug motivation; instead they can result in all or none responses (Bardo & Bevins, 2000). Despite these limitations, CPP can provide unique information about reward and conditioned approach associated with drug administration, as not all drugs that produce CPP are also self-administered (Prus, James, and Rosecran, 2009).

1.3.2. Drug-taking

Operant SA. The traditional SA model involves operant (instrumental) conditioning, where an emitted behaviour is modified by its consequences (Ator & Griffiths, 2003). SA procedures are often considered the “gold standard” in addiction research because they have
good construct and face validity in characterizing human drug taking (Ator & Griffiths, 2003). In this paradigm, animals are trained to perform an operant response (i.e., nose poke, lever press) to receive a motivationally significant stimulus (unconditioned stimulus; US) (Panlilio & Goldberg, 2007). A substance is reinforcing if it increases the likelihood of the operant behaviour (Panlilio & Goldberg, 2007). To assess different aspects of reinforcement, the schedule of reinforcement can be manipulated, for example: (1) continuous reinforcement schedule, where an animal performs a single action to receive a stimulus; (2) fixed ratio schedule, an animal performs a set number of actions to receive a stimulus; (3) progressive ratio schedule, the number of responses required for each reinforcer increases exponentially within or between sessions. The breakpoint (at which the animal no longer works to receive the reward) is used as an index of motivation (Richardson & Robert, 1996).

In the studies described in this dissertation, two types of SA methods are utilized, intravenous (IV) and intra-oral (IO). IV SA is extensively used in drug addiction research (for review see Sanchis-Segura & Spanagel, 2006; Ator & Griffiths, 2003). IO SA on the other hand has not been extensively utilized. IO SA was developed to examine whether rats would lever press to self-inject solutions directly into their oral cavity (Levy et al., 2015). Typically, in studies of food reinforcement, animals are required to voluntarily consume solid foods, drink solutions from a spout, or operantly respond for solid pellets or drops of fluid delivered in a receptacle. IO SA offers several advantages over its traditional counterparts. It allows for the testing of controlled quantities of any water-soluble food-additive. The use of passive IO infusions eliminates the delay between the operant response and reinforcer, which allows for quicker reward delivery. Finally, it allows for the measurement of orofacial reactions in response
to an IO infusion of a tastant, which can be used to assess whether the administration of certain drugs alter the tastant’s perceived palatability and reinforcing properties in SA. Typically, during SA, the reinforcer is paired with a brief presentation of a neutral stimulus (i.e., cue light, tone). After multiple pairings, the neutral stimulus acquires conditioned stimulus properties and its presentation may also elicit similar behavioural responses produced by the US. When it acquires the ability to do this, it is referred to as a conditioned stimulus (CS). Responses to the CS can then be investigated in tests of drug-seeking (extinction and reinstatement).

1.3.3. Drug seeking

While SA is considered a model of drug-taking behaviour, it is possible to modify the conditions in operant SA to measure drug-seeking, such as extinction and reinstatement (Millian, Marchant, & McNally, 2011; Bouton and Swartzentruber, 1989). There is evidence that the neural substrates mediating cue-induced seeking are different depending on whether seeking is assessed in a single extinction session or during reinstatement (McLaughlin & See, 2003; Koya et al., 2009).

**Extinction.** Extinction involves the gradual reduction of a previously learned behavior. However, prior to extinction, animals must be trained to respond for a drug reinforcer. Once reliable SA has been established during the acquisition phase, the primary reinforcer is removed, and animals undergo extinction. During extinction training, operant responding is no longer reinforced (Millian, Marchant, & McNally, 2011) and this leads to a reduction in responding on the reinforcer-paired lever. Extinction does not erase the drug memory (Bouton & Swartzentruber, 1989). In fact, extinction-based therapies for drug dependence are not successful as much of what was learned during acquisition remains intact after extinction, as evidenced by
the phenomenon of relapse (Bouton, 2004). Rather, extinction can be conceived of as new learning that involves active inhibition (Bouton & Swartzentruber, 1989). One of the ways in which this inhibition can be removed is through reinstatement.

**Reinstatement.** The most common procedure to study relapse behaviour in the form of drug-seeking has been the reinstatement model. Reinstatement refers to an increase in seeking behaviour precipitated by exposure to drug primes, drug-associated cues, or stress following periods of extinction (Shaham et al., 2003; Bouton and Swartzentruber, 1989). In this dissertation, reinstatement will be induced using both reinforcer-associated cues and primes. Stretch & Gerber (1973) discovered that a non-contingent injection of the previously self-administered drug (drug priming) in small doses can restore operant responding following extinction. This effect of priming was observed with DOA (for example: Stewart, 2003; Stewart & Wise, 1992) and non-drug reinforcers (see review Nair et al., 2009). Then, in 1976, Davis and Smith demonstrated that the reintroduction of previously drug-associated CS was sufficient to reinstate drug-seeking. Since then, a variety of environmental stimuli associated with drug or food SA have been found to reliably induce reinstatement (Shalev, Grimm & Shaham, 2002; Kenny et al., 2006; Hamilin et al., 2006).

**1.3.4. Locomotor sensitization**

Repeated exposure to DOA leads to progressive and long-lasting changes in locomotor responses (Stewart & Badiani, 1993). Altered locomotor behaviour can be observed several weeks or months following a drug free period (Robinson & Berridge, 1993). This process is important, because the sensitizing properties of DOA are implicated, in part, in the development of compulsive drug-seeking behaviour and drug-craving that characterizes addictive behaviours (De Vries et al., 2001). Initially, acute administration of a DOA may increase locomotor
behaviour (Sanchis-Segura & Spanagel, 2006). Following repeated intermittent exposure to that same drug of abuse, the locomotor response may progressively become enhanced (Sanchis-Segura & Spanagel, 2006). This process is referred to as sensitization and is characterized by enhanced psychomotor and reward response to a DOA (Steketee & Kalivas, 2011).

Both non-associative and associative processes can play a role in the development of sensitization of locomotor activity (Schausberger & Peneder, 2017). Associative learning occurs following multiple pairings of unrelated stimuli (biologically salient and neutral stimuli; Sanchis-Segura & Spanagel, 2006). By way of these repeated pairings, the environmental context in which the DOA is administered can acquire motivationally relevant properties that influence the development and expression of locomotor sensitization. In contrast, non-associative learning can occur in response to a single drug exposure (Schausberger & Peneder, 2017). The expression of associative and non-associative behavioural sensitization depends on enhanced mesolimbic DA signalling in the NAc (Di Chiara, 2002). Some researchers believe that the ability of a drug to induce a sensitized locomotor response is predicted by its ability to reinstate drug-seeking behaviour in rats (DeVris et al., 1998, 1999; Vandershuren et al., 1999).

1.4. Similarities between drugs of abuse and highly palatable food

1.4.1. Human

1.4.1.1. Behaviour.

In humans, similar responses can be elicited by DOA and highly palatable food. Cocaine dependent individuals have reported wanting cocaine as much as their favourite food (Goldstein et al., 2010). Similarly, Koeber et al. (2010) found that in heavy-smokers who were exposed to cigarette and palatable food cues, the intensity and the ability to resist food craving was comparable to that of cigarette craving. Traits such as high impulsivity also appear to be a shared
underlying factor precipitating vulnerability to drug abuse, developing obesity, and in the overconsumption of food (Braet et al., 2007; Nigg et al., 2006). Lastly, stress was found to magnify cravings for both DOA (Morley, Levine, Yim, Lowy, 1983) and highly palatable food (Zellner et al., 2006; Steptoe, Lipsey, & Wardle, 1998).

1.4.1.2. Neurobiological.

Consumption of highly palatable foods and, in some instances, the presence of obesity is shown to induce similar neurobiological alterations of the dopaminergic and opioid systems that are also observed following repeated intake of DOA. For instance, neuroimaging studies have revealed a decrease in DA D2 receptor availability in both chronic drug users and obese individuals (Wang et al. 2004; Nader et al., 2006; Volkow et al., 1993; Wang et al., 2001). Evidence suggests that decreased dopamine D2 (D2R) density in the striatum both contributes to the development of addictive behaviours (Dalley et al., 2007; Morgan et al., 2002; Volkow et al., 1999) and is a consequence of prolonged drug use (Nader et al., 2002; Porrino et al., 2004). These types of alterations may lead to decreased reward reactivity, which is believed to enhance consumption of DOA and palatable food as a compensatory mechanism (Johnson & Kenny, 2010; Volkow et al., 2002). This decrease in D2R is also associated with decreased metabolism in the prefrontal and orbitofrontal cortex (Volkow et al., 2008; Volkow et al., 1993). Such reduced activation of PFC and OFC are implicated in lack of top-down regulation of behaviour and may contribute to lack of inhibition of seeking and taking behaviour (Volkow et al., 2017).

Although results are not always consistent, there is evidence that obese individuals may have increased activation of several brain regions implicated in anticipation of consuming highly palatable food (Stice et al., 2008). These effects are specific to highly palatable foods and suggest a heightened effect on reward reactivity. This heightened reactivity is also observed with
food cues. Food and drug cues are shown to activate the same brain regions: amygdala, insula, OFC, and striatum (Tang, Fellows, Small, & Dagher, 2012; Volkow et al., 2001; Volkow & Fowler, 2000); these areas have been shown to regulate processes like incentive salience, inhibitory control, habit formation, and learning. In one study, normal and obese women were shown a picture of energy dense food and researchers found that different brain regions were activated in the two groups (Lutter & Nestler, 2009). In normal women, the dorsal caudate was activated upon presentation of energy dense food. However, in obese women the OFC, PFC, amygdala, dorsal and ventral striatum, insula, ACC, and hippocampus were activated. These data suggest that obese women have an altered evaluation of food reward (Lutter & Nestler, 2009).

1.4.2. Animal

1.4.2.1. Behavioural

There are important behavioural and neurobiological similarities between DOA and highly palatable food in animals as well. Koob and Volkow (2010) describe addiction as a chronically relapsing disorder that involves three stages: binge/intoxication, withdrawal/negative affect, and preoccupation/craving.

Binge/intoxication. One characteristic of “addiction” like behaviour is “bingeing”, which can be described as a high proportion of intake in a short period of time (Mathes et al., 2009). Over time, we see the development of bingeing behaviour at the onset of access to sugar (Bello et al., 2003; Wojnicki, Stine, & Corwin, 2007; Cottone, Sabino, Steardo, Zorilla, 2008; Colantuoni et al., 2001) and DOA (Gilpin, Karanikas & Richardson, 2012; Segal & Kuczenski, 1997). There is also continued drug or food-seeking despite negative consequences (i.e., administration of aversive footshock) (Deroche et al., 2004; Oswald et al., 2011; Teegarden & Bale, 2007). Like DOA, extended access to a palatable diet also induces an escalation of intake
(La Fleur et al., 2010; Colantuoni et al., 2001; Ahmed & Koob, 1998). Another characteristic of addiction is the development of tolerance (decreased effectiveness of a given drug with repeated administration) to one or more of the substance’s effects (Stewart & Badiani, 1993). Like DOA, overtime a gradual decrease in responsiveness to food reward is observed (Cottone et al., 2008; Colantuoni et al., 2001; O’Brien et al., 1996).

Withdrawal/negative affect. When DOA, like opioids, are discontinued or their effects are pharmacologically antagonized, withdrawal symptoms can develop (Minhas & Leri, 2014). Naloxone/naltrexone precipitated withdrawal in sucrose dependent animals leads to similar somatic withdrawal symptoms (i.e., anxiety, aggression, wet dog shakes) as those produced by opioid withdrawal (Colantuni et al., 2002; Avena et al., 2008). This suggests that sucrose induces opioid like neural adaptations (Avena et al., 2008; Nestler, 2005). Furthermore, the time spent in withdrawal has similar effects on “craving” for both sucrose and DOA. For example, following a 1- or 15-day withdrawal period after animals were trained to lever press for cocaine or sucrose, animals showed greater seeking responses 15 days after withdrawal compared to only 1 day after withdrawal from sucrose and cocaine (Grimm et al., 2002). This increase in reactivity to cues over time is known as “incubation of craving” (Grimm et al., 2002; Shalev et al., 2001; Grimm et al., 2005).

Preoccupation/craving. Lastly, both stress and conditioned cues can precipitate relapse to both drug- and sugar-seeking behaviours (Shepard, Bossert, Liu, Shaham, 2004; Lee, Tiefenbacher, Platt, Spealman, 2004; Ghitza et al., 2006; Stewart & de Wit, 1987; Crombag & Shaham, 2002; Davis & Smith, 1976; Grimm et al., 2007).
1.4.2.2. Neurobiological

DOA and palatable food also share a common neural circuitry; most notably, they enhance DA signaling between the ventral tegmental area (VTA) and the NAc to influence learning and the attribution of reinforcing value to the US as well as environmental stimuli that predict its availability (Volkow, Wang, Tomasi, Baler, 2013; Berridge, 2013; DiChiara, 2002; Roitman, Stuber, Phillips, Wightman & Carrelli, 2004). Chronic use of DOA is associated with decreases in the expression of D2 receptors in the striatum (Volkow et al., 1993). A similar reduction in D2R density is observed in the striatum of animals after consumption of a cafeteria-style diet (Johnson and Kenny, 2010) and those with intermittent access to sucrose or glucose (Avena et al., 2008; Colantuoni et al., 2001; Bello et al., 2002). Furthermore, during a sucrose binge, similar DA release and reduction of extracellular acetylcholine occurs in the NAc that is produced by morphine injections (Avena et al., 2008). Subsequent withdrawal from palatable food and DOA is accompanied by an increase in thresholds for reward stimulation. For example, using intracranial self-stimulation (ICSS), withdrawal from a 40-day cafeteria diet elevated brain reward threshold of rats for up to 14 days following the end of the diet (Johnson & Kenny, 2010). Similar alterations in reward threshold are observed during withdrawal from cocaine (Stoker & Markou, 2011), alcohol (Schulteis, Markou, Cole, & Koob, 1995), and heroin (Kenny et al., 2006). From these results it can be inferred that repeated consumption of DOA and palatable food can lead to a disruption in reward processing.

1.4.3. Drug and food interactions

1.4.3.1. Humans

Further evidence of similarities between DOA and highly palatable foods comes from studies that look at interactions between the two. Individuals with substance abuse disorder show
increased consumption of food during treatment (Hodgkins, Fros-Pinedou, & Gold, 2007; Rigolli et al., 2009). For example, an increased craving for sweet food is observed in methadone-maintained individuals (Zador et al., 1996; Nolan & Scaghlhm, 2007), detoxified alcohol abusers (Kampov-Polevoy et al., 2001) and in individuals giving up cigarettes (Toll et al., 2008). There also appears to be an association between heightened preferences for sucrose in individuals and a likelihood of developing cocaine abuse disorder (Janowsky et al., 2003). This relationship is also observed in the opposite direction, where individuals who reported problems with high sugar foods before weight loss surgery were more likely to have an onset of substance use disorders after surgery (Fowler et al., 2014). In fact, there is considerable co-morbidity between eating disorders and the use of illicit drugs and poly substance use (Root et al., 2010). Some studies report that up to 50% of individuals with an eating disorder meet the criteria for alcohol or illicit substance abuse or dependence (Holderness et al., 1994; National Center on Addiction and Substance Abuse, 2003).

1.4.3.2. Animals

Pre-clinical models of cross-sensitization likewise support the notion that exposure to palatable food and/or DOA can alter subsequent responding for incentive stimuli. Sensitization refers to an enhanced response for a stimulus which occurs with repeated intermittent exposure to that stimulus (Steketee & Kalivas, 2011). Therefore, cross-sensitization refers to the idea that pre-treatment with stimulus A results in sensitization to the effects of stimulus B (Callaway and Geyer, 1992; Yang et al., 2003; Vitale, Chen & Kanarek, 2003). The induction of sensitization involves brain structures common to those known to play a role in reward processes, which is the reason it is believed to be a good marker of neurochemical changes that underlie addiction (Sanchis-Segura & Spanagel, 2006). Cross-sensitization is observed across different classes of
drugs. For example, repeated injections of morphine enhance subsequent place preference for amphetamine and cocaine (Steketee & Kalivas, 2011). Additionally, injections of amphetamine into the VTA produce an increase in locomotor response to systemic injections of morphine (Vezina & Stewart, 1990). More recently, this phenomenon has also been observed with food reinforcers. For example, in animals, long-term intake of sucrose enhances the reinforcing properties of opioids (Kanarek et al., 1997; Vitale, Chen, & Kanarek, 2003), stimulants (Vitale, Chen, & Kanarek, 2003; Gosnell, 2005), alcohol (Avena et al., 2004), and nicotine (Clemens, Caille, & Cador, 2010). It also increases locomotor activity to a challenge of amphetamine/cocaine (Avena et al., 2008; LeMerrer & Stephens, 2006) or morphine (LeMerrer & Stephens, 2006).

Alternatively, there is also evidence that repeated exposure to DOA and palatable foods may blunt subsequent responding for incentive stimuli. For example, chronic consumption of a highly palatable diet decreased place preference for amphetamines (Davis et al., 2008), as well as operant responding for sucrose pellets (Gosnell et al., 2010), cocaine (Carroll, Lac & Nygaard, 1989) and ethanol (Carroll, Rodefer & Rawleigh, 1995) in SA studies. Morphine pre-treatment decreases saccharin consumption during withdrawal (Lieblich, Yirmiya & Liebeskind, 1991), although nicotine withdrawal was shown to increase intake of saccharin flavoured foods (Grunberg et al., 1985). Nonetheless, these findings indicate that palatable food and DOA share a unique ability to alter the same neural mechanisms responsible for reinforcement and learning. As a result, repeated exposure to one may consequently alter responding for the other, enhancing risk of abuse. Often, cross-sensitization and cross-tolerance is observed in substances that share similar pharmacokinetic properties; palatable foods are thought to be more potent than other foods because of their processing, which make them more drug-like (Monteiro et al., 2010).
1.5. Differences between drugs and food addiction

1.5.1. Behavioural

Despite similarities, differences between drug and food reinforced behaviour exist. Contrary to the belief that DOA are more powerful reinforcers than sweeteners, some pre-clinical research has revealed otherwise. For example, in choice procedures, when given the option between cocaine and saccharin-paired levers, animals tend to respond more for saccharin (Ahmed et al., 2013; Lenoir & Ahmed, 2008; Lenoir et al., 2007; Cantin et al., 2010) and saccharin-paired cues (Madsen & Ahmed, 2015). In fact, 85% of the animals preferred a sweet drink over cocaine (rats were not food deprived). The preference for sweeteners over DOA continues despite manipulations with short and long access models, as well as early and protracted withdrawal periods (Caprioli, Zeric, Thorndike & Venniro, 2015). In fact, preference for a sweet solution was not overcome by increasing the cocaine dose (Lenoir et al., 2007) or past history of drug consumption (Cantin et al., 2010). Only when concentrations of saccharin were reduced to very low levels, did researchers observe similar preferences for saccharin and cocaine. A preference switch for cocaine was only observed when rats had to expend more effort (i.e., eight times as many responses) to obtain saccharin (Cantin et al., 2010) or food pellets (Christensen et al., 2008). Rats also cease responding for cocaine faster than they do for food, although this was tested in food deprived rats (Christensen et al., 2008, 2009). A preference for food over DOA is also seen in humans; cocaine-dependent individuals noted that their liking and wanting for food was greater than that for cocaine (Goldstein et al., 2010). Taken together these findings indicate that the reinforcing value of palatable food in some cases is greater than that of DOA.
In rhesus monkeys, however, a preference for cocaine over food can be easily established (Nader & Woolverton, 1991, 1992; Aigner & Balster, 1978; Anderson, Velkey, 2002), even when the monkeys are food deprived (Nader & Woolverton, 1991). Additionally, in some cases, rats with extended access to cocaine are more resistant to the effects of an aversive conditioned stimulus (Kearns, Weiss, & Panlilio, 2002) or punishment (Pelloux, Everitt & Dickinson, 2007) than animals with extended access to sucrose, suggesting greater compulsive behaviour produced by DOA. Furthermore, in rats, long-term exposure to cocaine and food was shown to increase the reinforcing value of cocaine but not food (Christensen et al., 2008); although the reinforcing value of cocaine did not surpass the reinforcing value of food. Several studies have also revealed that once reinforcers are removed, drug cues, compared to food cues, result in greater persistence of seeking behaviour (Kearns, Gomez-Serrano, & Tunstall 2011; Cicciocioppo, Martin-Fradon, & Weiss, 2004); an effect which has been replicated in studies that examine the effect of stress on food- and drug-seeking (Ahmed & Koob, 1997; Buczek et al., 1999). Thus, while there are a few situations where highly palatable food is similar to or produces behavioural effects that are greater in magnitude to those observed with DOA, there are a number of situations where the opposite is also observed. Although, it is not entirely clear why this is observed.

1.5.2. Neurobiological

The differences between highly palatable foods and DOA likewise extend to underlying neurobiological mechanisms. It has been demonstrated that there is partial but not complete overlap in the neural mechanisms mediating food- and drug- taking and seeking behaviours (Nair et al., 2009; Tomasi et al., 2015). Carelli et al. (2000) found that different neural populations in the NAc encode information and respond to food and drug stimuli. Differences also arise in the extent to which brain regions are recruited for drug- and food-paired cues. In a recent study by
Yager et al (2015), rats were trained to self-administer the mu-opioid receptor agonist remifentanil or banana-flavoured food pellets over 10 sessions. It was found that rats exposed to food or remifentanil cues displayed greater Fos expression (an indication of increased neural activity; Chung, 2015) for food cues in the central nucleus of the amygdala, medial habenula, paraventricular thalamus, medial nucleus of the thalamus, and the basolateral amygdala (Yager et al., 2015). Hence, even though similar brain areas are recruited, the extent of their involvement in reinforcement may differ for drug and food cues. Furthermore, there are dissociations in the recruitment of brain sub regions in associative learning for drug and food stimuli. For example, within the basolateral amygdala (BLA), inactivation of the rostral BLA decreases drug (Kantak et al., 2002) but not sugar (McLaughlin & Floresco, 2007) seeking behaviour. Conversely, inactivation of the caudal BLA has no effect on drug-seeking (Kantak et al., 2002) but enhances responding for food-paired cues (McLaughlin & Floresco, 2007). A similar dissociation is seen in the PFC, where inactivation of the dorsal medial PFC decreases cocaine seeking but has no effects on food-seeking behaviour (McFarland & Kalivas, 2001).

Differences also arise in the extent to which neurotransmitter systems are involved. For example, the magnitude of DA released by administration of DOA is often greater than that induced by palatable food (Pothos et al., 1991; Wise et al., 1995; Volkow & Wise, 2005). Furthermore, DA release in response to exposure to DOA persists across multiple administrations of the drug, however DA release by repeated consumption of the same food does not persist once the food is no longer novel or the animal is satiated (Avena, Rada, & Hoebel, 2008).

There are also differences with respect to the effects of food and DOA on different DA receptor subtypes. For example, DA D3 receptor antagonism decreases drug-seeking, but not
food-seeking behaviour (Khaled, Pushparaj, Di Ciano, Diaz, & Le Foll, 2014). These differences between DOA and palatable foods also extend to other neurotransmitter systems. For instance, decreases in norepinephrine transmission also decrease the amount of work performed by rats to obtain cocaine but have no effect on operant responding for sucrose (Schroeder et al., 2013) and chow (Schroeder et al., 2010). And, glutamatergic N-methyl-D-aspartate (NMDA) antagonists such as memantine block place preference for morphine, but not food (Popik & Danysz, 1997). Furthermore, nicotinic acetylcholine receptor antagonists decrease responding for nicotine, but not food (Fowler & Kenny, 2011). Lastly, melanocortin 4 receptor activation increases cocaine reward (Hsu et al., 2005), but at the same time decreases food reward (Benoit et al., 2000).

Overall, these results suggest that food and drug reinforcement differ in many ways, which may in turn affect the abuse liability of them.

So, while many researchers have suggested that the overconsumption of highly palatable food and drug addiction are similar (Meule et al., 2014; Pursey et al., 2014), others posit that there is a lack of evidence for the food addiction construct (Ziauddeen, Farooqi, & Fletcher, 2012; Ziauddeen & Fletcher, 2012). One caveat remains that a large proportion of studies making comparisons between drug- and sugar-reinforcement have used experimental conditions that were not always equated, or experiments were conducted in separate cohorts of animals, thereby changing the overall pharmacological experience. Therefore, studies that include direct comparisons between food and drug-reinforced behaviours in the same animals may be more meaningful. Lastly, most studies are conducted using stimulants as the DOA; however, important differences exist between stimulants and other classes of drugs, such as, opiates (for review see Badiani et al., 2011). Therefore, to better understand and make generalizations about the
similarities and differences between palatable foods and DOA, it is important that we include other DOA as comparisons.

1.6. Reward Processing by the Opioid System

The opioid system is recognized as playing an important role in the regulation of both homeostatic and non-homeostatic (hedonic) feeding processes (Nogueiras et al., 2012). The former relating to changes in peripheral energy status that initiates hunger and the later non-homeostatic mechanisms motivated not by hunger, but rather eating for pleasure (Nogueiras et al., 2012). Systemic injections of an opioid agonist increase, whereas antagonists decrease, the intake of sugars (Holtzman, 1979; Mucha & Iversen, 1986; Rudski et al., 1997; Gosnell et al., 2010). This effect on food intake is mediated by changes in palatability. In fact, systemic administration of opioid receptor agonists increases hedonic orofacial reactions to sugar (Rideout & Parker, 1996), whereas antagonists decrease orofacial reactions to sugar (Parker et al., 1992) in rats. In the central nervous system (CNS), hedonic "hotspots” have been identified that mediate these orofacial reactions: the rostrodorsal nucleus accumbens medial shell and the caudal ventral pallidum (Kenny et al., 2005; Smith & Berridge, 2007). It was found that activation of mu-opioid receptors in the NAc shell (Pecina and Berridge, 2005) and the caudal ventral pallidum (Smith & Berridge, 2005) increased the number of tongue protrusions and paw licks in response to intra-oral infusions of sucrose. Interestingly, the stimulation of opioid receptors in areas surrounding the NAc "hotspot" trigger increased food intake but has no effect on orofacial reactions to taste (Smith & Berridge, 2005). This was replicated by Kenny et al. (2005) who demonstrated that rats injected with mu-opioid agonists in the NAc shell were more willing to work for a sucrose pellet even before tasting the reward. This would suggest a role for opioids not only in palatability, but modulating motivation.
There are three main receptor subtypes for opioids: mu, delta, and kappa (Ross & Smith, 1997). These receptors belong to the GTP binding regulatory proteins (Goodman & Gilman, 2001). Opioid receptors are expressed in regions of the brain involved in euphoria (NAc, VTA), pain perception and analgesia (i.e., dorsal horn of the spinal cord, periaqueductal grey etc.), as well as in peripheral organs (Basbaum & Jessell, 2013). Injection of selective opioid agonists into the NAc reveal different roles for the different opioid receptor subtypes. For instance, injections of mu- or delta-opioid agonists into the NAc or VTA stimulate feeding (Lowy & Yim, 1983; Ruegg, Yu & Bodnar, 1997; Gosnell & Majchrzak, 1990), while kappa-opioid agonists have no effect (Merrer, Becker, Befort, & Kieffer, 2009).

Opioids can also have downstream effects on dopamine systems and so in part the effects of opioids can also be mediated by dopamine release. Typically, mu-opioid receptors (MORs) modulate the release of DA by inhibiting the inhibitory effects of GABA interneurons on DA neurons in the VTA, thereby leading to an increase in DA release (Jalabert et al., 2011; Johnson & North, 1992). In fact, morphine (or heroin) CPP was shown to be attenuated by DA receptor antagonists or 6-hydroxydopamine lesions of the NAc (Shippenberg, Bals-Kubik, & Herz 1993), and D2R receptor blockade decreases the rewarding effects of morphine in opiate dependent rats (Laviolette et al., 2002). Conversely, blockade of striatal opioid receptors can also decrease amphetamine induced locomotion (Gonzalez-Nicolini et al., 2003; Wiskerke et al., 2011). And so, both DA and opioid neurotransmitter systems are thought to be involved in reward behaviour, an effect also seen in humans, where drug-dependent individuals often present with decreased D2R density in the striatum (Hietala et al., 1994; Volkow et al., 2012) and increased MOR density (Gorelick et al., 2005; Heinz et al., 2005). Alternatively, obese individuals display decreased MOR availability, but unaltered (Karlsson et al., 2015) or
decreased (Wang et al., 2012) D2R availability. The interaction between them appears to be necessary in treating obesity, as in weight loss pharmacotherapies, MOR or DA receptor antagonists alone do not lead to significant weight loss, however, combined, these drugs are effective at treating obesity by producing significant weight loss following long term use (Greenway et al., 2010; Plodkowski et al., 2009). Furthermore, cross-talk between MOR and DRD2 systems appears to be disrupted in obesity, which may underlie the altered reward processing often seen in obese individuals (Tuominen et al., 2015).

There is evidence that the rewarding effects of opioids are also mediated by DA independent mechanisms (Pert & Sivit, 1977; Kalivas et al., 1983; Pettit et al., 1984; Amalric & Koob, 1985; Hnasko et al., 2005; Olmstead et al., 1998; Bechara & VanderKoy, 1992; Swerdlow, Vaccarino, & Koob, 1985). For example, opioid reward can occur without normal DA function such as in the case of DA depleted mice that are still able to acquire morphine CPP (Hnasko et al., 2005). In another example, DAMGO (a MOR agonist) increased palatable food consumption, an effect which was not altered by prior infusions of DA D1 and D2, or AMPA antagonists (Will, Pratt, & Kelley, 2006) suggesting that food intake induced by MOR activation can be independent of DA and glutamate (GLUT) receptors. It is believed that GABA_A receptor signalling properties in the VTA are important in these DA-independent processes (Ting-A-Kee & van der Kooy, 2012).

### 1.7. The role of dopamine

DA is a neurotransmitter that arises primarily from neurons located in the VTA and substantia nigra, which has major projections to the NAc, PFC, amygdala, and the hippocampus (for review see Adinoff, 2004). DA receptors can be classified into two families: D1- and D2-like receptors (Brown & Makmami, 1972). The D1-like family contains D1 and D5 receptors...
(Sunahara et al., 1990, 1991); whereas the D2-like receptor family contains D2, D3, and D4 receptors (Sokoloff et al., 1990). These two classes of families can be differentiated by different pharmacological and functional properties (Missale et al., 1998). There are two distinct patterns of DA neural activity: DA firing at low frequencies (1-8 Hz), known as tonic activity which regulates motivational processes that occur over time (Grace, 1991), and DA neurons firing at high frequency bursts (>15 Hz), known as phasic firing, which is characterized by large transient increases in DA concentrations. Phasic firing is typically elicited by motivationally salient stimuli (i.e., drug or sugar reward, conditioned stimuli etc.) (Schultz, 1998). Typically, the more rapid the increase in DA release, the stronger the reinforcing effect of the stimulus (Volkow et al., 2007).

The effects of DA signalling are multifaceted, as they are involved in learning, prediction of rewards (Schultz & Dickinson, 2000), attribution of incentive salience to neural representations of rewards/cues (Berridge & Robinson, 1998), and lastly plays a role in attention, sensorimotor behaviour and effort (Ikemoto, & Panksepp, 1999; Horvit, 2000). Together, these responses converge to influence reward pursuit. In fact, mice that are DA deficient lack the motivation to perform tasks such as consuming food, and as a result die of starvation (Palmiter et al., 2008). However, replenishing DA in the dorsal striatum restores the motivation to consume food (Palmiter et al., 2008). Chronic consumption of sugar and DOA can lead to a dysfunctional DA system. Decreases in D2R expression in the ventral and/or dorsal striatum are observed following chronic sugar consumption (Bello et al., 2002; Spranger et al., 2004; Colantuoni et al., 2001), in obesity (Stice, Yokum, Blum, Bohon, 2010; Wang et al., 2001) and in those who repeatedly use DOA (Volkow et al., 2011).
1.8. Neurobiology of food intake

Both homeostatic and non-homeostatic mechanisms regulate food intake (Berthoud 2002, 2011). Homeostatic pathways regulate energy balance and food consumption by influencing the synthesis and release of nutrient sensing substrates (Berthoud, 2011). These substrates respond to changes in hunger, satiety, and adiposity signals to influence food intake.

During hunger, ghrelin is secreted from the stomach and in the CNS acts on the hypothalamus to increase appetite by stimulating the synthesis and release of orexigenic peptides (Popovic & Duntas, 2005). Peripheral, central, and intra-VTA administration of ghrelin increases the motivation to obtain food (Skibicka et al., 2012; King et al., 2011). These orexigenic properties are mediated by ghrelin secretagogue receptors (GHSR1A), which are expressed on neuropeptide Y (NPY) and agouti related protein (AgRP) neurons in the arcuate nucleus of the hypothalamus (Skibicka et al., 2012). When these neurons are activated by ghrelin they stimulate food intake (Crespo et al., 2014). Ghrelin also interacts with the mesolimbic reward system. GHSR’s are expressed in the VTA on DA neurons (Abizaid, 2009). When activated, they stimulate the release of DA in the NAc (Abizaid et al., 2006). Additionally, systemic administration of ghrelin interacts with opioid receptor signaling in the VTA to regulate the reward value of food (Kawahara et al., 2013) and mediate pain relief (Sibilia et al., 2006). Because of these interactions, it would then logically follow that ghrelin also influences responses to DOA; and in fact, we see that administration of ghrelin does increase place preference for cocaine (Davis, Wellman, & Clifford, 2007).

Following food consumption, the stomach releases leptin, an appetite suppressing hormone (Berthoud, 2005; Sobhani et al., 2000). Leptin is a hormone synthesized by white adipose tissue; its circulating levels are proportionate to adipose stores (Friedman & Halaas,
1998), and it functions to suppress food intake and stimulate metabolic processes to dissipate energy stores (Palmiter, 2007; Zhou & Rui, 2013). Leptin activates pro-opiomelanocortin (POMC) & cocaine amphetamine regulated transcript (CART) neurons, as well, it inhibits neuropeptide Y (NPY) and AgRP neurons to suppress feeding (Millington, 2007). Leptin also interacts with the mesolimbic DA system as its receptors are co-localized on DA neurons in the VTA (Figlewicz et al., 2003). When leptin receptors are activated, they inhibit DA neurons and decrease feeding (Blumental & Gold, 2010; Hommel, Trinko, Sears, George et al., 2006). It has also been observed that low doses of DA receptor antagonists injected systemically or into the NAc prolong or escalate food consumption (Bakshi & Kelley, 1991); effects thought to be modulated by DA influence in the ventromedial hypothalamus through actions on NPY and POMC neurons (Morton et al., 2006). Given such interactions between reward and homeostatic pathways for DOA and palatable food consumption, it is likely that the two systems interact to enhance reward related behaviour. Because palatable foods, like sugar, possess both orosensory and post-ingestive properties, it is likely that their consumption is driven by effects on these properties.

1.8.1. Orosensory

The orosensory features of food motivates, in part, the consumption of certain foods (Mela, 2006). Sweet foods typically have a sweet taste because they activate taste receptors (T1R2 and T1R3) on the tongue (Low, Lacy, and Keast, 2014). Their activation initiates a cascade of intracellular signalling that transmits the signal to peripheral nerves where it is carried to the nucleus of the solitary tract, parabrachial nucleus in the pons, the thalamus, and insular cortex (for review see McCaughey, 2008 and Lee & Owyang, 2017).
Sweet taste alone is also reinforcing. Animals are willing to work for and readily consume non-caloric but sweet solutions (i.e. they do not produce a post-ingestive effect) (Casen & Aston-Jones, 2013). Short-term licking, sham-drinking of sweeteners (Xenakis & Sclafani, 1981; Schneider et al., 1986; Hajnal et al., 2016) and the consumption of non-caloric sweeteners (i.e., saccharin) can promote DA release in the NAc (Mark et al., 1991; Scheggi et al., 2013), although these effects are typically very weak (Blackburn, Phillips, Jakubovic, & Fibiger, 1986). Similarly, in humans, researchers have found that sweet taste alone is sufficient to decrease food craving (Michener & Rozin, 1994). For example, in a study by Michener and Rozin (1994), chocolate cravers were presented with a box containing a milk chocolate bar, white chocolate bar (containing none of the pharmacological components of chocolate), capsules containing cocoa (containing the psychoactive component of chocolate) or capsules containing white chocolate. They found that only the consumption of white or milk bars reduced chocolate craving. The authors concluded that these results demonstrate that the orosensory rather than pharmacological effects were primarily involved in chocolate craving. Although, it might be reasoned that previous experience with chocolate consumption may have influenced reports of craving.

1.8.2. Post-ingestive

Independent of taste, the post-ingestive effects of caloric sweeteners are shown to mediate reinforcement. For example, mice lacking taste ion channels are still able to acquire a preference for sipper positions associated with nutrient intake (Zhang et al., 2003). In fact, sugar intake becomes the same within hours to the wild-type mice (intact taste ion channels). Moreover, gastric infusions of glucose promote intake of solutions and support flavour conditioning (Myers et al., 2013; Zukerman et al., 2011, 2013).
DA release is also observed in mice without taste ion channels (for review see de Arajo et al., 2012) and the magnitude of the extracellular DA release in the striatum is correlated with the caloric density of gut infusions (de Arajo et al., 2012). More specifically, the release of extracellular DA in the dorsal striatum increased in proportion to the caloric density of infusions and this was not associated with motor responses (i.e., dry licks) to obtain the intragastic infusions (Ferreira et al., 2012). Although, other studies have shown that the macronutrients in palatable food stimulate the brain reward system independently of their caloric value (Wang et al., 2004). Flavour-nutrient conditioning by gut-glucose infusions can be blocked or attenuated by D1 receptor antagonist injections into the NAc, amygdala, medial PFC, and LH (for review see de Arajo et al., 2012). Tellez et al. (2016), found that taste and nutrition separately control DA levels. Specifically, they found that while taste modulates DA release in the ventral striatum, DA release in the dorsal striatum is associated with metabolic changes (Tellez et al., 2016).

1.9. High Fructose Corn Syrup

The over consumption of high fructose corn syrup (HFCS) in drinks and food is believed to be a factor that has contributed to the development of the current obesity epidemic (Bray, Nielsen, & Popkin, 2004). In countries that consume high rates of HFCS, there is a higher rate of type 2 diabetes compared to countries that mostly consume sucrose (Goran et al., 2013). HFCS is a caloric sweetener that is derived from corn starch, most commonly found in the form of 55% fructose, 42% glucose, and 3% other polysaccharides (HFCS-55 type; White, 2008). Over the years, HFCS has replaced sucrose (table sugar) in most products. Typically, HFCS-55 is perceived to be approximately as sweet as sucrose with uses primarily in soft drinks, cereals, juices, and desserts (Moeller et al., 2009; Bocarsly et al., 2010). The food industry has increased its use of HFCS due to: (1) the inexpensive nature of its production; (2) improved shelf-life via
improved moisture; (3) superior consistency; (4) enhancement of other flavours (HFCS’s sweetness is detected quickly, which results in a clear and more crisp perception of other flavours); and (5) maintains its structural stability over a range of temperatures and acidity levels (Moeller et al., 2009). Because of its increased prevalence in the modern diet, it is important to understand the behavioural, physiological, and neurobiological effects of HFCS.

One primary difference between HFCS and sucrose is that the fructose-glucose (FG) ratios in HFCS are monosaccharides (FG molecules are free floating), whereas in sucrose they are disaccharides (bond linking FG). As a result, the enzymes in the intestine must break sucrose’s disaccharide bond to allow for absorption (Moeller et al., 2009). Additionally, this difference results in alterations in osmotic pressure generated by the smaller FG monosaccharides (in HFCS) compared with the FG disaccharide (in sucrose), which can affect the amount of fluid secreted in the stomach (Soenen & Westerterp-Plantenga, 2007; Bray, Nielsen, Popkin, 2004); this can ultimately result in differences in the intestinal absorption of HFCS and sucrose.

Following the cleaving process, gram for gram HFCS-55 (55%) has a slightly higher level of fructose than sucrose (50%). Individually, fructose and glucose differ in digestion, absorption, and metabolism leading to different acute metabolic effects (Melanson, 2008; Riby, Fujisawa, & Kretchmer, 1993). For example, although, fructose stimulates insulin synthesis it does not release it, most likely because there is a lack of fructose transporters on the beta-cells of the pancreas (Elliott, Keim, Stern, Teff, Havel, 2002; Curry, 1989). Glucose, on the other hand, increases insulin release from the pancreas (Vilsboll et al., 2003). Insulin release is important because it is involved in appetite regulation, inhibits eating, and also increases leptin which inhibits food intake (Schwarts et al., 2000). Furthermore, suppression of the appetite-stimulating
hormone ghrelin is reduced following the consumption of beverages sweetened with fructose relative to glucose (Wylie-Rosett, Segal-Isaacson, Segal-Isaacson, 2004; Teff et al., 2004). These effects of glucose and fructose then regulate satiety and eating.

Consumption of fructose relative to glucose may also be more lipogenic. Fructose metabolism promotes greater de novo lipogenesis in the liver and is associated with the development of non-alcoholic fatty liver disease (Ouyang et al., 2008). Fructose consumption is also associated with elevating triglyceride synthesis and uric acid levels (Angelopoulos et al., 2009), which are implicated in the development of metabolic syndrome (Nakagawa et al., 2006). Stanhope et al. (2008) also report that isocaloric fructose-sweetened, but not glucose-sweetened, beverage consumption leads to enhanced visceral adiposity and lipids and decreased insulin sensitivity in overweight and obese individuals. These differences in hepatic metabolism between fructose and glucose may influence the increased energy intake observed with high fructose products and could theoretically contribute to the different short and long-term effects of fructose compared to glucose.

Only a handful of studies have explored differences between HFCS and sucrose; and those studies have found mixed results. Generally, it is believed that the small difference in fructose content between HFCS and sucrose is not relevant from a health perspective (White, 2008). In support of this, several studies examining responses to consuming HFCS and sucrose have found a lack of difference in total energy intake (Soenen & Westerterp-Plantenga, 2007), macronutrient intake (Melanson et al., 2007), taste (White, 2008), satiety (Monsivais, Perrigue, Drewnowski, 2007; Soenen & Westerterp-Plantenga, 2007), thirst (Melanson et al., 2007), circulating insulin and leptin (Lowndes et al., 2012; Yu, Lowndes & Rippe, 2013; Melanson et al., 2007), glucose (Monsivais, Perrigue, Drewnowski, 2007; Melanson et al., 2007), uric acid
(Angelopoulus et al., 2009; Akhavan & Anderson, 2007), and ghrelin (Monsivais, Perrigue, Drewnowski, 2007; Melanson et al., 2007). And so, it has been argued that there is no unique relationship between HFCS and obesity (Rippe et al., 2012). One important consideration in studies reporting no differences in peripheral and central responses to fructose and glucose is that most investigated the acute effects of HFCS and sucrose, rather than long-term effects.

Contrary to the belief that there are no differences between HFCS and sucrose, a subset of research has revealed distinctions. More specifically, these studies have found that: (1) female mice fed mixtures of HFCS in amounts comparable to the American diet were less likely to reproduce and more likely to die early (Ruff et al., 2014); (2) rats given water sweetened with HFCS gained more weight than rats given water sweetened with sucrose (Bocarsly, Powell, Avena, & Hoebel, 2010; Light et al., 2009); (3) HFCS increased fat accrual and circulating levels of triglycerides (Levy et al., 2015; Light et al., 2009; Bocarsly et al., 2010); (4) chronic HFCS consumption led to memory impairments during memory tests, which was not found with sucrose; (5) HFCS significantly increased proinflammatory cytokines (interluken 6 and interluken 1B) in the dorsal hippocampus (Hsu et al., 2015); (6) there was an increased preference for HFCS to sucrose in naïve rats (Ackroff & Sclafani, 2011); and lastly (7) the consumption of HFCS pellets was associated with significant decreases in Fos density in areas that regulate food intake (periforinial area of the LH and arcuate nucleus), a result that was not observed with sucrose (Levy et al., 2015). Taken together, these results are consistent with the idea that sucrose and HFCS can engender different neural and physiological changes that may uniquely contribute to HFCS’s deleterious effects on reward and metabolic disease.

There is little research on the contribution of the 3% polysaccharide in HFCS on physiological, behavioural, and neurobiological responses. One exception is that Ackroff and
Sclafani (2011) report that the palatability of HFCS is enhanced by the inclusion of the 3% glucose polymers that make up HFCS, when compared to a similar concentration of a FG mixture. This is notable because it suggests that even small additions of saccharides may alter consummatory behaviour and palatability of solutions. Hence, the extra 5% fructose in HFCS could have effects that uniquely contribute to its “addictive” potential.

1.10. Oxycodone (subsections from Minhas & Leri, 2017)

Prescription opioids are important therapeutic agents in the treatment of pain. However, the over-prescription of opioids, in combination with escalating rates of illegal sharing and their high potential for abuse and addiction, has led to a public health crisis. In a World Drug Report published by the United Nations, Canada and the United States have the highest rate of prescription opioid use in the world (United Nations, 2016). The increase in opioid abuse appears to be concurrent with the increase in the number of prescription opioid medications written and dispensed (NIDA, 2016).

Of particular concern is the rising rate of oxycodone use. It is reported that the consumption of oxycodone increased almost 500% from 1999-2011 (Jones, 2013). The National Survey on Drug Use and Health in 2015 revealed that in the United States approximately 27.9 million people aged 12 or older (10.4% of the population) used oxycodone products (Hughes et al., 2015). From this, 4.3 million people aged 12 or older reported misusing these products in the past year, representing 1.6% of the population aged 12 or older. At the same time, the increase in opioid abuse has increased the number of emergency room non-medical visits, overdose deaths, and incidences of neonatal abstinence syndrome (SAMSA, 2011; Patrick et al., 2012; Sproule, Brands, Li, & Biro, 2009; Canadian Centre on Substance Abuse, 2015; Gomes & Jurlink, 2016).
Oxycodone (6-deoxy-7,8-dihydro-14-hydroxy-3-O-methyl-6-oxomorphine) is a semisynthetic opioid analgesic that is a derivative of the opioid alkaloid thebaine, which is found in opium poppies. Some common street names for oxycodone include hillbilly heroin, perc, and OC. It is an active ingredient in a number of formulations which include intravenous injections, immediate release solutions/capsules (Percocet, Percodan, OXY IR, OXY FAST) and extended release preparations (OxyContin). Similar to other opioids, oxycodone induces pharmacological effects that include analgesia, euphoria, feelings of relaxation (Walsh et al., 2008; Jones et al., 2011; Colucci et al., 2014) and adverse side effects like respiratory depression, constipation, cough suppression, and sedation (Drug Enforcement Administration, 2014; Walsh et al., 2008; Jones et al., 2011; Colucci et al., 2014).

Although oxycodone is an efficacious pain reliever, the abuse of and addiction to oxycodone has become a major public health concern. It is a semisynthetic opioid analgesic that acts as a mu- and kappa-opioid agonist (Gallego, Barón, & Arranz, 2007; Ross & Smith, 1997) and has reinforcing properties in both humans and animals (Leri & Burns, 2005; Grella et al., 2011; Walsh et al., 2008). It possesses characteristics that are common to most addictive substances, including: reinforcement, relapse, and neurobiological alterations.

Reinforcement. All DOA can act as reinforcers; that is, they increase the strength of the response that produces them (Everitt & Robbins, 2005). For example, if an individual takes a drug that results in a positive experience, they are then likely to repeat that behaviour. Various studies have shown that non-dependent recreational opioid users who received oxycodone reported drug-liking, euphoria, and a willingness to take the drug again (Colucci et al., 2014; Zancy & Drum, 2010; Stoops et al., 2010; Walsh et al., 2008). When drug use is chronic it can
lead to a loss of control over drug taking, and over time becomes compulsive and habitual (Ostlund & Balleine, 2008).

Oxycodone produces reinforcing effects similar in magnitude to other opioids like heroin and morphine with substantially fewer negative effects (Comer et al., 2008). The differences in the positive and negative effects produced by the different classes of opioids may differentially contribute to their addiction liability. This is supported by drug choice procedures, which have found that when given the choice between high doses of morphine and oxycodone, non-dependent participants strongly prefer oxycodone (Comer et al., 2013). Similarly, Babalonis et al. (2013) found that if given the chance to work for money and oxycodone, non-dependent prescription opioid abusers worked significantly harder to obtain high doses of oxycodone. And at lower doses of oxycodone the amount of work performed to obtain oxycodone and money was similar (Babalonis et al., 2013). In the same individuals, oxycodone produced greater ratings of “drug liking” and “highs,” compared to placebo and codeine (Babalonis et al., 2013). These data suggest that, although oxycodone is similar to other opioids in many ways, it possesses reinforcing properties that set it apart; this may then contribute to its increased abuse potential.

Oxycodone also has reinforcing properties in non-human animals. It is readily self-administered in rodents (Leri & Burns, 2005; Zhang et al., 2009; Wade et al., 2015; Secci et al., 2016) and monkeys (Beardsley et al., 2004). Similar to other opioids, self-administration with oxycodone shows an escalation of lever pressing over time and increases in the amount of work performed (motivation) to obtain the reinforcer (Wade et al., 2015). It also produces a robust CPP (Kirkpatrick & Bryant, 2015; Campbell et al., 2012; Rutten et al., 2011). Furthermore, repeated treatment with oxycodone facilitates psychomotor sensitization (Leri & Burns, 2005; Niikura et al., 2013; Liu et al., 2005; Emery et al., 2015, 2016). The risk of relapse during
periods of abstinence poses a major challenge in the treatment of opiate drug addiction (Gossop et al., 1989; Gossop et al., 2002). Given the magnitude of the oxycodone abuse problem, it is surprising that little research has been performed to characterize oxycodone relapse. In the research available, similar to other DOA, oxycodone in the CPP paradigm induces relapse after exposure to drug paired cues, stress, and/or re-exposure to the drug itself (Leri and Burns, 2005; Campbell et al., 2012; Grella et al., 2011).

The opioid system is heavily linked to the mesolimbic dopamine (DA) system (Johnson & North, 1992). One of the G-protein coupled receptors, the µ-opioid receptor, is found throughout the mesolimbic system and modulates DA release in the nucleus accumbens, affecting responses to drug reward (Le Merrer et al., 2009). Opioids act on the mesolimbic DA system through inhibition of GABA interneurons in the ventral tegmental area (VTA), which exert an inhibitory control on DA neurons in the NAc. Hence, opioids indirectly activate the DA system through GABA inhibition (Johnson & North, 1992).

Weele and colleagues (2014) profiled differences in how oxycodone and morphine affected DA activity. They utilized fast-scan cyclic voltammetry and microdialysis combined with mass spectrometry to reveal that during the first minute following a morphine infusion there was an increase in extracellular DA and GABA; however, this increase in GABA concentration was not observed after an oxycodone infusion. Since, GABA inhibits DA activity, it offers an explanation as to why phasic DA release activity for morphine returned to baseline following the first minute but persisted for oxycodone throughout the 15 minute measurement period; it would also explain why there was a greater amplitude of phasic DA (DA transients) for oxycodone (Weele et al., 2014). Increases in DA transients are thought to be associated with increased addiction liability and so it is possible that oxycodone has greater addictive liability, which
would also explain the earlier study reported where oxycodone was more reinforcing than morphine in a drug-choice procedure (Comer et al., 2013). This increased responsiveness to DA has also been demonstrated in studies where mice pretreated with oxycodone showed greater locomotor supersensitivity to quinpirole (a D2/D3 DA receptor agonist) than morphine pre-treated mice (Emery, Bates, Wellman, Eitan, 2015). Overall, these results suggest oxycodone exerts effects on the DA system that are different from other opioids. Although there has been a rise in the abuse of oxycodone, only a few studies are specifically aimed at identifying its abuse potential.

1.11. Rationale and objectives

In a subset of vulnerable individuals, overconsumption of highly palatable foods occurs in a manner that is similar to DOA (Volkow et al., 2012; Tomasi et al., 2015). This has led to the food addiction hypothesis. The food addiction hypothesis suggests that highly palatable food can promote “addictive” like behaviours by increasing sensitivity to food reward and induce eating patterns that lead to excessive and compulsive intake of food (Ludwig et al., 1999). The current dissertation addressed the question of whether palatable food can engender “addictive”-like responses using high fructose corn syrup (HFCS). HFCS is a widely used sweetener in the North American diet (White, 2008). It is believed to have properties that uniquely contribute to its “addictive potential.” It was compared with oxycodone (OXY), a widely used and abused prescription opioid. In brief, the aim of this thesis was to investigate behavioural and pharmacological processes underlying HFCS and OXY reinforcement and the interactions between them. The proposed studies should further clarify parallels and points of divergence between behavioural regulation behind sugars and DOA by using various models of abuse liability. To this end, three experiments were designed to examine:
1. **The effect of HFCS exposure on responses to OXY**

To examine the impact of HFCS exposure on subsequent responses to OXY, male Sprague Dawley rats drank HFCS (0% or 50%) in home cages. Following a sugar-free period, responses to OXY conditioned approach, locomotion, and DA release in the NAc were assessed. The food addiction hypothesis would predict that if HFCS and OXY act on similar systems in the brain, then HFCS pre-exposure should alter responses to OXY.

2. **Behavioural patterns of responding and interactions between HFCS and OXY using self-administration.**

Operant SA procedures were employed to study the similarities and differences in behaviours reinforced by HFCS and OXY consumption. First, animals were trained to self-administer HFCS (8, 25, or 50%) or OXY (0.05, 0.1, or 0.2 mg/kg) (Single SA). Responses were then assessed during acquisition of SA, extinction, and during cue and prime induced reinstatement. The food addiction hypothesis would postulate that palatable rewards can lead to addictive behaviours because they act on systems of learning and reinforcement that are targeted by DOA. This leads to the prediction that “taking” and “seeking” behaviours reinforced by palatable rewards and DOA should be comparable in a situation where their availability, or availability of their conditioned stimuli, is similar.

Then, to examine interactions between HFCS and OXY, both HFCS (50%) and OXY (0.2 mg/kg) were self-administered in the same animals (Dual SA). Responses were then assessed during acquisition of SA, extinction, and during cue- and prime-induced reinstatement. The food addiction hypothesis would predict that if HFCS and OXY act on
similar systems, then we should observe interactions between the reinforcers; where the self-administration of one would change responses to the other.

3. The effect of bupropion on HFCS and OXY cue-induced reinstatement

Operant SA procedures were employed to investigate the effect of bupropion on cue-induced reinstatement. Animals were trained to self-administer both HFCS (50%) and OXY (0.2 mg/kg) (Dual SA). Following extinction of instrumental responding, animals were pre-treated with bupropion (0, 10, 30 mg/kg) and responses were measured on tests of cue-induced reinstatement. The food addiction hypothesis would predict that if HFCS and OXY act on similar systems of learning and reinforcement, that pre-treatment with BUP should alter responses to cues in a similar manner.
Chapter 2

The effect of high fructose corn syrup pre-exposure on oxycodone-induced conditioned approach, locomotion, and dopamine release in the nucleus accumbens
2.1. Abstract

Introduction. Opioids are important therapeutic agents in the treatment of pain. However, increasing use of prescription and non-prescription opioids has led to an increase in abuse/addiction and opioid-related mortality in North America. Therefore, it has become important to identify factors that might increase vulnerability to opioid abuse. One of these factors may be our diets. There is evidence that highly palatable foods (i.e., high in fat and sugar) act on reward pathways in the brain similar to DOA. This leads to the prediction that sugars and DOA interact at both neural and behavioural levels. Therefore, we investigated whether chronic HFCS pre-exposure would affect responses to oxycodone, a widely used and abused prescription opioid. Methods. Male Sprague-Dawley rats received in their home cages: 0% HFCS 24h a day, 50% HFCS 24h a day (continuous access group, CONT), or 50% HFCS 12h a day (intermittent access, INT) for 26 days. Following a 9-day sugar free period, animals were tested on one of the following: (1) place conditioning (biased design) involving: pre-test, place conditioning (0, 0.16, or 2.5 mg/kg OXY SC; 3 pairings each over 6 days), and a test of preference; (2) locomotion involving context-dependent treatment with OXY (0, 0.16, or 2.5 mg/kg SC) over 5 days, followed by a 9-day drug-free period, following which locomotor activity was measured in the drug-paired context, both in the absence and, 24 hrs later, in the presence of OXY; (3) in vivo microdialysis of extracellular DA in the nucleus accumbens prior to, during, and following an injection of OXY (0 or 2.5 mg/kg SC). Results. It was found that 0.16 and 2.5 mg/kg OXY produced a place preference, but this was not modified by HFCS pre-exposure. Furthermore, HFCS pre-exposure decreased OXY-induced, but not context-induced locomotion. Lastly, microdialysis data revealed that HFCS pre-exposure decreased the OXY-induced dopaminergic response in the NAc. Conclusion. These results suggest that opioids and sugars interact at both...
behavioural and neural levels, indicating that nutrition has the potential to influence some responses to opioids, and this may be relevant to the licit and illicit use of these drugs.
2.2. Introduction

Prescription opioids are important therapeutic agents in the treatment of pain (Minhas & Leri, 2017). However, the over prescription of opioids, in combination with escalating rates of illegal sharing and their high potential for abuse has led to a public health crisis, as it raises health care costs, rates of crime, and most importantly leads to a loss of productivity and quality of life for those affected (SAMHSA, 2016). Therefore, it is important to identify factors that may contribute to increased vulnerability to opioid abuse and addiction.

One factor that might alter the effects of opioids is our diet. It is known that highly palatable foods, such as those high in sugar, act on neural substrates that are similar to drugs of abuse (DOA). For example, both enhance dopamine (DA) signalling between the ventral tegmental area and the nucleus accumbens (NAc) (Volkow et al., 2013; Everitt & Wolf, 2002). If sugar and DOA similarly affect neural systems, it can be hypothesized that sugars and DOA will interact at both behavioural and neural levels. There is evidence for this interaction in both clinical and pre-clinical studies. In clinical studies, it was found that giving up cigarettes resulted in increased sweet cravings (Toll et al., 2008). Similarly, in opioid dependent individuals there was a shift in preference from foods that were rich in fat/protein to foods high in sugar (Nolan & Scagnelli, 2007).

Further support of sugar-drug interactions comes from pre-clinical studies, where long-term intake of sucrose enhances the rewarding properties of stimulants (Vitale, Chen, & Kanarek, 2003) and increases motor activity after a challenge with stimulants (Avena et al., 2008). One caveat of these studies is that, for sucrose to produce these enhanced neural and behavioural responses that are similar to DOA, it is necessary to induce “bingeing” through an intermittent access regimen, which involves alternating cycles of repeated food deprivation. For
food, bingeing refers to an escalated intake during a short-defined time period, where there is more eating than would be typically consumed (Corwin et al., 2011; Rada, Avena & Hoebel, 2005). A few confounds of using food deprivation have been identified: (1) it may only characterize situations where there is food deprivation, followed by bingeing; (2) consumption is likely driven by a need to overcome energy deficits; and (3) food deprivation is a form of stress that may increase the rewarding properties of stimuli (Carr, 2002; Frank et al., 2005; Holsen et al., 2012). Therefore, it is important to characterize the effects of sugars in the absence of food deprivation/restriction paradigms.

Most research investigating the effects of sugars uses sucrose (Vitale, Chen & Kanarek, 2003; Gosnell, 2005; Avena and Hoebel, 2003; Avena et al., 2004, 2005; Avena et al., 2008; Vucetic et al., 2010; Rada et al., 2005). However, there is evidence that different sugars (i.e., high fructose corn syrup) affect behavioural and neural markers in different ways (Levy et al., 2015). Because high fructose corn syrup (HFCS) is a widely used sugar in our diet (White, 2008), it is important to investigate its effects. Previous research in our lab indicates that HFCS is consumed in a manner that is similar to DOA and produces neural alterations also similar to DOA, and greater in magnitude to those observed with sucrose (Levy et al., 2015).

Lastly, a majority of previous research has explored the effect of highly processed sweeteners on stimulant consumption (Ahmed et al., 2013; Lenoir & Ahmed, 2008; Lenoir et al., 2007; Cantin et al., 2010). Although there are commonalities in how both psychostimulants and opiates affect brain and behaviour, there are also considerable distinctive features of each; for example, they have different primary mechanisms of action in the body and brain (for review see Badiani et al., 2011). Furthermore, sugars activate both opioid and DA systems in regions of the brain involved in reward (Colantuoni et al., 2001; Bello et al., 2002; Spangler et al., 2004), and,
considering the opioid epidemic, it would be interesting to see how certain nutrients in our diet can influence responses to opiates. Therefore, the current study, explored whether pre-exposure to HFCS (55% fructose to 45% glucose) altered responses to oxycodone (OXY), a widely used and abused prescription opioid (Minhas & Leri, 2017). More specifically, this study investigated whether HFCS pre-exposure would affect OXY-reward (Experiment 1), OXY-induced locomotion (Experiment 2), and OXY-induced elevation of DA concentrations in the NAc (Experiment 3), a region of the brain linked to reward functions (Olsen, 2011). If sugars and DOA similarly affect neural systems, it can be predicted that HFCS pre-exposure will alter both behavioural and pharmacological responses to OXY.

In Experiment 1, we tested the effect of continuous (CONT) and intermittent (INT) HFCS access on OXY reward. However, because there was no effect of CONT and INT HFCS access on OXY reward, INT HFCS access was not assessed in Experiments 2 and 3; instead in Experiments 2 and 3, the effects of 0% HFCS CONT and 50% HFCS CONT on responses to OXY were assessed.

2.3. Materials and Methods

2.3.1. Subjects

Male Sprague Dawley rats (Charles River Laboratories, St-Constant, QC, Canada), weighing approximately 200-250 g at the beginning of the experiment, were housed individually in standard rat cages (polycarbonate; L 50.5 X 48.5 X 20cm) with bedding and environmental enrichment on a reverse 12-h light/12-h dark cycle (lights on at 7 am; off at 7 pm) and behavioral testing occurred during their active cycle. All experiments were approved by the Animal Care Committee of the University of Guelph and were carried out in accordance with the recommendations of the Canadian Council on Animal Care.
2.3.2. Apparatus

2.3.2.1. Home-cage self-administration

No-drip 100 mL water bottles (Thermo Scientific, Waltham, MA, USA) with rubber stoppers (Fisher Scientific, Ottawa, ON, Canada) and metal spouts (Ancare, Bellemore, NY, USA) were made available in the home cage of rats alongside standard water bottles to measure drinking of 0 or 50% HFCS solutions. HFCS consumption was measured daily by quantifying differences in HFCS remaining from the previous day. Water (1mL/gram) and food (Tecklbad Global 18% protein, 4% fat Rodent diet; Envigo RMS Inc, Indianapolis, IN, USA; 3.1 kCals/gram) were made available ad libitum. Daily measurements of HFCS, water, chow, and weight were recorded.

2.3.2.2. Conditioned place preference

Place conditioning was conducted in boxes (custom made, 6 in total) constructed of semitransparent Plexiglas that included two compartments of equal size (30 x 40 x 26 cm) separated by a removable insert (dark gray PVC) with or without a small square opening (10 x 10 cm) at the back. The compartments differed in both visual (i.e., marbled white and black pattern on the wall of one compartment and vertical white and black stripes on the other compartment wall; objects external to the boxes such as cabinets, tables and computer) and tactile cues; one compartment contained a black ceramic floor tile (30 x 30 x 0.5 cm). Each box was covered by black wire mesh to allow automatic video tracking (EthoVision v3, Noldus, The Netherlands). Using this software, it was possible to create a virtual transition zone (approximately the size of a 400 g rat) when the inserts with openings were used for place conditioning testing.
2.3.2.3. Locomotion

Locomotion chambers were boxes (custom made, 12 in total) constructed of semitransparent Plexiglas (30 x 40 x 26 cm) lit by individual LED lights (42 dodes). Each box was covered by black wire mesh to allow automatic video tracking (EthoVision v11.5, Noldus, Wageningen, Netherlands). Distance moved was defined as total horizontal movement (expressed in cm).

2.3.2.4. Microdialysis

Microdialysis boxes were four Plexiglass chambers (model ENV-008CT, Med Associates, Georgia, VT), each enclosed in a larger sound-attenuating plexiglass chamber (model ENV-018M, Med Associates). Infusion pumps (Model Pump 11 Elite, Harvard Apparatus) for the delivery of artificial cerebrospinal fluid (aCSF) were positioned outside the sound-attenuating chamber.

A microdialysis probe (custom made 2 mm length of semipermeable dialysis membrane [Spectra/Por in vivo Microdialysis Hollow Fibers, 240 µm OD, 13 000 MW cutoff], closed at one end and attached to a 23 mm long, 26-gauge piece of stainless steel tubing, protruding 2 mm from the guide shaft) was used to reach the intended site in the NAc.

Probes were inserted 1 week after surgery, and perfused with an aCSF (NaCl 147 mM, KCl 2.8 mM, CaCl₂ 1.2 mM, MgCl₂; pH 7.4) solution with a flow rate of 0.6 µL/min. There was a 2-hour habituation period. Following this, dialysate samples were collected in 20-minute intervals, with 12 µL used for analysis. Samples were immediately put on dry ice following collection, and then stored in -80 C until DA was quantified using high-performance liquid chromatography (HPLC).
**HPLC detection of dopamine.** The dialysate from the samples were analyzed for DA using the Eicom HTEC-510 HPLC/ECD system (Eicom USA). Each sample was extracted from the vial and loaded on a C-18 reverse-phase column (PP-ODS II, 4.6 x 30 mm, Eicom USA) using a manual injection port (Rheodyne 9725i; 20 µL loop). The column was maintained at a temperature of 25°C with a mobile phase (0.1 M Phosphate buffer pH5.4 including 1.5% methanol, 500 mg/L Decansulfonate sodium salt (DSS) and 50 mg/L 2Na-EDTA) set at a flow rate of 500 uL/min. Electrochemical detection of DA was determined using a graphite working electrode (WE-3G, Eicom USA) maintained at a potential of +400 mV relative to an Ag/AgCl reference electrode (RE-500, Eicom USA). The concentration of DA in the dialysate samples was converted to percent baseline (determined by the mean pg/µl of the first three baseline readings).

### 2.3.3. Surgical procedures

For microdialysis experiments, a subset of the rats underwent stereotaxic surgery during which a stainless-steel guide cannula (21G, 8mm below pedestal; C312G/SPC from Plastics One) was implanted unilaterally to a position just above the NAc using the following coordinates relative to bregma: AP: 2.1mm; LM 1.2mm; DV 7.8mm (Paxinos and Watson, 2005) from the skull surface. The cannulae were fastened with dental acrylic and anchored with 4 stainless steel screws to the skull. Animals were allowed to recover from the surgery for 7-8 days before microdialysis testing.

### 2.3.4. General procedures

#### 2.3.4.1. Pre-exposure

In addition to regular chow and water, three different groups of animals received in their home cages: 0% HFCS \(n = 70\) 24h a day; 50% HFCS 24h a day (continuous access group,
CONT; n = 84); or 50% HFCS 12h a day (intermittent access beginning 4 h into the dark cycle, INT; n = 28) for 26 days. After 26 days, HFCS bottles were removed and animals received a 9-day sugar-free period. A 9-day sugar free period has previously been utilized in studies demonstrating significant changes in behavioural and neural responses following sucrose exposure (Hoebel et al. 2009; Avena et al., 2008). More specifically, it was observed that repeated consumption of DOA or palatable food lead to long lasting neuroplastic changes that take time to develop. These changes are not apparent 1 day following withdrawal, however manifest around 7 days after withdrawal and can sometimes last 1-3 months following last exposure (Grimm et al., 2005; Lu et al., 2004). Following the 9-day sugar free period, animals were tested on one of the following experiments: place conditioning, locomotor conditioning, or microdialysis.

In a subset of animals, to assess whether INT access to HFCS produced bingeing behaviour, a phenomenon that is hypothesized to lead to the neural alterations that cause enhancement in motor responses and reward (Rada et al., 2005), we measured HFCS and food consumption at 3 different time points: 10 am, 5 pm, and 9 pm (see Figure 1 for details of home cage measurements).

2.3.4.2. Experiment 1: Place conditioning

Place conditioning included three phases: pre-test, conditioning, and test of conditioning. Pre-test: Animals had free access to both compartments for 20 min, and time spent in each was measured. The compartment in which each animal spent less time (at least 1 second) was designated to be paired with the drug (i.e., biased design). Conditioning: 24 hours later, rats were injected with vehicle or OXY (0, 0.16 or 2.5 mg/kg), and were immediately confined to the vehicle- or drug-paired compartment, for 30 min. Over 6 days, rats received a total of three
vehicle and three OXY compartment pairings, counterbalanced, each 24 h apart. Test of conditioning: 24 hrs following the last conditioning session, animals were given free access to both compartments for 20 min. No injections were given prior to the conditioned place preference (CPP) test. See graphs for sample sizes.

2.3.4.3. Experiment 2: Locomotion testing

Habituation (Day 1). Animals were placed into locomotion chambers for 30 minutes and activity measured. Based on the test results, within each pre-exposure condition (0% HFCS or 50% HFCS) rats were re-assigned into three groups (6 groups it total) so that locomotor activity between the groups was not significantly different from each other.

Treatment sessions (Day 2-6). Treatment sessions occurred over 5 consecutive days. Rats were injected with OXY (0, 0.16, 2.50 mg/kg; SC) and immediately transferred to locomotion chambers for 120 minutes where horizontal activity was measured. Opioids have been reported to have biphasic effects on locomotor activity (suppression followed by stimulation; Babbini & Davis 1972; Vasko & Domino, 1978; Iwamoto, 1984), therefore based on previous studies animals were tested in the appropriate context for 120 minutes. Groups were as follows: (1) 0% HFCS + 0 mg/kg OXY, (2) 0% HFCS + 0.16 mg/kg OXY, (3) 0% HFCS + 2.50 mg/kg OXY, (4) 50% HFCS + 0 mg/kg OXY, (5) 50% HFCS + 0.16 mg/kg OXY, or (6) 50% HFCS + 2.50 mg/kg OXY.

Drug-free period (Days 7-15). Following treatment with OXY (0, 0.16, or 2.50 mg/kg SC) across 5 days, animals received a 9-day drug free period.

Test of context-induced locomotion (Day 16). Animals were placed into the drug-paired context and locomotor activity was measured (60 minutes), to assess the impact of context-cues on locomotion (Walter & Kuschinsky, 1989; Wei & Li, 2014; Li et al., 2010; Bardo &
Neisewander, 1987; Rauhut, Gehrke, Phillips & Bardio, 2002). It is typical that context-induced locomotion tests are shorter in duration than tests with the drug on board is because effects of the context on locomotion are typically observed within the first 30-40 minutes of testing (Walter & Kuschinsky, 1989; Wei & Li, 2014; Li et al., 2010; Bardo & Neisewander, 1987; Rauhut, Gehrke, Phillips & Bardio, 2002).

Test of locomotion after challenge with oxycodone (Day 17). Twenty-four hours later, rats received an injection of OXY (0, 0.16, or 2.50 mg/kg SC) at the same dose that was administered during treatment sessions and were immediately placed into locomotion chambers and locomotor activity was measured for 120 minutes. This test was performed to assess the impact of HFCS pre-exposure on OXY induced behavioural sensitization.

2.3.4.4. Experiment 3: Microdialysis

On the day of sampling, animals were acclimatized to chambers for a period of 120 mins, following which baseline dialysate samples were collected in 20-minute intervals for 60 mins. Animals were then injected with OXY (0 or 2.5 mg/kg; SC) and samples were collected at 20-minute intervals for approximately 140 minutes.

Microdialysis is an in vivo sampling technique, useful in the assay of extracellular fluid. A semi-permeable membrane is stereotaxically implanted into a discrete brain region. The dialysis buffer (aCSF) is perfused through the membrane through tubing attached to a syringe pump. Substances in the brain that are low concentration in buffer and that are small enough to pass through dialysis membrane (includes all neurotransmitters and some peptides) will diffuse into dialysis membrane and will flow through another tube for collection (Torregrossa & Kalivas, 2008). The contents of the dialysis membrane can then be determined by several methods that included high performance liquid chromatography coupled with a detector. The biggest
advantage of this technique is the ability to measure neurotransmitters and their metabolites in awake, freely moving animals both pre-and post-drug administration (Darvesh et al., 2011).

2.3.5. Histology

The histological identification of cannula tracts and probe placements was performed as follows: Following the completion of microdialysis testing, rats were sacrificed and perfused as described by Leri et al. (2007) before having their brains removed. Coronal sections (60 µm) of NAc were taken using a cryostat and mounted on glass slides. This was followed by cresyl violet staining, and placements were examined using a microscope.

2.3.6. Drugs and High Fructose Corn Syrup

Oxycodone (Noramco Inc., Wilmington, DE) was dissolved in 0.9% physiological saline and administered subcutaneously (SC) at a dose of 0, 0.16, or 2.5 mg/kg/ (Leri & Burns, 2005; Grella et al., 2011; Niikura et al., 2013).

Pure HFCS (55-formula for human consumption, 0 g fat, 0 g protein and 77 g carbohydrate per 100 g serving; Ingredion, Mississauga, Canada) was prepared in a 50% solution with reverse-osmosis water. This concentration was chosen because rats readily consume it, and it engenders “liking” responses as measured by tongue protrusions in taste reactivity (Levy et al., 2014), and rats stabilize its intake after approximately 3 weeks of access (Levy et al., 2014).

2.3.7. Statistical analyses

One, two, or three-way factor Analyses of Variance (ANOVA) were used as appropriate. In cases of significant interactions or main effects, individual mean differences were identified by multiple comparisons using the Student-Newman-Keuls method ($\alpha = 0.05$). Analyses were performed using SigmaStat (3.5 for Windows, Systat Software, Inc).
2.4. Results

2.4.1. Home cage consumption data

Figure 2 represents mean (SEM) total weight gain (g) in animals given 0% CONT, 50% CONT and 50% INT HFCS access over 26 days of exposure. An ANOVA comparing the effects of Pre-exposure (0% CONT, 50% CONT and 50% INT HFCS) on total weight gain identified no significant main effect of Pre-exposure. Additionally, there were no differences in total caloric intake (chow + HFCS) in animals (data not shown).

Figure 3 represents mean (SEM) HFCS intake in animals given pre-exposure to CONT and INT HFCS (50%) at different time points throughout the day (10 am, 5 pm, 9 pm). An ANOVA comparing total HFCS consumption at the different time points throughout the day revealed a significant main effect of Pre-exposure at every time point [10 am: \( F(1, 39) = 17.37, p < 0.001 \), 5 pm: \( F(1, 39) = 7.87, p < 0.01 \), 9 pm: \( F(1, 39) = 9.86, p < 0.01 \)]. Animals with INT 50% HFCS exposure drank more HFCS than animals in the CONT 50% HFCS group. However, an analysis on total HFCS consumed revealed no differences (data not shown).

2.4.2. Experiment 1: Place conditioning

Figure 4 represents the mean (SEM) difference in time spent (sec) in the OXY paired compartment from pre-conditioning to test of conditioning. An ANOVA comparing the effects of Pre-exposure (0% CONT, 50% CONT, 50% INT HFCS) and Dose (0, 0.16, and 2.50 mg/kg OXY) identified a significant main effect of Dose \([F(2, 97) = 14.59, \ p < 0.001]\). Multiple comparisons indicated that animals conditioned with 0.16 or 2.50 mg/kg OXY spent significantly more time in the drug-paired compartment than those injected with vehicle. There was no significant main effect of Pre-exposure or Dose X Pre-exposure interaction.
2.4.3. Experiment 2: The effect of Pre-exposure on OXY-induced locomotion

Figure 5A represents mean (SEM) distance moved (cm) across session on Day 5 of 0 mg/kg OXY treatment. The ANOVA identified a significant main effect of Time [$F(11, 110) = 13.31, p < .001$]. No main effect of Pre-exposure or Time by Pre-exposure interaction was observed. Multiple comparisons revealed that there was a decrease in locomotor activity across the session.

Figure 5B represents mean (SEM) distance moved (cm) across session on Day 5 of 0.16 mg/kg OXY treatment. The ANOVA identified a significant main effect of Time [$F(11, 110) = 24.32, p < .001$]. No main effect of Pre-exposure or Time by Pre-exposure interaction was observed. Multiple comparisons revealed a decrease in locomotor activity across the session.

Figure 5C represents mean (SEM) distance moved (cm) across session on Day 5 of 2.5 mg/kg OXY treatment. The ANOVA identified a significant main effect of Time [$F(11, 110) = 22.77, p < .001$] and Pre-exposure [$F(1, 10) = 8.93, p < .005$]. No Time by Pre-exposure interaction was observed. Animals that previously has exposure to 50% HFCS displayed reduced locomotor activity when compared to those with 0% HFCS exposure.

Figure 6A represents mean (SEM) distance moved (cm) during a test of conditioned locomotion (60 min) in animals pre-treated with 0 mg/kg OXY and pre-exposure to 0% or 50% HFCS. In animals pre-treated with 0 mg/kg OXY, the ANOVA revealed a significant main effect of Time [$F(5, 50) = 50.39, p < 0.001$]. There was no significant main effect of Pre-exposure or Time by Pre-exposure interaction. Multiple comparisons on marginal means indicated that over time there was a decrease in the distance moved.

Figure 6B represents mean (SEM) distance moved (cm) during a test of conditioned locomotion (60 min) in animals pre-treated with 0.16 mg/kg OXY and pre-exposure to 0% or
50% HFCS. In animals pre-treated 0.16 mg/kg OXY the ANOVA revealed a significant main effect of Time \(F(5, 50) = 50.14, p < 0.001\). There was no significant main effect of Pre-exposure or Time by Pre-exposure interaction. Multiple comparisons on marginal means indicated that over time there was a decrease in the distance moved.

Figure 6C represents mean (SEM) distance moved (cm) during a test of conditioned locomotion (60 min) in animals pre-treated with 2.50 mg/kg OXY and pre-exposure to 0% or 50% HFCS. In animals pre-treated with 2.5 mg/kg OXY, the ANOVA revealed a significant main effect of Time \(F(5, 50) = 28.16, p < 0.001\). There was no significant main effect of Pre-exposure or Time by Pre-exposure interaction. Multiple comparisons on marginal means indicated that over time there was a decrease in the distance moved.

Figure 7A represents mean (SEM) distance moved (cm) across the test of OXY-induced locomotion in animals pre-exposed to 0% or 50% HFCS following a challenge with 0 mg/kg OXY. The ANOVA identified a significant main effect of Time \(F(11, 110) = 16.09, p < .001\). No main effect of Pre-exposure or Time by Pre-exposure interaction was observed. Multiple comparisons on marginal means revealed a decrease in locomotor activity across the session.

Figure 7B represents mean (SEM) distance moved (cm) across the test of OXY-induced locomotion in animals pre-exposed to 0% or 50% HFCS following a challenge with 0.16 mg/kg OXY. The ANOVA identified a significant main effect of Time \(F(11, 110) = 29.84, p < .001\). No main effect of Pre-exposure or Time by Pre-exposure interaction was observed. Multiple comparisons revealed a decrease in locomotor activity across the session.

Figure 7C represents mean (SEM) distance moved (cm) across the test of OXY-induced locomotion in animals pre-exposed to 0% or 50% HFCS following a challenge with 2.5 mg/kg OXY. The ANOVA identified a significant main effect of Time \(F(11, 110) = 16.76, p < .001\).
No main effect of Pre-exposure or Time by Pre-exposure interaction was observed. Multiple comparisons on marginal means revealed a decrease in locomotor activity across session.

### 2.4.4. Experiment 3: Microdialysis

Figure 8 represents mean (SEM) dopamine levels over 140 mins. The ANOVA identified a significant Dose by Pre-exposure interaction \([F(1, 28) = 4.55, p < .05]\) and main effect of Dose \([F(1, 28) = 9.33, p < .01]\). There was no main effect of Pre-exposure, Time, Time by Pre-exposure interaction, Time by Dose interaction, or Time by Pre-exposure by Dose interaction. Multiple comparisons revealed that DA concentrations in animals pre-treated with 0% HFCS + 2.5 mg/kg OXY were significantly higher than those in animals pre-treated with 0% HFCS + 0 mg/kg OXY and 50% HFCS + 2.5 mg/kg OXY.

### 2.5. Discussion

Various factors contribute to increased vulnerability for opioid abuse and addiction (Whitesell et al., 2013; Aghaii, Kamaly, Esfahani, 2012). One of these factors may be the consumption of specific foods, such as those high in sugar. In the current study we examined whether the consumption of high fructose corn syrup (HFCS), a commonly added sugar in our diet, altered responses to oxycodone (OXY), a widely used and abused prescription opioid. To this end, animals were pre-exposed to 0 or 50% HFCS for 26 days. Animals then underwent a 9-day sugar free period and were subsequently tested on one of the following: (1) place conditioning with OXY (0, 0.16, or 2.50 mg/kg SC); (2) locomotion involving context-dependent treatment with OXY (0, 0.16, or 2.50 mg/kg SC); or (3) in vivo microdialysis of extracellular dopamine (DA) in the nucleus accumbens (NAc) prior to, during, and after an injection of OXY (0 or 2.50 mg/kg SC). It was found that 0.16 and 2.50 mg/kg OXY produced a significant place preference, but this was not modified by HFCS pre-exposure. Furthermore, HFCS pre-exposure
decreased OXY-induced locomotion (at the high dose) but not context induced locomotion. Lastly, microdialysis data revealed that HFCS pre-exposure decreased the OXY-induced dopaminergic response in the NAc. Taken together, these experiments indicate that HFCS and OXY interact at both behavioral and neural levels and that HFCS has the potential to influence behavioral and pharmacological responses to OXY.

HFCS pre-exposure blunted the OXY induced DA response in the NAc. In animals that were pre-exposed to 0% HFCS, an injection of 2.50 mg/kg OXY significantly increased DA concentrations in comparison to animals that received 0 mg/kg OXY. Conversely, in animals that were pre-exposed to 50% HFCS, an injection of 2.50 mg/kg OXY did not increase DA concentrations in the NAc. These data are consistent with those studies that reveal decreased DA turnover in the NAc following consumption of a high fat diet (Carlin, Hill-Smith, Lucki, & Reyes, 2013; Davis et al., 2004). Conversely, our results are not consistent with studies that have demonstrated increases extracellular DA release in response to systemic injections of psychostimulants following consumption of high fat and/or sugar diets (Pathos, 2001; Gosnell, 2004; Rada, Avena & Hoebel, 2005; DeSousa, Bush, & Vaccarino, 2000). It is likely that these differences in the direction of DA dysfunction are a result of differences in methodology. Those studies that report increased DA turnover often utilize food restriction procedures which have shown to increase drug reinforced behaviour (Avena et al., 2008; Carr, Tsimberg, Berman, & Yamamoto, 2003; de Vaca & Carr, 1998); an effect possibly mediated by DA’s interaction with the stress system (Wichmann et al., 2017).

Other DA system alterations following repeated consumption of palatable food have also been observed. For example, Johnson and Kenny (2010) found that repeated access to a cafeteria diet was associated with a decrease in D2R expression in animals that displayed the “obese
phenotype”. Similarly, in obese individuals there is decreased activation of reward circuits during the consumption of food (Stice et al., 2008) and decreased D2R availability in reward related brain regions (Wang et al., 2001). It is believed that a blunted striatal dopaminergic response is associated with overeating as a means to compensate for the reduced activation (Stice et al., 2008), which may then contribute to diet induced obesity. That being said, in the current study there was no significant difference in weight gain between the 0% and 50% HFCS group. This is an interesting finding because these results support the notion that, independent of weight gain, excessive consumption of HFCS is sufficient to drive pharmacological and behavioural dysfunction. A dysfunctional DA system is also observed with DOA, where repeated drug use is associated with decreases in D2R density in the NAc (Georges et al., 1999; Turchan et al., 1997).

It was found that 50% HFCS pre-exposure decreased locomotion in animals treated with 2.5 mg/kg OXY. More specifically, on day 5 of treatment with 2.5 mg/kg OXY, the locomotion in animals pre-exposed to 50% HFCS was significantly reduced in comparison to animals pre-exposed to 0% HFCS (an effect not observed on day 1 of OXY treatment; data not shown). Although, there was no significant interaction, examining locomotion across the session suggested that the stimulatory effects of 2.5 mg/kg OXY were suppressed in the 50% HFCS group; leading to the observed decrease in locomotor activity. DOA that are administered non-contingently result in the development of hyperlocomotion over time (Babbini & Davis, 1972; Vasko & Domino, 1978). However, this development of hyperlocomotion was not observed in animals pre-treated with 50% HFCS. This suggests that exposure to 50% HFCS prevented the release of locomotor inhibition. These results are consistent with our microdialysis DA data. The locomotor activating properties of DOA are thought to depend on increases in NAc DA transmission (Kalivas & Stewart, 1991), so a blunted DA system would prevent the development
of this sensitization response. It is also possible that HFCS pre-exposure affected the striatonigral pathway. Using DREADDs it was shown that the inhibition of neurons in the striatonigral pathway blocked the emergence of a sensitized locomotor response to amphetamine (Ferguson et al., 2011). Future studies should ascertain whether exposure to HFCS can lead to long-term changes in the striatonigral pathway.

It was found that CPP was not altered by HFCS pre-exposure. The CPP paradigm measures appetitive learning, where an association is created between the interoceptive effects of the drug with a unique environment (Sanchis-Segura & Spanagel, 2006). Our results suggest that HFCS pre-exposure did not alter appetitive learning as measured by CPP. This finding was surprising as a majority of the drugs that produce place preference affect the mesolimbic system (Prus, James & Rosecrans, 2009). Because, pre-exposure to 50% HFCS decreased DA release in the current study, it was expected that CPP would be reduced; especially in light of studies that have shown that decreases in NAc DA are associated with decreases in reward motivated behaviour, such as decreases in amphetamine-induced conditioned approach and operant responding for sucrose (Davis et al., 2004). Other studies have also shown that a high fat diet attenuates reward-related behaviours associated with cocaine SA (Wellman, Nation, & Davis, 2007). That being said, the rewarding effects of opioids are partially thought to be mediated by DA-independent mechanisms (Pert & Sivit, 1977; Kalivas et al., 1983; Pettit et al., 1984; Amalric & Koob, 1985; Hnasko et al., 2005; Bechara & VanderKoy, 1992; Vaccarino et al., 1986). Opioid reward can occur in DA depleted mice, which are still able to acquire morphine conditioned place preference (Hnasko et al., 2005). Additionally, prior infusions of DA D1 and D2 antagonists were not able to alter opioid induced increases in palatable food consumption
(Will, Pratt, & Kelley, 2006). Therefore, it is possible that OXY was able to induce a place preference as a result of these DA-independent mechanisms.

Although, Vitale et al. (2003) found that repeated sucrose exposure without food restriction significantly enhanced the development of opioid CPP. It is possible that differences between our results and those of Vitale et al. (2003) may be attributable to differences in methodology. In the present study, animals were tested in a sugar free state (9 days following the cessation of 0 or 50% HFCS consumption), whereas in the study by Vitale et al. (2003) sucrose was on board during testing. Additionally, they employed a three-choice procedure in CPP, whereas in the current experiment the CPP apparatus is a forced choice between two compartments, where the animal picks either the vehicle or drug paired side. It is possible that our apparatus was less sensitive in detecting differences. This interpretation is further supported by our results which show there were no differences in the magnitude of place preference between 0.16 mg/kg OXY and 2.50 mg/kg OXY. Although, it is also possible that the 0.16 mg/kg dose was not low enough, which is the reason for a similar magnitude of place preference.

Lastly, our results reveal that “bingeing” behaviour does not necessarily lead to the development of enhanced reward responses. In a subset of rats, we explored the effect of intermittent (12hr, INT) and continuous (24hr, CONT) sugar access on conditioned place preference. Although, animals in the INT access group displayed the “bingeing” phenotype (Figure 3), INT access did not alter place preference (Figure 4). This perhaps suggests that food restriction has a unique effect, whereby it increases reactivity to rewarding stimuli; an effect that has previously been reported (Carr, 2007; Holsen et al., 2012). Again, it is possible that the doses of OXY utilized were too high and this effect may have revealed itself with lower doses of OXY.
It may be useful to conduct the present study using a self-administration (SA) paradigm. There is evidence that CPP and SA are not redundant measures of common processes (for review see Bardo & Bevins, 2000). In fact, studies have shown that SA procedures are more sensitive than CPP to reinforcement (Bardo et al., 1999; Deroche et al., 1999). Since repeated consumption of highly palatable food alters reward sensitivity (Shin et al., 2012; Johnson & Kenny, 2011), and coupled with results from the current study which demonstrate that 50% HFCS exposure alters the NAc DA response and the locomotor activating properties of OXY, we expect to see changes in the underlying appetitive learning processes that would affect attribution of significance to cues and responses. Hence, these studies should be repeated using SA which would be more sensitive to the differences in reinforcing behaviour, if they exist.

2.6. Conclusion

Overall, these results indicate that an HFCS rich diet can induce alterations in mesolimbic DA functioning and locomotor responses to OXY, independent of weight gain. Moreover, these results suggest that HFCS and OXY interact at both behavioral and neural levels. This may be relevant to the licit and illicit use of these drugs.
**Figure 1:** Time of daily measurements of chow, high fructose corn syrup (HFCS), and water intake in animals with continuous (CONT) and intermittent (INT) access to 50% high fructose corn syrup (HFCS). Measurements were taken at the end of M1, M2, & M3; where M1 represents the amount of intake between pre-exposure groups following 1 hour of HFCS access for the INT group. M2: represents the amount of HFCS consumption during the remainder of the dark cycle. M3: represents the amount of consumption during 4 hours following the start of the light cycle.
Figure 2: Mean (SEM) total weight gain (grams) in animals that had pre-exposure to 0% CONT (n = 58), 50% CONT (n = 71), or 50% INT HFCS (n = 28) across the 26 days of pre-exposure.
Figure 3: Mean (SEM) HFCS consumption (mL) during exposure to continuous (CON, n = 13) or intermittent (INT, n = 28) access to 50% HFCS across various time points throughout the day.

* Significantly different from continuous access
Figure 4: Mean (SEM) difference score of time (min) spent in drug-paired compartment after conditioning with 0, 0.16, or 2.50 mg/kg (SC) oxycodone (OXY) in animals given pre-exposure to 0% continuous high fructose corn syrup (0% HFCS CONT; n = 32), 50% continuous high fructose corn syrup (50% HFCS CONT; n = 46), and 50% intermittent high fructose corn syrup (50% HFCS INT; n = 28) access.
Figure 5: Mean (SEM) locomotion (cm) in animals that were pre-exposed to 0% or 50% high fructose corn syrup (HFCS) following an injection of 0 (Panel A), 0.16 (Panel B), or 2.50 mg/kg (Panel C) oxycodone (OXY) on day 5 of OXY conditioning ($n = 6$ per group).
**Figure 6:** Mean (SEM) locomotion (cm) to drug-context in animals that were pre-exposed to 0% or 50% high fructose corn syrup (HFCS) on the test of context induced locomotion (n = 6 per group).
Figure 7: Mean (SEM) locomotion (cm) in animals that were pre-exposed to 0% or 50% high fructose corn syrup (HFCS) following an injection of 0 (Panel A), 0.16 (Panel B), or 2.50 mg/kg (Panel C) oxycodone (OXY) during the test of locomotion after challenge with OXY.
Figure 8: Mean (SEM) dopamine % of baseline prior to (BL) and following an injection of 0 or 2.5 mg/kg oxycodone (OXY) in rats with 0% (Panel A) or 50% (Panel B) high fructose corn syrup (HFCS) pre-exposure ($n = 7-10$ per group).
Figure 9: Histological representation of microdialysis probe placements in the nucleus accumbens shell (AcbS) and core (AcbC) of the rat brain (represented on coronal sections)
Chapter 3

Dual self-administration of oxycodone and high fructose corn syrup in rats
3.1. Abstract

*Rationale:* The hypothesis of “food addiction” postulates that palatable foods can lead to addictive behaviors because they act on systems of learning and reinforcement that are targeted by drugs of abuse (DOA). This leads to the prediction that there should be interactions between palatable foods and DOA, and that “taking” and “seeking” behaviours reinforced by palatable foods and DOA should be comparable in a situation where their availability or availability of their conditioned stimuli is similar. This study investigated the taking and persistence of seeking behaviour of oxycodone (OXY) and high fructose corn syrup (HFCS) by the same subjects; as well as the interactions between them. *Methods:* In Experiment 1, male, Sprague-Dawley rats were implanted with an intra-oral (IO) or intravenous (IV) cannula and allowed to self-administer IO infusions of HFCS (8, 25, or 50%) or IV infusions of OXY (0.05, 0.1, or 0.2 mg/kg/inf) across 16 alternating days for 3 hrs/day. Following acquisition, animals were tested in extinction conditions by presenting the associated paired lever in the absence of the primary reinforcer and conditioned light stimulus. Following extinction of responding, animals received a test of cue-induced reinstatement during which lever presses activated the appropriate HFCS- or OXY-paired conditioned light stimulus. Following extinction to cues, animals were given a priming injection of HFCS (8%, 25%, or 50%) or OXY (0.05, 0.01, or 0.2 mg/kg) and responding was assessed on HFCS- or OXY-paired levers. In Experiment 2, rats were implanted with both IO and IV cannulas and trained to self-administer IO HFCS (50%) and IV OXY (0.2 mg/kg/inf), over 16 alternating days, 3 hours/day, on a continuous reinforcement schedule, by operating two different levers presented in the same experimental context. Following acquisition, all animals were tested in extinction conditions by simultaneously presenting HFCS- and OXY-
paired levers. Rats then received a test of cue-induced reinstatement during which lever presses activated the appropriate HFCS- and OXY-paired conditioned stimuli. Following extinction to cues, animals were given a priming injection of HFCS (50%, IO) and OXY (0.2 mg/kg, IV) on alternating days and responding was assessed on HFCS- and OXY-paired levers. Results: At the concentration and doses tested, it was found that there was greater operant responding for HFCS than for OXY. However, during tests of extinction and cue-induced reinstatement there was more responding on the OXY-paired lever than the HFCS-paired lever. Furthermore, co-self-administration of OXY and HFCS enhanced the effects of an OXY-prime on OXY-lever responding. Conclusion: Overall, the findings indicate that an opioid reinforcer engenders a greater persistence of behaviour both in the presence and absence of conditioned reinforcers and that the consumption of a caloric sweetener can enhance some effects of opioids.
3.2. Introduction

Are all rewards the same? The food industry has developed food in such a way that it has led to food with increasingly higher “rewarding properties.” Highly palatable food (i.e., high in fat and sugar) is thought to carry an “addictive” potential by increasing sensitivity to food reward and is described as inducing patterns of consumption that lead to overeating and obesity (Berthoud et al., 2011). A growing body of evidence finds that repeated overconsumption of highly palatable foods induces behavioural and neurobiological changes that are similar to those observed with DOA (Volkow et al., 2012; Tomasi et al., 2015). These similarities extend to similar “bingeing” behaviour (Bello et al., 2003; Wojnicki, Stine, & Corwin, 2007; Colantuoni et al., 2001; Gilpin, Karanikas & Richardson, 2012; Segal & Kuczenski, 1997), continued use despite negative consequences (Deroche et al., 2004; Oswald et al., 2011; Teegarden & Bale, 2007), an escalation of intake overtime (La Fleur et al., 2010; Colantuoni et al., 2001; Ahmed & Koob, 1998), development of withdrawal symptoms (Colantuni et al., 2002; Avena et al., 2008), craving for the substance (Goldstein et al., 2010; Koeber et al., 2010; Morley, Levine, Yim, Lowy, 1983; Zellner et al., 2006; Steptoe, Lipsey, & Wardle, 1998), shared underlying trait factors such as high impulsivity (Braet et al., 2007; Nigg et al., 2006), and shared underlying neurobiological mechanisms (Wang et al. 2004; Nader et al., 2006; Volkow et al., 1993; Wang et al., 2001; Kenny et al., 2006; Johnson & Kenny, 2010; Avena et al., 2008).

Despite similarities, there are also differences in the ability of DOA and sugars to reinforce behaviour. For instance, in choice procedures where one lever is paired with cocaine and another with saccharin, animals tend to respond more for saccharin (Ahmed et al., 2013; Lenoir & Ahmed, 2008; Lenoir et al., 2007; Cantin et al., 2010) and saccharin-paired cues
(Madsen & Ahmed, 2015). The preference for sweet solutions over DOA continues despite manipulations with short and long access models, as well early and protracted withdrawal periods (Caprioli et al., 2015). In contrast, a preference for cocaine over food is easily established in rhesus monkeys (Nader & Woolverton, 1991, 1992; Aigner & Balster, 1978; Anderson, Velkey, & Woolverton, 2002), even during periods of food deprivation (Nader & Woolverton, 1991). Moreover, animals with extended access to cocaine are more resistant than animals with extended access to sucrose to the effects of an aversive conditioned stimulus (Kearns, Weiss, & Panlilio, 2002) or punishment (Pelloux, Everitt & Dickinson, 2007). A number of studies have also revealed that once reinforcers are removed, drug cues result in greater persistence of seeking (Kearns et al., 2011; Ciccioioppo, Martin-Fradon, & Weiss, 2004); an effect that has been replicated in studies that examine the effect of stress on food- and drug-seeking behaviour (Ahmed & Koob, 1997; Buczek et al., 1999). So, while there are a number of situations where highly palatable foods are similar to or engender behavioural effects that are larger in magnitude than those obtained with DOA, there are a number of situations where the opposite is also observed.

Furthermore, differences between highly palatable food and DOA also extend to the recruitment of underlying neurobiological mechanisms (Nair et al., 2009; Carelli et al., 2000; Yager et al., 2015; McLaughlin & Floresco, 2007; McFarland & Kalivas, 2001; Khaled, Pushparaj, Di Ciano, Diaz, & Le Foll, 2014). And so, while there are similarities between highly palatable foods and DOA (Meule & Gearhardt., 2014; Pursey et al., 2014), there are also various differences (Ziauddeen, Farooqi, & Fletcher, 2012; Ziauddeen & Fletcher, 2013), which has kept the debate of “food addiction” alive (Meule & Gearhardt., 2014; Pursey et al., 2014; Ziauddeen, Farooqi, & Fletcher, 2012; Ziauddeen & Fletcher, 2013).
One feature lacking from some comparative studies is a direct comparison of sugar and drug-reinforced behaviours in the same animal. A large proportion of studies making comparisons between drug- and sugar-reinforcers have utilized experimental conditions that were not always equated, or experiments were conducted in separate cohorts of animals, thereby changing the overall pharmacological experience. Studies that include direct comparisons between food and drug-reinforced behaviours in the same animals may be more meaningful because they eliminate differences in pharmacological history and carry out comparative studies in the same animals to directly assess the similarities and differences between drug and sugar reinforcers.

Therefore, to investigate how drug and sugar reinforcers interact and affect persistence of seeking behaviors, we combined the methods of intra-oral (IO) and intravenous (IV) self-administration (SA) to create a dual self-administration model. Here, animals were trained to co-self-administer IO infusions of high fructose corn syrup (HFCS), a widely added sugar in our diet linked to the obesity crisis (Bray et al., 2004), and IV infusions of oxycodone (OXY), a widely used and abused prescription opioid (Minhas & Leri, 2017), by operating two different levers presented in the same experimental context but on alternate days. Responding for both reinforcers was then assessed during tests of seeking on extinction, cue- and prime- induced reinstatement.

3.3. Materials and Methods

3.3.1. Subjects

Subjects were adult male Sprague-Dawley rats (Charles River, QC) weighing 250-300 g at the beginning of experiments. Rats were individually housed, maintained on a reverse light/dark cycle (7:00 AM lights off; 7:00 PM light on) and behavioural testing occurred during
their active cycle. All rats had ad lib access to food and water except during testing. All procedures were approved by the Animal Care Committee of the University of Guelph and were carried out in accordance with the recommendations of the Canadian Council on Animal Care.

3.3.2. Apparatus

Operant IO and IV SA of HFCS and OXY, respectively, occurred in 20 operant chambers (model ENV-008CT, Med Associates, Lafayette, IN) equipped with two retractable active levers, two stationary inactive levers, two cue lights, and a house light. One of each type of lever was located on the opposite walls of the chamber and above each active lever there were two white lights that served as a condition stimulus (CS) to be associated with the infusion of a particular primary reinforcer. The retractable active levers were connected to infusion pumps that were located outside the sound-insulating chamber for delivery of primary reinforcers when they were pressed.

3.3.3. Intravenous and intraoral surgery.

All animals were implanted with an intraoral (IO) and/or intravenous (IV) cannula as previously described in Leri et al. (2009) and Levy et al. (2014). In the case of Dual SA, both IO and IV surgeries were conducted on the same day; where the IV surgery was conducted prior to the IO surgery.

3.3.4. General Procedures

3.3.4.1. Experiment 1: Single self-administration

The primary objective of this Experiment was to provide a rationale for selecting experimental parameters for Experiment 2.
Self-administration

Using a between groups design, a total of 91 rats were trained to lever press for IO infusions of HFCS (8, 25, or 50%) (and a cue light as a conditioned stimulus) or IV infusions of OXY (0.05, 0.1, or 0.2 mg/kg/inf) (and a cue light as a conditioned stimulus) across 16 alternating days on a continuous schedule of reinforcement for 3 hours/day. Each session was initiated by the activation of the house light that remained on for the duration of the session, entry of the levers (one of the two retractable levers) was introduced and the corresponding light stimulus (continuous or blinking) located above the active lever was illuminated for 30 seconds. Subsequently, each press on the active lever resulted in the delivery of a 150 µL infusion of OXY (or a 90 µL of HFCS) and illumination of the stimulus light for 30 seconds. During these 30 second periods responses on the active lever were recorded but did not lead to further infusions. Depression on the inactive levers had no consequences, but all presses were recorded. Whether HFCS or OXY commenced on day one, as well as the lever light (solid or blinking)-drug associations were counterbalanced across rats and groups. On days animals did not self-administer a primary reinforcer they were put into the operant chamber, but no active levers or cue lights were present (see Table 1 for SA design).

Extinction

Following an OXY or HFCS-free period (4 days), animals received a minimum of 4 sessions (3 hrs each) of extinction over 4 days (or until a criterion of less than 15 responses/3 hrs was achieved). During these 3 hr daily sessions, the retractable lever was introduced. During extinction, presses on the previously active lever were without consequences. Rats were then free to press the active and inactive levers; responding on the active lever was not reinforced by OXY (or HFCS) and light stimulus.
**Tests of cue reinstatement**

Following extinction, animals were tested for 3 hours during which responding on the active lever was reinforced by the appropriate light stimulus (continuous or blinking) but did not lead to any infusions of OXY or HFCS.

**Tests of prime induced reinstatement**

Animals extinguished responding to conditioned cues. Once a low level of responding (<15 responses) was achieved, animals received a second test of prime-induced reinstatement where animals received an injection of OXY (IV) or HFCS (IO) at the dose (1 mL/kg IV; 0.05, 0.1, 0.2 mg/kg OXY) or concentration (IO 1 mL/kg; 8%, 25%, 50%) that was previously self-administered. Immediately following the prime, animals were tested for 3 hours during which responding on the active lever activated the appropriate light stimulus (continuous or blinking) but did not lead to any infusions of OXY or HFCS.

**3.3.4.2. Experiment 2: Dual self-administration**

**Self-administration**

On the basis of the dose-response results of Experiment 1 (see below), a total of 19 rats were trained to lever press for IO infusions of HFCS (50%) and IV infusions of OXY (0.2 mg/kg/inf) on a continuous reinforcement schedule across 16 alternating days for 3 hours/day. Each session was initiated by the activation of the house light, entry of one of the two active levers, and illumination of the stimulus light located above the active lever for 30 seconds. Subsequently, each press on the active lever resulted in the delivery of OXY or HFCS, and continuous illumination of a stimulus light for 30 seconds; responses on the second lever the following day, delivering the alternate reinforcer, were associated with a light that flashed at a rate of 1 Hz. This allowed the reinforcers to be paired with different cues. The light stimulus
(continuous or blinking) was counterbalanced across reinforcers and start date (see Table 1 for SA design). During these 30 seconds periods, responses on the active lever were recorded but did not lead to further infusions.

**Extinction**

Following training, animals underwent a 4-day OXY and HFCS free period, which was followed by extinction whereby both active levers were introduced into the chamber and lever presses no longer resulted in infusions of HFCS, OXY, and paired light cues.

**Tests of cue reinstatement**

Once responding was extinguished (i.e., < 15 responses) on both levers, in order to assess the impact of reinforcer-paired cues on seeking, the session was initiated by the activation of both stimulus lights (30 seconds) located above each respective active lever. Thereafter, responses on the active lever activated the appropriate light conditioned stimulus (continuous or blinking) for 30 seconds; this test lasted 3 hours.

**Tests of prime reinstatement**

Following the test of cue reinstatement, responding was again extinguished to establish a baseline and then animals were given experimenter-delivered infusions of OXY (1 mL/kg IV; 0.2 mg/kg) and HFCS (1 mL/kg IO; 50%) to test seeking; known as prime-induced reinstatement. Each test lasted 3 hours and took place once day across a 2-day period, where one reinforcer was tested the first day and the second reinforcer was tested 24 hrs later. The day (1st or 2nd) the reinforcer was tested was counterbalanced across reinforcer type.

**3.4. Drugs and food**

Oxycodone (OXY) was dissolved in 0.9% physiological saline and self-administered by animals at doses, 0.05, 0.10, or 0.20 mg/kg/inf. These doses of OXY were utilized as they have
shown to support intravenous self-administration (Leri & Burns, 2005; Grella et al., 2011).

High Fructose Corn Syrup (HFCS-55% fructose and 42% glucose) solutions were diluted in reverse osmosis water to concentrations of 8%, 25%, or 50%. These concentrations were utilized as they have shown to support IO self-administration (Levy et al., 2014).

The priming doses of OXY and HFCS were based on previous dose or concentration the animals self-administered at 1mL/kg and previous route of administration IV or IO. Previously, an intravenous prime has shown to reinstate responding for heroin (deWit & Stewart, 1983).

3.5. Statistical Analyses

Experiment 1 was performed in several replications over the course of several months. In each replication, animals self-administered 0.05 (n = 13), 0.1 (n = 14), or 0.2 mg/kg (n = 11) OXY or 8% (n = 12), 25% (n = 13), or 50% (n = 10) HFCS. At the conclusion of data collection, it was noted that not all rats acquired self-administration. It was deemed inappropriate to include animals that never acquired self-administration in tests of relapse, so a criterion for exclusion from data analysis was utilized (see below). The exact values of non-significant results are not reported.

Single SA.

Self-administration. For each of the three groups for HFCS (8%, 25%, 50%) and OXY (0.05, 0.1, 0.2 mg/kg), we compared the number of infusions (and total number of responses emitted) across the 8 sessions of SA using analyses of variance (ANOVAs) with one within-subjects variable (i.e., session) and one between-subjects variable (i.e., Concentration or Dose). Here and elsewhere when there were significant interactions or significant main effects, we isolated specific group differences using the Student Newman-Keuls multiple comparisons procedure, with alpha = .05.
Extinction. For each of the three groups for HFCS (8, 25, 50%) and OXY (0.05, 0.1, 0.2 mg/kg), we compared the number of responses emitted by the three groups across the extinction period on the HFCS- or OXY-associated lever using analyses of variance (ANOVAs) with one within-subjects variable (i.e., session) and one between-subjects variable (i.e., Concentration or Dose). A second ANOVA was conducted on the total number of responses across 4 sessions of extinction for each of the three groups for HFCS (8, 25, 50%) and OXY (0.05, 0.1, 0.2 mg/kg).

Cue Reinstatement. Animals that did not meet the criterion of <15 responses did not move on to cue-induced reinstatement (only 3 animals were removed). For each of the three groups for HFCS (8, 25, 50%) and OXY (0.05, 0.1, 0.2 mg/kg), we compared the number of responses emitted by the three groups during cue-induced reinstatement on the HFCS- or OXY-associated lever using analyses of variance (ANOVAs) with one within-subjects variable (i.e., session) and one between-subjects variable (i.e., Concentration or Dose).

Prime Reinstatement. Animals that did not meet the criteria of <15 responses did not move onto prime-induced reinstatement. For each of the three groups for HFCS (8, 25, 50%) and OXY (0.05, 0.1, 0.2 mg/kg), we compared the number of responses emitted by the three groups during prime-induced reinstatement on the HFCS- or OXY-associated lever using analyses of variance (ANOVAs) with one within-subjects variable (i.e., session) and one between-subjects variable (i.e., Concentration or Dose).

Dual SA.

Self-administration. The analysis compared the mean number of active lever responses emitted during acquisition on the OXY- and HFCS-associated levers using an ANOVA with two within-subject variables (lever and session).
Extinction. The analysis compared the mean number of responses emitted during extinction on the OXY- and HFCS-associated levers using ANOVAs with two within subject variables (lever and session).

Cue Reinstatement. The analysis compared the mean number of responses emitted during cue-induced reinstatement on the OXY- and HFCS-associated levers using ANOVAs with two within subject variables (lever and session).

Prime Reinstatement. The analysis compared the mean number of responses emitted during prime-induced reinstatement on the OXY- and HFCS-associated levers using ANOVAs with two within subject variables (lever and session).

Comparisons between Single and Dual SA.

Self-administration. We compared total infusions and responses emitted for OXY and HFCS during self-administration using an ANOVA with one between-subjects variable (Self-administration; Single and Dual).

Extinction. We compared total responses emitted for OXY and HFCS during extinction using an ANOVA with one between-subjects variable (Self-administration; Single and Dual).

Cue-induced reinstatement. We compared total responses emitted for OXY and HFCS during cue-induced reinstatement using an ANOVA with one between-subjects variable (Self-administration; Single and Dual).

Prime-induced reinstatement. We compared total responses emitted for OXY and HFCS during prime-induced reinstatement using an ANOVA with one between-subjects variable (Self-administration; Single and Dual).
3.6. Results

3.6.1. Exclusion criteria

Because it was deemed inappropriate to include animals that never acquired self-administration in tests of relapse, a criterion for exclusion from data analysis had to be established. Therefore, operant responding was assessed in a different group of rats \((n = 7)\) that was tested as described above, but that did not receive IV or IO surgery, and that did not self-administer OXY or HFCS (animals were put into operant chambers where responses on the active lever resulted in the illumination of a light cue). Total number of responses made by these rats on the active lever during the last 5 days of testing was calculated and used as an exclusion threshold: Any rat that did not exceed this threshold by at least one response was excluded from data analysis. Using this criterion, 8 and 10 rats in the OXY and HFCS groups were excluded, respectively, from Single SA; and three rats were excluded from Dual SA. In these rats, there was no increase of responding on the active lever during acquisition, and no evidence of discrimination between the active and the inactive levers.

3.6.2. Experiment 1: Single self-administration

*Self-administration: HFCS and OXY showed dose and concentration dependent effects on the number of infusions obtained and/or responding*

Figure 10A represents mean (+SEM) infusions obtained during OXY SA (0.05, 0.1, and 0.2 mg/kg). The ANOVA identified a significant main effect of Session \([F(7, 252) = 8.19, p < .001]\). There was no significant main effect of Dose or Dose X Session interaction. Comparisons on marginal means indicate that number of infusions obtained increased across self-administration sessions.
Figure 10B represents mean (±SEM) responses obtained during OXY (0.05, 0.1, and 0.2 mg/kg) SA. The ANOVA identified a significant Dose X Session interaction \[ F(14, 252) = 2.39, p < .01 \] and a significant main effect of Session \[ F(7, 252) = 10.42, p < .001 \]. No significant main effect of Dose was identified. Multiple comparisons revealed that responding for 0.2 mg/kg/inf OXY was significantly lower than 0.05 mg/kg/inf OXY on sessions 5-8 and lower than 0.1 mg/kg/inf OXY on Sessions 5 and 8.

Figure 10D represents mean (±SEM) infusions obtained during HFCS (8, 25, or 50%) SA. The ANOVA identified a significant Concentration X Session interaction \[ F(14, 224) = 2.86, p < .001 \], and significant main effects of Concentration \[ F(2, 32) = 3.82, p < .05 \] and Session \[ F(7, 224) = 2.58, p < .05 \]. Multiple comparisons revealed that animals that received 25% HFCS obtained more infusions than animals that received 8% HFCS on sessions 4 and 5, and more infusions than animals that received 50% HFCS on sessions 5 and 7.

Figure 10E represents mean (±SEM) responses obtained during IO SA of HFCS (8, 25, or 50%). The ANOVA identified a significant Concentration X Session interaction \[ F(14, 224) = 3.04, p < .001 \] and significant main effect of Session \[ F(2, 32) = 2.79, p < .01 \]. There was no significant main effect of Concentration. Multiple comparisons revealed that there was significantly more responding when animals were given 25% HFCS than when they were given 8% HFCS.

Figure 11A represents mean (±SEM) responses obtained across the session on days 1 and 8 for .05 mg/kg/inf OXY SA. The ANOVA identified a significant Time X Day interaction \[ F(17, 204) = 2.63, p < .001 \], as well as a significant main effect of Time \[ F(17, 204) = 2.91, p < .001 \] and Day \[ F(1, 12) = 8.78, p < .05 \]. Multiple comparisons revealed significantly greater responding on the active lever on Day 8 at 10, 80, and 180 mins.
Figure 11B represents mean (+SEM) responses obtained across the session on days 1 and 8 for 0.1 mg/kg/inf OXY SA. The ANOVA identified a significant Time X Day interaction \[ F(17, 221) = 3.40, p < .001 \] as well as a significant main effect of Time \[ F(17, 221) = 4.79, p < .001 \] and Day \[ F(1, 13) = 26.55, p < .001 \]. Multiple comparisons revealed significantly greater responding on the active lever on Day 8 at 10, 20, 40, 70, 170, and 180 mins.

Figure 11C represents mean (+SEM) responses obtained across the session on days 1 and 8 for 0.2 mg/kg/inf OXY SA. The ANOVA identified a significant Time X Day interaction \[ F(17, 170) = 5.75, p < .001 \] as well as a significant main effect of Time \[ F(17, 170) = 8.52, p < .001 \] and Day \[ F(1, 10) = 10.98, p < .01 \]. Multiple comparisons revealed significantly greater responding on the active lever on Day 8 at 10 and 140 mins.

Figure 11D represents mean (+SEM) responses obtained across the session on days 1 and 8 for 8% HFCS SA. The ANOVA identified a significant Day \[ F(1, 9) = 5.50, p < .05 \]. No significant main effect of Time or Time X Day interaction was identified. Comparisons on marginal means revealed significantly greater responding on the active lever on Day 8 than on Day 1.

Figure 11E represents mean (+SEM) responses obtained across the session on days 1 and 8 for 25% HFCS SA. The ANOVA identified a significant Time X Day interaction \[ F(17, 170) = 3.89, p < .001 \] as well as a significant main effect of Time \[ F(17, 170) = 5.62, p < .001 \]. No significant main effect of Day was identified. Multiple comparisons revealed significantly greater responding on the active lever on Day 8 at 10-40 mins.

Figure 11F represents mean (+SEM) responses obtained across the session on days 1 and 8 for 50% HFCS SA. The ANOVA identified a significant Time X Day interaction \[ F(17, 153) = 2.19, p < .01 \] as well as a significant main effect of Time \[ F(17, 153) = 2.12, p < .01 \]. No
significant main effect of Day was identified. Multiple comparisons revealed significantly greater responding on the active lever on Day 8 at 10-30 mins.

*Extinction: The previously self-administered OXY dose or HFCS concentration had no effect on magnitude of extinction responding.*

Figure 12A represents mean (+SEM) active lever responses obtained during extinction for OXY. The ANOVA identified a significant main effect of Session \([F(3, 105) = 33.25, p < .001]\). There was no Session by Dose interaction or main effect of Dose. Comparisons on the marginal means for Sessions revealed that active lever responding decreased across session.

Figure 12C represents mean (+SEM) responses obtained during extinction for HFCS. The ANOVA identified a significant main effect of Session \([F(3, 99) = 36.78, p < .001]\). No significant Concentration X Session or main effect of Concentration was identified. Comparisons on marginal means revealed that across sessions active lever responding decreased for all groups.

*Cue-Induced Reinstatement: OXY, but not HFCS cues reinstated lever responding*

Figure 13A represents mean (+SEM) active lever responses during the last session of extinction and cue induced reinstatement on the OXY-paired lever. The ANOVA identified a significant Dose by Session interaction \([F(2, 25) = 5.72, p < .01]\) and significant main effect of Dose \([F(2, 25) = 4.73, p < .01]\) and Session \([F(1, 25) = 22.01, p < .001]\). Comparisons revealed that there was significantly more responding during cue induced reinstatement than extinction for both 0.1 and 0.2 mg/kg OXY.

Figure 13B represents mean (+SEM) active lever responses during the last session of extinction and cue induced reinstatement on the HFCS-paired lever. The ANOVA identified a significant main effect of Session \([F(1, 26) = 12.18, p < .01]\). There was no significant Dose X
Session interaction or main effect of Concentration. Comparisons on marginal means revealed that there was greater responding during cue induced reinstatement than during extinction.

*Prime-induced reinstatement: An HFCS, but not OXY prime induced a reinstatement of responding*

Figure 14A represents mean (+SEM) active lever responses for OXY during prime induced reinstatement. There was no significant Session X Dose interaction, nor main effects of Dose and Session.

Figure 14B represents mean (+SEM) active lever responses for HFCS during prime induced reinstatement. The ANOVA identified a significant Session X Dose interaction \[ F(2, 26) = 6.53, p < .01 \]. There was no significant main effect of Dose or Session. Multiple comparisons revealed that there was a significant increase in responding from extinction to prime induced reinstatement in animals that received 50% HFCS.

### 3.6.3. Experiment 2: Dual self-administration

*Self-administration: There was more instrumental responding for HFCS than for OXY*

Figure 15 represents mean (+SEM) active lever responses obtained during self-administration on HFCS- and OXY-paired levers. The ANOVA identified a significant main effect of Session \[ F(7, 105) = 2.83, p = .01 \] and Lever \[ F(1, 15) = 9.99, p < .01 \]. There was no Session X Lever interaction. Multiple comparisons on marginal means revealed that there was a significant increase in responding from Session 1 to Session 8. As well, there was significantly more responding on the HFCS lever than the OXY lever.

*Extinction: Greater responding on the OXY lever during extinction.*

Figure 16 represents mean (+SEM) active lever responses obtained during extinction on the HFCS- and OXY-paired levers. The ANOVA identified a significant Session X Lever
interaction [$F(4, 60) = 5.96, p < .001$], as well as significant main effect of Session [$F(4, 60) = 32.34, p < .001$] and Lever [$F(1, 60) = 10.28, p < .01$]. Overall responding decreased across sessions on the OXY and HFCS active levers. Multiple comparisons indicated that there was significantly more responding on the OXY lever than the HFCS lever on extinction sessions 1 and 2.

**Cue Reinstatement: OXY cues engendered greater responding on paired levers than HFCS cues**

Figure 17 represents mean (+SEM) active lever responses during the last session of extinction and cue induced reinstatement on the HFCS- and OXY-paired levers. The ANOVA identified a significant Session X Lever interaction [$F(1, 15) = 5.62, p < .05$] and main effect of Session [$F(1, 15) = 40.03, p < .001$]. There was no significant main effect of Lever. Multiple comparisons revealed that during cue induced reinstatement, there was significantly more responding on the OXY lever than the HFCS lever. Furthermore, there was significantly more responding on both HFCS and OXY levers during the test of cue induced reinstatement than during extinction.

**Prime Reinstatement: A priming injection of OXY selectively reinstated responding on the OXY-lever; no effect on the HFCS lever following an HFCS prime**

Figure 18 represents mean (+SEM) active lever responses during the last session of extinction (before prime induced reinstatement) and prime induced reinstatement on HFCS- and OXY-paired levers. The ANOVA identified a significant Session X Lever interaction [$F(2, 16) = 14.94, p < .001$] and main effect of Lever [$F(1, 8) = 8.63, p < .05$]. There was no significant main effect of Session. Multiple comparisons revealed that there was significantly more responding on the OXY lever during the OXY prime than during extinction; an effect not observed on the HFCS paired lever following a HFCS prime.
3.6.4. Comparison between Single and Dual self-administration

Self-administration

An ANOVA comparing mean (SEM) total infusions obtained during Single and Dual Self-administration for OXY or HFCS did not identify a main effect of Self-administration Condition for either OXY or HFCS.

An ANOVA comparing mean (SEM) total responses obtained during Single and Dual Self-administration for OXY or HFCS identified a significant main effect of Self-administration Condition for HFCS \(F(1, 24) = 5.11, p < .05\). There was less responding for HFCS in Dual SA than during Single SA. The ANOVA did not identify a main effect of Self-administration Condition for OXY.

Extinction

An ANOVA comparing mean (SEM) total active lever responses obtained during Single and Dual SA for HFCS or OXY across 4 sessions of extinction did not identify a main effect of Self-administration Condition for either OXY or HFCS.

Cue-induced reinstatement

An ANOVA comparing mean (SEM) active lever responses obtained during Single and Dual SA during OXY and HFCS cue-induced reinstatement revealed main effect of Self-Administration Condition for either OXY or HFCS.

Prime-induced reinstatement

An ANOVA comparing mean (SEM) active lever responses obtained during Single and Dual SA for OXY or HFCS prime induced reinstatement revealed a significant main effect of Self-Administration Condition for OXY \(F(1, 26) = 4.33, p < .05\). There was significantly more
responding on the OXY-lever during Dual SA than Single SA. The ANOVA revealed no significant main effect of Self-administration Condition for HFCS.

3.7. Discussion

Highly palatable foods (i.e., processed to be high in sugar and fat) and drugs of abuse (DOA) act on common neural circuitry related to reward processing (Volkow et al., 2013; Berridge, 2013; Deroche et al., 2004; Johnson & Kenny, 2010; Kober et al., 2010). This has led to the “food addiction” hypothesis. The “food addiction” hypothesis postulates that palatable food rewards can lead to addictive behaviors because they act on systems of learning and reinforcement that are targeted by DOA. This leads to the prediction that “taking” and “seeking” behaviours reinforced by palatable rewards and DOA should be comparable in a situation where their availability, or availability of their conditioned stimuli, is similar. Second, it would predict that highly palatable food and DOA interact at both behavioral and neural levels. To test these predictions, we combined intra-oral (IO) and intravenous (IV) self-administration (SA) and studied acquisition, extinction, cue- and prime- induced reinstatement of lever pressing reinforced by IO high fructose corn syrup (HFCS) and/or IV oxycodone (OXY). There are three primary findings: (1) HFCS and OXY engender similar patterns of self-administration and extinction responding; (2) OXY was associated with more persistent responding during tests of extinction and cue-induced reinstatement; and (3) co-self-administration of OXY and HFCS enhanced the effects of an OXY-prime on OXY-lever responding and the effects of an HFCS cue on reinstatement. Overall, the findings indicate that an OXY reinforcer engenders a greater persistence of behaviour and that the consumption of one reinforcer affects some responses to the other.
3.71. Self-administration (SA)

_Single SA_. HFCS and OXY showed dose and concentration dependent effects on the number of infusions obtained and/or responding during SA (Figure 10). Animals that received OXY displayed less responses/infusions for 0.2 mg/kg OXY than for 0.05 or 0.10 mg/kg OXY. This pattern of SA for OXY as a function of maintenance dose, where total responses per session decreases with increasing unit of maintenance dose, has previously been described in animals self-administrating opioids (Leri & Burns, 2005; Carroll & Boe, 1984; Leri & Stewart, 2001; Dai et al., 1989) and cocaine (Carroll & Lac, 1992). Responding for HFCS was on an inverted U curve, where animals that self-administered 8% and 50% concentrations of HFCS responded less than animals that self-administered 25% HFCS. These findings are in accordance with those previously obtained in food restricted animals (Levy et al., 2014).

There was also the development of a loading effect with all doses of OXY (0.05, 0.1, 0.2 mg/kg/inf) and HFCS (8%, 25%, 50%) (Figure 11). The loading effect is an increase in intake/concentrations of responses at the onset of access that develops over time (Ahmed & Koob, 1998; Rada et al., 2005; Avena et al., 2006). This escalation of intake is thought to be a critical factor in the transition from voluntary drug use to compulsive intake (Koob & LeMoal, 2001).

_Dual SA_. There was more instrumental responding for HFCS than for OXY in Dual SA (Figure 15). Because opioids produce a transient decrease in locomotor activity (Babbini and Davis, 1972; Buxbaum et al., 1973), they have rate-limiting effects on operant responding and so the results were not surprising.
3.7.2. Extinction

Single SA. The previously self-administered OXY dose or HFCS concentration had no effect on magnitude of extinction responding. During SA training, animals self-administered different doses/concentrations of OXY (0.05, 0.1, or 0.2 mg/kg) or HFCS (8%, 25%, or 50%); when subsequently tested in extinction conditions, the previously self-administered dose or concentration did not alter the magnitude or persistence of responding (Figure 12). Likewise, other studies have also reported that the magnitude of extinction responding was not affected by maintenance doses of cocaine (Comer, Lac, Wyvell, Curtis, & Carroll, 1995; de Wit and Stewart 1981; Comer et al. 1993) and methamphetamine (Yokel and Pickens 1976). From an incentive-motivational view it might be predicted that stimuli paired with different training doses might acquire different conditioned values and therefore their associated levers would maintain different responding during extinction (Markou et al., 1993). However, our results fail to support this interpretation. Although, in some procedures like the runaway task, the magnitude of reinforcement during training does alter resistance to extinction for sucrose or food rewards (Wilton, 1966; Zeaman, 1949; Hill & Spear 1962; Likely, Little, & Mackintosh, 1971), an effect that has been replicated with DOA in SA (Grant & Johanson, 1987). And therefore, differences in findings are related to differences in methodology and/or procedures employed.

Dual SA. There was a greater persistence of responding on the OXY lever during extinction. Animals that were trained to co-self-administer OXY (0.2 mg/kg) and HFCS (50%), when moved to extinction conditions, demonstrated significantly more responding on the OXY lever on extinction days 1 and 2 than on the HFCS lever (Figure 16). This is especially interesting in light of the finding that during SA, there was significantly more responding for HFCS than there was for OXY. It is possible that there were differences in the acquired incentive
motivational properties for the OXY and HFCS lever conditioned stimuli (CS). This interpretation is supported by studies that demonstrate preferences for DOA over food reinforcers (Nader & Woolverton, 1991, 1992; Aigner & Balster, 1978; Anderson, Velkey, & Woolverton, 2002) and those studies, which have revealed a greater persistence of seeking for drug cues than food cues (Kearns et al., 2011; Caccioccioppo, Martin-Fradon, & Weiss, 2004; Ahmed & Koob, 1997; Buczek et al., 1999). Although this effect may be driven by a release of locomotor inhibition, in which case perhaps -taking behavior is not always reflective of how a substance may affect subsequent seeking behaviour.

It is also possible that the neural plasticity induced by OXY strengthens stimulus-reward associations and facilitates memory consolidation in ways that are different from HFCS reinforcers. In fact, behavioural and neurochemical evidence exists to support the notion that DOA induce neuroplastic changes that are different from those observed with sugar reinforcers. For example, in studies of incubation of craving, where there are time-dependent increases in cue-seeking behaviour during an abstinence period (Pickens et al., 2011), it was found that the conditioned reinforcing effects of cues can last up to 3 months after cocaine exposure, but only 1 month after sucrose exposure (Grimm et al., 2001, Li & Frantz, 2009; Li & Frantz, 2010). Furthermore, Chen et al. (2010) found that SA of cocaine, but not sucrose, was associated with a long-lasting enhancement of glutamatergic function in VTA DA neurons; in other words, cocaine SA produced persistent long-term potentiation in the VTA. In the same experiments, a yoked control revealed that the enhancement of synaptic potentiation in the VTA was specific to the drug-cue associative learning process and not a result of exposure to only cocaine (Chen et al., 2010). Therefore, in the current set of experiments, it is tempting to suggest that differences in responding in extinction were a result of differences in learning and memory mechanisms.
It could also be argued that during acquisition the temporal distance between the response and reinforcement is smaller for HFCS than that for OXY, which may contribute to faster extinction. A faster delivery of the reinforcer is associated with a faster extinction (Gibson et al., 1965). This would explain the decrease in persistence of responding for HFCS. Although a few studies have also shown that the delay of reinforcement does not affect resistance to extinction (Pankseep & Trowill, 1967). Furthermore, because HFCS consumption is accompanied by immediate sensory feedback, it might be predicted that in the absence of this sensory feedback, extinction would be more rapid.

3.7.3. Cue-reinstatement

_**Single SA.**_ OXY cues produced a dose dependent effect on cue-induced reinstatement, whereas HFCS cues did not reinstate responding. Animals that previously self-administered 0.1 or 0.2 mg/kg OXY during training showed a significant increase in responding on tests of cue-induced reinstatement (Figure 13). This increase in responding to an associated light cue was not observed at any concentration of HFCS. Our finding is consistent with that of Cicciocioppo, Martin-Fardon, and Weiss (2004) who performed a study where animals learned to lever press for sweetened condensed milk or cocaine; they found that a discriminative stimulus associated with sweetened milk or cocaine energized seeking behaviour for cocaine but not for sweetened condensed milk. Other studies have also found that a cue associated with a food reinforcer is not capable of energizing behaviour to the same extent as a drug reinforcer (Grimm et al., 2001; Li & Frantz, 2010). Additionally, the findings from the current study also demonstrate that responding during extinction and reinstatement was not simply a function of the amount of responding emitted during training; a finding similar to that of other studies (Leri & Stewart, 2001).
Dual SA. OXY cues engendered greater responding on paired levers than HFCS cues. Once responding was extinguished in rats that co-self-administered HFCS and OXY, animals received a test of cue-induced reinstatement. There was a significant increase in responding to both HFCS and OXY paired cues; and the magnitude of reinstatement for OXY paired cues was larger than that for HFCS paired cues (Figure 17). These findings are consistent with choice procedures in primates where a larger preference for stimulants than for palatable food is observed (Nader et al., 1991, 1992; Aigner & Balster, 1978; Anderson, Velkey, Woolverton, 2002; Negus & Mello, 2003; Aigner & Balster, 1978). It is also similar to studies demonstrating that drug cues induce a greater persistence of seeking than food cues (Cicciocioppo, Martin-Frandon & Weiss, 2004). The difference in the magnitude of “seeking” responses is perhaps related to differences in acquisition of incentive values for the cues. As previously mentioned, this is perhaps related to differences in learning and memory for drug paired and sugar paired cues. Chen et al. (2010) showed that, in animals trained to self-administer cocaine, there was enhanced synaptic plasticity in the VTA after extensive extinction training and after cue-induced reinstatement; an effect not observed with sucrose trained rats. Our research is also consistent with human data that indicates that drug use occurs in the presence of alternate reinforcers, and drug-seeking behaviour is chosen over non-drug seeking behaviours (Hyman & Malenka, 2001; Ahmed, Lenoir, & Guillem, 2013). These results suggest that cues associated with DOA become “stamped” in ways that make them more enduring and may contribute to the difficulty associated with resisting them. Our results would support this interpretation. In fact, since animals self-administer both OXY and HFCS in the current study, yet differences are observed in cue reactivity, we might be able to suggest that different processes occur during the encoding of drug
and sugar cues. Although, it is unclear which exact mechanisms are affected by these enduring changes.

The results of our experiment are not in agreement with rodent choice procedure studies. During discrete-trial choice studies, rats prefer saccharin over an intravenous dose of cocaine (Lenoir et al., 2007; Cantin et al., 2010; Augier et al., 2012) or heroin (Lenoir, Cantin, Vanhille, Serre, & Ahmed, 2013; Madsen & Ahmed, 2015); a finding that also extends to saccharin-paired cues (Madsen & Ahmed, 2015). One explanation for the discrepancy between our results and those in the rodent literature may be due to differences in methodology. In the present study, rats were trained to self-administer each reinforcer on alternating days. So only the HFCS lever was available on Day 1 and only the OXY lever was available on Day 2. This was different from the discrete-choice operant procedures, where both choices are available, but levers are typically retracted for a period after each reinforcer is delivered; therefore, taking one reinforcer limits the intake of the other. Also, during our choice phase, we employed a free-operant choice procedure, where both choices are available continuously throughout the cue-reinstatement test, allowing the animal to allocate behaviour between the alternatives, and so responding for one conditioned reinforcer did not interfere with the ability to respond for the alternate conditioned reinforcer.

It may possible that the “sweet taste” of HFCS becomes a conditioned stimulus that acquires properties of an incentive stimulus; and so, in the absence of a sweet stimulus, there was a decrease in the magnitude of responding. Also, related to taste, it is possible that the sensory cue of taste interfered with attention to the light cue during conditioning, such that the taste cue resulted in an overshadowing effect (Cannon et al., 1985). Overshadowing occurs when a stimulus of a greater salience reduces the ability of the animal to learn cue light-stimulus associations (Kamin, 1969; Schachtman et al., 1992). This phenomenon can be observed with
both taste and odours. For instance, Taukulis & George (1982) reported that when a strong odour and a distinctive environmental context are paired with lithium, the odour interferes with conditioning to environmental cues. Similarly, in studies of taste aversion when a taste and a distinctive environmental context are paired with lithium, the taste interferes with conditioning to environmental cues (Meachum, 1990). Taken together we can conclude that conditioned cues associated with OXY induce more persistent seeking behaviour than cues associated with HFCS.

Comparison. The induction of reinstatement by HFCS cues during Dual SA, despite lack of reinstatement to HFCS cues during Single SA, suggests that the co-self-administration of HFCS with OXY resulted in an interaction between the reinforcers. This supports the hypothesis that DOA and sugar reinforcers act on overlapping pathways in the brain to affect reward processes (Volkow et al., 2012; Tomasi et al., 2015). Interestingly, seeking responses for OXY and HFCS remained the same in both Single and Dual SA even though there was concurrent access to both conditioned cues. Typically, during concurrent access to reinforcers, access to the palatable food leads to a devaluation of the other reinforcer (Panlilio, Hogarth, and Shoaib, 2015). Instead, we found that, overall animals were more energized, emitting more total responses during the test of cue reinstatement.

3.7.4. Prime-induced reinstatement

Single SA. An HFCS, but not OXY prime induced a reinstatement of responding. Following self-administration of OXY or HFCS, animals were administered an OXY- or HFCS-prime that was similar in dose/concentration to the maintenance dose/concentration during training. An OXY (0.05, 0.1, 0.2 mg/kg IV) prime did not produce a reinstatement in responding in Single SA. Alternatively, HFCS (50%) produced a significant reinstatement of responding (Figure 14). The lack of reinstatement after an OXY prime was surprising, given that non-
contingent priming injections of the self-administered drug typically lead to a reinstatement in responding (deWit & Stewart, 1981, 1983). It is assumed that priming injections induce incentive motivational states that energize motivated behaviour (Stewart, 1983) and lead to approach and interactions with stimuli previously associated with those effects resulting in reinstatement (Stewart & de Wit, 1987). Although, the magnitude of prime-induced reinstatement can vary depending on the priming dose and the pharmacological class of the priming drug (Gerber and Stretch 1975; de Wit and Stewart, 1983; Slikker et al. 1984; Comer et al. 1993; Worley et al. 1994). It is possible that the doses of OXY employed during reinstatement were not large enough to induce prime-induced reinstatement. Similarly, Stewart & Vezina (1988) found that injections of morphine into the VTA did not always reinstate responding. It might also be possible that although priming infusions may reinstate behaviour by predicting the availability of the drug, animals quickly learn that priming infusions during extinction do not lead to further drug availability. And, since they have gone through extinction learning twice, extinction learning would be strong.

There was a significant increase in responding following an IO infusion of HFCS (50%). Similarly, other studies have shown that non-contingent delivery of food increased response rate over baseline (Deluty, 1976; Eiserer, 1978; Pankseep & Trowill, 1967). As previously mentioned the lack of reinstatement to the 8% and 25% HFCS solutions may be a concentration effect. It is also possible that the feedback arising from the accumulation of food (by ad lib chow access in the home cage) in the gut outweighed the taste and early post-ingestive signals, and so it was not enough to induce reinstatement for 8% and 25% HFCS. In support of this interpretation, Cornell, Rodin, & Weingarten (1989) found that satiated subjects following a prime of ice-cream showed significantly reduced consumption of ice-cream compared to hungry subjects. Lastly, it is
important to mention that the increase in responding after 50% HFCS was modest and could be a fortuitous effect.

**Dual SA.** A priming injection of OXY selectively reinstated responding on the OXY-lever, whereas a HFCS prime did not engender a similar increase in responding on the HFCS lever (Figure 18). This finding is an example of discriminative stimulus control of responding by the drug. In other words, infusion of the self-administered reinforcer re-established the stimulus conditions that were present when responding was reinforced in the SA sessions and so responding was reinstated for OXY. Reinstatement produced by other drugs typically only occurs when the drug’s stimulus properties are known to resemble the self-administered drug (Ando & Yanagita, 1978). The lack of prime-induced reinstatement for a HFCS prime might suggest that animals can discriminate when an HFCS reinforcer will not be available, which might be a result of a lack of immediate sensory feedback.

**Comparison.** A comparison of responding during tests of OXY- and HFCS- induced reinstatement during both Single and Dual SA revealed that the effects of an OXY prime were enhanced when OXY was co-self-administered with HFCS. In fact, during Single SA an OXY prime did not increase responding for the OXY lever. This would suggest that HFCS and OXY must act on overlapping reinforcement pathways in order for HFCS to magnify the effects of OXY. The effect was not driven by differences in extinction responding prior to tests of OXY-induced reinstatements. And, since the number of pairings with OXY was similar across both Single and Dual SA, it suggests that the addition of HFCS specifically during training magnified the reinforcing effects of OXY.
3.8. Conclusion

Taken together our results indicate that when both sugars and DOA are self-administered by the same animal, the reinforcers interact and modify responses to one another. More specifically, OXY and HFCS alter responses to each other when self-administered together. Furthermore, the persistent choice for OXY over HFCS during tests of seeking behaviour supports the notion that sugar reinforcers may not be as strong as opioid reinforcers. Perhaps the stimulus-cue associations for DOA that were learned during training are stamped in ways that make DOA unique in their ability to cause a persistence of responding in the absence of the DOA. Determining the similarities and differences in the processes underlying opioid and sugar reinforcement and seeking behaviour can help us understand the process involved in each. Elucidation of differences could lead to different approaches for creating effective treatments for abuse and relapse.
<table>
<thead>
<tr>
<th>Session</th>
<th>Single self-administration for HFCS</th>
<th>Single self-administration for OXY</th>
<th>Dual Self-administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HFCS SA with lever and cue</td>
<td>OXY SA with lever and cue</td>
<td>OXY SA with lever 1 and cue1</td>
</tr>
<tr>
<td>2</td>
<td>No reinforcer, lever, or cues</td>
<td>No reinforcer, lever, or cues</td>
<td>HFCS SA with lever 2 and cue 2</td>
</tr>
<tr>
<td>3</td>
<td>HFCS SA with lever and cue</td>
<td>OXY SA with lever and cue</td>
<td>OXY SA with lever 1 and cue 1</td>
</tr>
<tr>
<td>4</td>
<td>No reinforcer, lever, or cues</td>
<td>No reinforcer, lever, or cues</td>
<td>HFCS SA with lever 2 and cue 2</td>
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<tr>
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</tr>
<tr>
<td>15</td>
<td>HFCS SA with lever and cue</td>
<td>OXY SA with lever and cue</td>
<td>OXY SA with lever 1 and cue 1</td>
</tr>
<tr>
<td>16</td>
<td>No reinforcer, lever, or cues</td>
<td>No reinforcer, lever, or cues</td>
<td>HFCS SA with lever 2 and cue 2</td>
</tr>
</tbody>
</table>

*Table 1.* Experimental design for Single and Dual self-administration (SA) of oxycodone (OXY) and/or high fructose corn syrup (HFCS). Animals were placed into operant chamber boxes for 3 hrs for all sessions. For Single SA, the day SA was initiated (Day 1 or 2) and cue (blinking or continuous) was counterbalanced for HFCS and OXY. Similarly, for Dual SA, the type of reinforcer (OXY or HFCS) self-administered on Day 1 and cue (blinking or continuous) was also counterbalanced.
<table>
<thead>
<tr>
<th>Oxycodone Dose (mg/kg/inf)</th>
<th>Infusions</th>
<th>Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>151.3 (23.4)</td>
<td>491.2 (113.5)</td>
</tr>
<tr>
<td>0.1</td>
<td>150.8 (23.4)</td>
<td>380.6 (78.0)</td>
</tr>
<tr>
<td>0.2</td>
<td>101.9 (12.6)</td>
<td>207.3 (22.6)</td>
</tr>
</tbody>
</table>

Table 2. Mean (±SEM) total infusions and responses across all self-administration sessions of oxycodone on a fixed ratio schedule of reinforcement during Single self-administration.

<table>
<thead>
<tr>
<th>HFCS Concentration (%)</th>
<th>Infusions</th>
<th>Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>252.8 (72.4)</td>
<td>786.0 (218.8)</td>
</tr>
<tr>
<td>25</td>
<td>466.3 (73.2)</td>
<td>1688.5 (394.9)</td>
</tr>
<tr>
<td>50</td>
<td>250.2 (24.4)</td>
<td>990.0 (193.2)</td>
</tr>
</tbody>
</table>

Table 3. Mean (±SEM) total infusions and responses across all self-administration sessions of high fructose corn syrup (HFCS) on a fixed ratio schedule of reinforcement during Single self-administration.
Figure 10. Mean (SEM) intravenous or intraoral infusions and responses (active and inactive) for oxycodone (0.05, 0.1, or 0.2 mg/kg IV; Panel A-C) and high fructose corn syrup (8%, 25%, or 50% IO; Panel D-F) self-administered on a continuous reinforcement schedule across 8 days of Single self-administration.
Figure 11. Mean (SEM) intravenous or intra-oral active lever responses for oxycodone (0.05, 0.1, 0.2 mg/kg/inf; panel A-C) and high fructose corn syrup (8%, 25% 50%; panel D-F) on a continuous reinforcement schedule across session (10 min bins) on self-administration Sessions 1 and 8.
Figure 12. Mean (SEM) responses on the oxycodone- or high fructose corn syrup-paired active (Panel A, C) and inactive levers (Panel B, D) on tests of extinction in animals that previously self-administered intravenous oxycodone (0.05, 0.1, or 0.2 mg/kg IV) or intraoral high fructose corn syrup (8%, 25%, or 50%).
Figure 13. Mean (SEM) responses on the oxycodone (OXY; Panel A) or high fructose corn syrup (HFCS; Panel B) paired active lever on the last session of extinction and on a test of cue induced reinstatement in animals that previously self-administered intravenous oxycodone (0.05, 0.1, or 0.2 mg/kg IV) or intraoral high fructose corn syrup (8%, 25%, or 50%).

* significantly different from extinction
Figure 14: Mean (SEM) responses on the oxycodone (OXY; Panel A) or high fructose corn syrup (HFCS; Panel B) paired active lever on the last day of extinction and on a test of prime induced reinstatement in animals that previously self-administered intravenous oxycodone (0.05, 0.1, or 0.2 mg/kg IV) or intraoral high fructose corn syrup (8%, 25%, or 50%).

* significantly different from extinction
Figure 15: Mean (SEM) intra-oral and intravenous active lever responses for oxycodone (OXY; 0.2 mg/kg/inf) or high fructose corn syrup (HFCS; 50%) across 16 alternating sessions.
Figure 16. Mean (SEM) responses on the simultaneously present high fructose corn syrup (HFCS)- and oxycodone (OXY)-paired active levers across extinction sessions (n = 16).

* Different from HFCS
*Figure 17.* Mean (SEM) responses on the active lever during extinction and cue-induced reinstatement on simultaneously present high fructose corn syrup (HFCS)- and oxycodone (OXY)- paired active levers in animals that previously dual self-administered ($n = 16$).

* represents significant differences from extinction

# represents significant difference from the HFCS lever
Figure 18. Mean (SEM) responses on the simultaneously present oxycodone (OXY)- or high fructose corn syrup (HFCS)- paired active levers during extinction, and HFCS- and OXY-induced prime reinstatement in animals that previously dual self-administered HFCS (50%) and OXY (0.2 mg/kg) (n = 16).

# significantly different from HFCS prime

*significantly different from Extinction
Chapter 4:

The effect of bupropion on cue-induced reinstatement of oxycodone and high fructose corn syrup
4.1. Abstract

*Introduction*: Bupropion (BUP) is a candidate medication for treating drug dependence and obesity. There are indications that it may be useful in treating craving. The objective of this study was to investigate the effects of BUP on cue-induced reinstatement for HFCS and OXY in the same animal. *Methods*: Each male Sprague-Dawley rat was implanted with both intra-oral (IO) and intravenous (IV) cannulas and trained to self-administer IO infusions of HFCS (50%) and IV infusions of OXY (0.2 mg/kg), over 16 alternating days, 3 hrs/day, on a continuous reinforcement schedule, by operating two different levers presented in the same context. Following acquisition of self-administration, all animals were tested in extinction conditions by simultaneously presenting both HFCS- and OXY-paired levers. Once responding reached low levels, rats were pre-treated with BUP (0, 10, and 30 mg/kg SC) and received a test of cue-induced reinstatement, during which lever presses activated the HFCS- or OXY-paired conditioned stimulus. *Results*: It was found that BUP (30 mg/kg) increased the magnitude of cue reinstatement for both OXY and HFCS; however, an examination of responding across the session revealed greater responding for OXY than HFCS cues. *Conclusions*: Taken together the results suggest that acute BUP does not reduce craving for reward cues. Instead, it increases reward reactivity to conditioned cues; particularly those associated with opioids.
4.2. Introduction

Opioid abuse disorders have become a public health burden. Their high potential for abuse has led to a decrease in the quality of life for those affected (SAMHSA, 2013). Opioid abuse disorders often co-occur with eating disorders (Root et al., 2010). It was reported that 36% of heroin users and 43.1% of methadone users were overweight (Rajs, 2009). Similarly, Nolan and Scagnelli (2007) found that methadone-maintained patients were more likely to have higher BMIs, and a preference for high sugar food, compared to controls. This is perhaps related to shared underlying behavioral and neurobiological processes (Volkow et al., 2012; Tomasi et al., 2015). A common feature among both DOA and palatable foods is the ability of environmental stimuli to elicit “craving” or “seeking” behaviour (Shalev, Grimm & Shaham, 2002; Kenny, 2006; Hamilin et al., 2006). This is a result of reinforcers being repeatedly paired with environmental stimuli; over time the stimuli become imbued with incentive properties that lead to increased “desire” for them and can contribute to increased relapse vulnerability (Berridge & Robinson, 2016). Multiple attempts involving behavioural (i.e., extinction therapy) and pharmacological strategies have been employed to decrease relapse vulnerability (Kantak & Dhonnchadha, 2011). More recently, one of these attempts has involved the use of bupropion (BUP).

BUP is a medication that has been prescribed for smoking cessation, obesity, depression, and ADHD (Stahl et al., 2004; Dwoskin et al., 2006; Holm & Spencer, 2000). It acts by inhibiting dopamine (DA) and norepinephrine (NE) reuptake and facilitates vesicular uptake of DA, resulting in increased DA and NE levels in the brain (Dwoskin et al., 2006). In humans, it
decreases appetite (Chouinard, 1983; Crone & Gabriel, 2004; Weisler et al., 1994; Dwoskin et al., 2006) and produces weight loss in overweight and obese women with binge eating disorder (White & Grilo, 2013; Anderson et al., 2002). In part, these effects are thought to be mediated by stimulation of POMC cells in the hypothalamus, which produces melanocyte-stimulating hormone, an agonist of melanocortin-4-receptors, that is associated with decreased appetite, increases in energy expenditure, and alterations in reward mediated eating (Brady, Smith, Gold, & Herkenham, 1990; Fan, Voss-Androal, Coa, & Marrison, 2005). These effects map onto pre-clinical studies, where BUP decreases the consumption of a palatable food reward (Levy et al., 2018; Randall et al., 2015; Zarrindast & Hosseini-Nia, 1988), while increasing the amount of work performed on a progressive ratio (PR) schedule to obtain a palatable reinforcer (Levy et al., 2018; Randall et al., 2015).

In addition to its use in treating obesity and eating disorders, there has been an increase in the use of BUP for nicotine and methamphetamine use disorder (Richmond and Zwar, 2003). BUP reduced withdrawal symptoms and cue-induced craving for methamphetamine (Newton et al., 2006; Berigan & Russell, 2001) and nicotine (Weinstein et al., 2009; Brody et al., 2004; Budzynska & Biaa, 2011; Shiffman et al., 2000; Strong et al., 2009). These effects are thought to be mediated by a reduction in activation in the ventral striatum, medial orbitofrontal cortex, and anterior cingulate cortex during craving in humans (Culberton et al., 2011). In pre-clinical studies, BUP pre-treatment is associated with decreases in nicotine SA on a fixed-ratio schedule (Rauhut, Dwoskin, & Bardo, 2005; Bruijnzeel & Markou, 2003; Glick et al., 2002; Liu et al., 2008), although increases in nicotine SA following BUP have also been observed (Shoaib, Sidhpura & Shafait, 2003). On a PR schedule BUP pre-treatment does not alter responding for nicotine but increases nicotine-seeking on tests of reinstatement (Liu et al., 2008). In animals
trained to self-administer stimulants, BUP pre-treatment reduced intake of amphetamine and methamphetamine (Reichel et al., 2008; Rauhut, et al., 2003) and decreased methamphetamine-induced stereotypy in mice (Muley et al., 1984). However, if methamphetamine preceded BUP treatment, methamphetamine induced stereotypy was augmented (Muley et al., 1984). And so, the effects of BUP appear to be affected by testing conditions (i.e., pre vs post treatment, schedule of reinforcement).

Only a few studies have investigated the effects of BUP on both food and DOA. Reichel, Murray, Grant, and Bevins (2009) demonstrated that BUP decreased intake of methamphetamine at various doses and decreased sucrose intake but only at the high dose. In another study using an effort-based task, Bruijnzeel & Markou (2003) found that BUP increased responding for food, while not altering responding for nicotine on a PR schedule. These findings suggest dissociation between the effects of BUP on responding for DOA and foods. However, even though the procedures were similar, these studies were conducted in separate groups of animals and so; it is possible that the results may be impacted by batch effects, or different pharmacological experiences.

One avenue to directly test the hypothesis that BUP differentially controls food- and drug- maintained responding is through the use of choice procedures. In choice procedures, an animal has access to two or more concurrently available reinforcers (Jones & Comer, 2013), and the animal allocates responses between the two. In using these models, BUP has shown to shift choice and increase tendency to work for palatable food (measured on PR schedule), while decreasing consumption of concurrently available chow (Randall et al., 2015). When given the choice between cocaine and a non-drug reinforcer (i.e., money), BUP pre-treatment reduces cocaine choice (Stoops, Lile, Glaser, Hays, & Rush, 2012). Lastly, Banks & Blough (2015)
found that BUP pre-treatment did not alter choice between methamphetamine and food in rhesus monkeys. A finding consistent with human lab choice studies (Stoops et al., 2015).

In the current experiment, we tested the hypothesis that BUP differentially controls behaviour controlled by DOA and foods. More specifically, we employed Dual SA to examine whether BUP pre-treatment affected choice for high fructose corn syrup (HFCS) and oxycodone (OXY) associated cues during a test of cue-induced reinstatement. We might expect that since BUP is shown to decrease cue-elicited craving for DOA and decrease appetite, that this will decrease the incentive value for the cues associated with HFCS and OXY and there will be a reduction in responding for both cues.

4.3. Methods and Materials

4.3.1. Subjects

Subjects were adult male Sprague-Dawley rats (Charles River, QC) weighing 250-300 g at the beginning of experiments. Rats were individually housed, maintained on a reverse light/dark cycle (7:00 AM lights off; 7:00 PM light on) and behavioural testing occurred during their active cycle. All rats had free access to food and water except during testing. All procedures were approved by the Animal Care Committee of the University of Guelph and were carried out in accordance with the recommendations of the Canadian Council on Animal Care.

4.3.2. Apparatus

See Chapter 3 for details of apparatus.

4.3.3. Intravenous and Intraoral surgery and self-administration.

All animals were implanted with an intraoral (IO) and intravenous (IV) cannula as previously described in Leri et al. (2009) and Levy et al. (2014). In the case of Dual SA, both IO
and IV surgeries were conducted the same day; where the IV surgery was conducted prior to the IO surgery.

4.3.4. General Procedures

4.3.4.1. Self-administration.

The procedure is similar to that described for the Dual SA paradigm in Experiment 3. A total of 33 rats were trained to lever press for IO infusions of HFCS (50%) and IV infusions of OXY (0.2 mg/kg/inf) on a continuous reinforcement schedule across 16 alternating days for 3 hours/day. Each press on the active lever resulted in the delivery of OXY (0.2 mg/kg/inf; 150 μL infusion) or HFCS (50%; 90 μL infusion) and continuous illumination of a stimulus light for 30 seconds; responses on the second lever the following day (delivering the alternate reinforcer) were associated with a light that flashed at a rate of 1 Hz. This allowed the reinforcers to be paired with different cues.

4.3.4.2. Extinction.

Following training, animals underwent a 4-day OXY and HFCS free period, following which they underwent extinction whereby both active levers were introduced into the chamber, and lever presses no longer resulted in infusions of HFCS, OXY, and paired light cues.

4.3.4.3. Tests of cue reinstatement.

Once responding was extinguished (i.e., ≤ 15 responses), to assess the impact of BUP on cue-induced reinstatement, animals were injected with BUP (0, 10, or 30 mg/kg SC) immediately before being put into operant chamber boxes. Presses on the active lever activated the appropriate light conditioned stimulus (continuous or blinking). This test lasted 3 hours.

4.4. Drugs and high fructose corn syrup
Bupropion hydrochloride (Toronto Research Chemicals, Toronto, ON, Canada) was dissolved in 0.9% physiological saline and administered within a range of doses known to differentially affect operant responding for food (Levy et al., 2018) and drug reward (Palmatier et al., 2009; Reichel, et al., 2009).

4.5. Statistical Analyses

This experiment was performed in several replications over the course of several months. In each replication, animals received 0 (n = 10), 10 (n = 10), or 30 mg/kg (n = 13) BUP prior to a test of cue-induced reinstatement. Data were analyzed using mixed analysis of variance (ANOVA). Significant interactions and main effects were assessed by multiple comparisons using the Student-Newman-Keuls method (alpha = .05). Exact values of non-significant results are not reported, and all analyses were performed using SigmaStat (v.3.5 for Windows).

4.6. Results

4.6.1. Exclusion criteria

At the conclusion of data collection, it was noted that not all rats acquired self-administration. Because it was deemed inappropriate to include animals that never acquired self-administration in tests of relapse, a criterion for exclusion from data analysis had to be established (see Chapter 3). A total of 9 animals were excluded.

4.6.2. Experimental results

4.6.2.1. Self-administration: There was more instrumental responding for HFCS than for OXY

Figure 19 represents mean (SEM) active lever responses obtained during SA on HFCS- and OXY-paired levers. The ANOVA identified a significant Session X Lever interaction \([F(7, 205) = 4.44, p < .01]\) and a significant main effect of Lever \([F(1, 31) = 5.76, p < .05]\). There was
no main effect of Session. Multiple comparisons revealed that there was significantly more responding on the HFCS lever than the OXY lever on Sessions 1, 2 and 4.

4.6.2.2. **Extinction: Greater responding on the OXY lever during extinction.**

Figure 20 represents mean (SEM) active lever responses obtained during extinction on the HFCS- and OXY-paired levers. The ANOVA identified a significant Session X Lever interaction \[F(3, 93) = 7.02, p < .001\], as well as significant main effect of Session \[F(3, 93) = 108.31, p < .001\] and Lever \[F(1, 31) = 12.07, p < .01\]. Multiple comparisons indicated that there was significantly more responding on the OXY lever than the HFCS lever on extinction session 1 and that overall responding decreased across sessions on the OXY and HFCS active levers.

4.6.2.3. **Tests of cue reinstatement: 30 mg/kg BUP significantly increased responding on the OXY and HFCS lever.**

An ANOVA comparing mean (+SEM) responses obtained during cue reinstatement following pre-treatment with BUP (0, 10, and 30 mg/kg) identified a significant main effect of Dose \[F(2, 30) = 3.97, p < .05\]. No significant Dose X Lever interaction or Lever. Multiple comparisons on marginal means revealed that animals pre-treated with 30 mg/kg BUP displayed significantly more responding than animals pre-treated with 0 or 10 mg/kg BUP.

Figure 21A represents mean (SEM) active lever responses obtained over the session during cue induced reinstatement for OXY and HFCS after pre-treatment with 0 BUP. The ANOVA identified a significant Time by Lever interaction \[F(17, 153) = 2.06, p < .05\] and main effect of Time \[F(17, 153) = 2.25, p < .01\]. No main effect of Lever was identified. Multiple comparisons revealed that there was a significant difference between HFCS and OXY responding at 10, 60, and 120 mins.
Figure 21A (embedded graph) represents mean (SEM) active lever responses obtained during extinction and cue reinstatement for OXY or HFCS after pre-treatment with 0 BUP. The ANOVA identified a significant effect of Session for both OXY \([F(1, 9) = 14.96, p < .01]\) and HFCS \([F(1, 9) = 7.55, p < .05]\).

Figure 21B represents mean (SEM) active lever responses obtained over the session during cue-induced reinstatement for OXY and HFCS after pre-treatment with 10 BUP. The ANOVA identified a significant main effect of Time \([F(17, 153) = 1.90, p < .05]\). No significant main effect of Lever or significant Time by Lever interaction was identified. Multiple comparisons revealed that across the session, responding on the active lever decreased.

Figure 21C represents mean (SEM) active lever responses obtained over the session during cue induced reinstatement for OXY and HFCS after pre-treatment with 30 BUP. The ANOVA identified a significant Time by Lever interaction \([F(17, 204) = 1.88, p < .05]\) and significant main effect of Time \([F(17, 204) = 1.81, p < .05]\). No main effect of Lever was observed. Multiple comparisons revealed that there was significantly more responding on the OXY lever than the HFCS lever at 30 and 60 mins.

Figure 21C (embedded graph) represents mean (SEM) active lever responses obtained during extinction and cue reinstatement for OXY or HFCS after pre-treatment with 30 BUP. The ANOVA identified a significant effect of Session for both OXY \([F(1, 12) = 5.31, p < .05]\) and HFCS \([F(1, 11) = 18.58, p = .001]\).
4.7. Discussion

Bupropion (BUP) has been a candidate medication for treating drug dependence and obesity. There are indications that it may be useful in treating craving associated with rewarding stimuli. In this study we investigated the effect of BUP on cue-induced reinstatement for high fructose corn syrup (HFCS) and oxycodone (OXY) in the same animal. To investigate this, male Sprague-Dawley rats were implanted with intra-oral (IO) and intravenous (IV) cannulas and trained to co-self-administer OXY (0.2 mg/kg) and HFCS (50%) on alternate days by operating one of two levers presented in the same experimental context. Following 16 days of self-administration (SA), animals underwent extinction training, where they were simultaneously presented with HFCS- and OXY-paired levers. Following extinction, animals were administered an injection of BUP (0, 10, or 30 mg/kg SC) and received a test of cue reinstatement. It was found that BUP (30 mg/kg) increased the magnitude of cue reinstatement for both OXY and HFCS; furthermore, there was more responding for OXY than HFCS cues. Taken together, our results do not support the conclusion that BUP reduces craving for reward cues. Instead, the results suggest that acute BUP increases reward reactivity to conditioned cues.

BUP (30 mg/kg) increased in the magnitude of reinstatement for OXY cues more than for HFCS cues. Following extinction of responding in animals trained to co-self-administer OXY (0.2 mg/kg) and HFCS (50%), animals were tested in cue reinstatement after pre-treatment with BUP (0, 10, or 30 mg/kg SC). In animals that were pre-treated with 30 mg/kg BUP, there was significantly more responding at 30 and 60 minutes for OXY cues than HFCS cues. Although there was a lot of individual variability for responses on the OXY lever, there was a trend for
more responding for OXY than HFCS. These changes in responding were independent of changes on the inactive lever (data not shown) and so we can conclude that the increase in responding was selective and unlikely to be affected by changes in activity level during the session.

These results were surprising in light of studies that have demonstrated that BUP reduces craving induced by cues (Richmond & Zwar, 2003; Newton et al., 2006; Berigan & Russell, 2001; Budzynska & Biala, 2011). Although, similar to our findings, Liu et al. (2008) found that BUP pre-treatment increased seeking for nicotine cues. And, considering the high recidivism rates within abstinent smokers on BUP treatment (Hurt et al. 1997; Jorenby et al. 1999; Hurt et al. 2003; Killen et al. 2006), our findings may indicate that BUP has little benefit for the reduction of reactivity to conditioned cues. That being said, in our studies we administered BUP acutely, whereas in clinical studies administration of BUP occurs across several sessions. Also, BUP functions as an inhibitor of dopamine (DA) and norepinephrine (NE) transporters, thereby increasing the level of these neurotransmitters in the brain (Nomikos et al. 1989; Li et al. 2002); it has been shown that drugs that increase DA and NE promote increased responding to cues (De Vries et al. 1999), which may explain the observed effects.

Lastly, we predicted that if HFCS and OXY cues were controlled by the exact same neural mechanisms, there would be similar alterations in responding on both levers. We did observe an increase in responding for both HFCS and OXY, however there was a larger increase in responding for OXY, suggesting some specificity in BUP’s actions on conditioned reinforcers, a finding confirmed by other studies (Reichel, et al., 2009). Additionally, BUP is self-administered above a vehicle in animals (Lamb & Griffiths, 1990), therefore it is possible that
BUP has discriminative stimulus effects which closely resemble those of OXY’s, which would have lead to a cross drug reinstatement effect.

4.8. Conclusion

Taken together the results of this study suggest that acute administration of BUP might not be effective in reducing relapse vulnerability for reward cues, since it increased reactivity to cues paired with HFCS and OXY. Additionally, differences in reactivity to OXY and HFCS cues elicited by BUP indicate that rather than a general reinforcement pathway being recruited, there are parallel, but perhaps different pathways controlling cue reactivity to opioid and sugar cues.
Figure 19: Mean (SEM) intra-oral and intravenous active lever responses for high fructose corn syrup (HFCS; 50%) or oxycodone (OXY; 0.2 mg/kg/inf) across 16 alternating sessions ($n = 32$).

*Significantly different from HFCS
Figure 20. Mean (SEM) responses on the simultaneously present high fructose corn syrup (HFCS)- and oxycodone (OXY)-paired active levers across extinction sessions ($n = 32$).

*Significantly different from HFCS
Figure 21: Mean (SEM) active lever responses on the simultaneously present oxycodone (OXY)- or high fructose corn syrup (HFCS)-paired levers over the test for cue induced
reinstatement following pre-retreatment with an intraperitoneal injection of 0 (Panel A; \( n = 10 \)),
10 (Panel B; \( n = 10 \)), or 30 mg/kg (Panel C; \( n = 12 \)) bupropion. Embedded graphs represent
Mean (SEM) total active lever responses on the simultaneously present oxycodone (OXY) - or
high fructose corn syrup (HFCS)-paired levers for the test for cue induced reinstatement.
* significantly different from HFCS
Chapter 5

General Discussion
The excessive consumption of highly palatable food shares behavioural characteristics of substance use disorders and involves common neurobiological pathways of reward (Volkow et al., 2012; Tomasi et al., 2015). These similarities have led to the “food addiction” hypothesis which predicts that food processed to be highly palatable can promote “addictive”-like behaviours. One key prediction of this hypothesis is that sugar and drug reinforcers will interact to influence responses to each other; second their behaviours will be comparable in a situation where their availability, or the availability of their conditioned stimuli (CS), is similar. To address these questions, high fructose corn syrup (HFCS), a widely added sweetener in the North American diet, and oxycodone (OXY), a widely used and abused prescription opioid, were utilized in this dissertation. Collectively, the data demonstrate that opioids and caloric sweeteners act on similar systems of learning and reinforcement, however opioids have an ability to create stimulus-reinforcer associations that are more persistent. These studies further clarify the parallels and points of divergence between sugar and drug reinforcers.

5.1. Summary of the results

The research reported in this dissertation generated three main results of interest:

1. **The effect of high fructose corn syrup pre-exposure on responses to oxycodone**

   The experiments in Chapter 2 found that, opioids and sugars interact at both behavioural and pharmacological levels; and that nutrition has the potential to influence some, but not all responses to opioids. More specifically, HFCS pre-exposure decreased the OXY-induced dopaminergic response in the NAc and decreased OXY-induced locomotion; however, it did not modify OXY-induced place preference.
2. Dual self-administration of oxycodone and high fructose corn syrup

The experiments in Chapter 3, revealed that sugars and opioids differentially control motivated behaviour expressed by the same subjects, whereby OXY engenders more persistent seeking behaviour than HFCS. Furthermore, the consumption of one reinforcer changes behavioural responses to the other during tests of seeking. More specifically, during tests of extinction and cue reinstatement there was more responding on the OXY-paired lever than the HFCS-paired lever. Additionally, co-self-administration of OXY and HFCS enhanced the effects of HFCS cue reinstatement and the effects of an OXY-prime on OXY-lever responding.

3. The effect of bupropion on cue-induced reinstatement

Finally, the experiments in Chapter 4 demonstrated that bupropion (BUP) does not reduce reactivity to OXY- and HFCS-paired cues. Instead, it increased the magnitude of cue reinstatement for both OXY and HFCS. Furthermore, the magnitude of reinstatement was higher for OXY than HFCS cues.

5. 2. Similarities between oxycodone and high fructose corn syrup as reinforcers

Chapters 2, 3, and 4 provide evidence that several behavioural and pharmacological similarities exist between highly processed sugars like HFCS and opioids such as OXY. These similarities are presented as similar behavioural phenotypes and pharmacological responses, such as: the “loading effect,” dose-response curves, extinction responding, and reactivity to BUP. Furthermore, similarities also reveal themselves as interactions between HFCS and OXY behavioural and pharmacological responses.

Loading effect. The “loading effect” is a characteristic often observed with DOA (Ahmed & Koob, 1998). It is defined as an increase that occurs over time in the intake/responses
following the onset of access to the drug reinforcer (Ahmed & Koob, 1998; Rada et al., 2005; Avena et al., 2006). In Chapter 3, a comparison of responding over the first hour of access to OXY and HFCS revealed the development of a loading effect for all doses and concentrations. More specifically, there was greater responding for, and consumption of, OXY and HFCS on Session 8 than on Session 1 of SA. In 1998, Ahmed and Koob examined the influence of the loading effect on relapse in rats trained to self-administer cocaine for 1 hr/day (Short Access) or 6 hours/day (Long Access). An examination of the first hour of intake in both groups revealed that long access exposure to cocaine led to the development of an escalation of intake; an effect not observed in animals with short access. Subsequently, during tests of relapse, they found that prior history of drug escalation (i.e., loading) facilitated relapse to cocaine (Ahmed & Koob, 1998). The development of the “loading effect” is thought to co-occur with neural alterations that lead to an enhancement of motor responses and reward (Avena et al., 2003, 2004, 2008). It is believed that the loading effect is indicative of a transition between voluntary drug use to compulsive intake (Koob & LeMoal, 2001; Ifland et al., 2009). It is considered a hallmark of substance abuse disorders and criterion for abuse liability (Cummins & Leri, 2008).

**Dose-response curve.** Another characteristic of self-administration of DOA is that drug intake is controlled by the dose of each drug infusion. Varying the dose of DOA can lead to an inverted U curve when animals self-administer on continuous reinforcement schedules (Yokel, 1987; Carney et al., 1976; Mello & Negus, 1996); low doses of a drug reinforcer fail to maintain a high level of responding (ascending limb of the curve) and then within a certain range of doses (descending limb of the curve) responding progressively decreases as the dose increases in order to maintain a constant level of intake (Ahmed & Koob, 1999; Lynch & Carroll, 2001; Carter & Griffiths, 2009). It has been reported that animals will self-administer a substance to keep DA
levels in the NAc at a constant level (Kenny et al., 2003). Similar to DOA, HFCS (8%, 25% & 50%) produced an inverted-U concentration response curve, where 8% and 25% made up the ascending limb and 50% made up the descending limb. This pattern of responding has previously been observed in food-restricted animals (Levy et al., 2015) as well. It appears that for OXY (0.05, 0.1, and 0.2 mg/kg), only the descending limb of the dose response curve was captured.

**Extinction of responding.** Extinction training involves situations whereby the response is no longer followed by a reinforcer, or a conditioned stimulus is no longer paired with the unconditioned stimulus (Millian, Marchant, & McNally, 2011). Repeated presentations of a non-reinforced cue leads to a dissipation or “extinguishing” of operant conditioned responses over time (Taylor et al., 2009). Extinction is believed to involve new learning rather than erasing initial learning (Bouton, 2004). In Chapter 3, animals decreased their responding on both OXY and HFCS active levers. The similar expression of extinction responding might suggest that opioids and sugar seeking involve parallel pathways of extinction learning (Millan, Marchant & McNally, 2011).

Additionally, it was found that for both OXY and HFCS the previous dose or concentration self-administered did not alter the magnitude of extinction responding. There is some evidence that the expression of extinction to instrumental responding is different than extinction of drug-paired cue memory; an effect that may be related to the differential involvement of infralimbic PFC in the two behaviours (Peters et al., 2008; Koya et al., 2009). This may explain in part why the magnitude of extinction responding was not altered by previous dose/concentration.

**Bupropion.** In Chapter 4, it was found that BUP increased responding for both HFCS and OXY cues, thereby suggesting the recruitment of parallel processes. BUP increases DA and NE
levels in the brain (Dwoskin et al., 2006), and so these results are consistent with other studies that show that DA agonists increase responding for cues (du Hoffman & Nicola, 2016).

**Interactions.** An interesting empirical contribution of this dissertation is the exploration of interactions between HFCS and OXY. In Chapters 2 and 3, it was found that HFCS and OXY were able to alter responses to each other. In Chapter 2, previous exposure to HFCS blunted the NAc DA response elicited by OXY, as well decreased the stimulatory properties of high dose OXY on locomotor behaviour. Given that previous HFCS consumption reduced DA release by OXY, it is not surprising that animals displayed a blunted locomotor response. The activation of midbrain DA is believed to be necessary in motor responses (Kalivas & Stewart, 1991; Cador et al., 1995). Our behavioural results support this interpretation.

This phenomenon of cross-tolerance (cross-dependence), where repeated exposure to one stimulus causes tolerance to the effects of another stimulus, is observed with sugars and DOA (Carroll, Rodefer, Rawleigh, 1995; Carroll, Lac, Nygaard, 1989; Davis et al., 2008). For example, animals with a long history of sucrose consumption become tolerant to the analgesic effects of morphine (D’Anci, Kanarek, & Marks-Kaufman, 1996). There is also evidence that repeated exposure to a palatable diet decreases place preference for amphetamine (Davis et al., 2008), as well as operant responding for sucrose pellets (Davis et al., 2008), cocaine (Carroll, Lac, Nygaard, 1989) and ethanol (Carroll, Rodefer, Rawleigh, 1995). Cross-tolerance or cross-sensitization effects are often observed between substances that share similar pharmacokinetic properties. This is consistent with the idea that highly palatable foods are more potent than other foods because their processing makes them more “drug-like.”

In Chapter 3, using a different procedure where animals co-self-administered OXY and HFCS (Dual SA), it was found that the consumption of HFCS and OXY subsequently increased
the effect of HFCS cues on seeking behaviour during tests of cue reinstatement. Additionally, co-self-administration increased the effects of an OXY prime on seeking behaviour. These effects are more akin to a cross-sensitization effect, where pre-treatment with one stimulus results in an enhancement in the effects of another stimulus (Callaway and Geyer, 1992; Yang et al., 2003; Vitale, Chen & Kanarek, 2003; Stewart & Badiani, 1993). The observed cross-sensitization effect is supported by previous literature that demonstrates that long-term intake of sucrose enhances the locomotor or operant responses to opioids (Kanarek et al., 1997; Vitale, Chen, & Kanarek, 2003), stimulants (Vitale, Chen, & Kanarek, 2003; Gosnell, 2005), alcohol (Avena et al., 2004), and nicotine (Clemens, Caille, & Cador, 2010). Taken together, our results indicate that DOA and highly palatable food have a unique ability to alter mechanisms responsible for reinforcement and learning and, as a result, repeated exposure to one may consequently alter responding to the other, perhaps enhancing the risk of abuse.

**5.3. Differences between Oxycodone and High Fructose Corn Syrup as reinforcers**

Although Chapters 3 and 4 provide evidence that OXY and HFCS recruit similar learning and reinforcement systems, the extent to which they engage these systems are different. In Chapter 3, during Single SA, only OXY cues were able to increase seeking behaviour. In Dual SA, a comparison of responding in the same animals revealed greater operant responding for HFCS than for OXY during SA. However, in tests of extinction and cue-induced reinstatement there was more responding on the OXY-paired lever than the HFCS-paired lever. Furthermore, co-self-administration of OXY and HFCS only enhanced the effects of an OXY-prime on OXY-lever responding, an effect not observed with HFCS. In Chapter 4, BUP increased seeking during a test of cue-induced reinstatement for both OXY and HFCS, however a comparison of responding over the session revealed that the magnitude of seeking was greater for OXY.
The finding that DOA exert effects on reinforcement that are greater in magnitude than those observed with sugar sweeteners is supported by primate studies, where a preference for cocaine over food is easily established (Nader & Woolverton, 1991, 1992; Aigner & Balster, 1978; Anderson, Velkey, & Woolverton, 2002). As well, similar to the results of our study, drug cues have shown to lead to a greater persistence of seeking behavior (Cicciocioppo, Martin-Fradon, & Weiss, 2004; Ahmed & Koob, 1997; Buczek et al., 1999). These results would be consistent with studies of food addiction in humans that show limited evidence of food addiction (Westwater, Fletcher, & Ziauddeen, 2016). Taken together, data suggest that while HFCS produces behaviour that is similar to OXY, there are long-lasting associations formed that appear to be stronger with OXY. Since the experiments in Chapters 3 and 4 make comparisons between drug- and sugar-reinforcers in the same animal, and using similar experimental conditions, the argument that there are important differences in how highly processed sugars and opioids control motivated behaviour becomes even more convincing.

5.4. Cross-tolerance vs cross-sensitization

Chapters 2 and 3 appear to reveal opposing results: In Chapter 2, it was found that exposure to HFCS induced tolerance-like effects for OXY, whereas in Chapter 3 co-self-administration with HFCS led to a sensitization of OXY’s effects on prime-induced reinstatement. One explanation for these contradictory results is that animals had different amounts of exposure to the primary reinforcers. In Chapter 2, animals had access to HFCS for 24 hrs/day, whereas in Chapter 3 animals only had access for 3 hours/day. It is possible that the home cage model (24 hrs/day, Chapter 2) is more akin to a continuous exposure paradigm, whereas the SA model (3 hrs/day, Chapter 3) is more akin to an intermittent access paradigm. It is well known that intermittent access and continuous access have different effects on tolerance
and sensitization (Calipari et al., 2013; Post, 1980). More specifically chronic continuous stimulation is associated with the development of pharmacological tolerance (Calipari et al., 2013), whereas intermittent stimulation is associated with the development of sensitization (Post, 1980). Nevertheless, both experiments demonstrate that opioids and sugars can influence responses to one another, suggesting a recruitment of similar underlying processes.

5.5. Implications of a dysregulated DA system

In Chapter 2, previous exposure to HFCS reduced the OXY-induced DA response. It has been shown that prolonged DA receptor stimulation from a high fat/sugar diet leads to reduced DA turnover in the NAc and reduced levels of striatal D2 receptors (D2R; Hamdi et al., 1992; Hajnal et al., 2008). It is believed that these changes reduce reward sensitivity (Johnson & Kenny, 2010). Alterations in DA related processes have only been observed in animals after significant weight gain (Johnson & Kenny, 2010) or in animals on food restriction diets (Rada et al., 2005). Weight gain or obesity induces changes to lipid profiles and endocrine appetite markers (de Oliveira et al., 2014; Machado et al., 2012; Johnson & Kenny, 2010; Stice et al., 2010), which have effects on DA responsiveness and food restriction is associated with increased DA sensitivity (Thanos et al., 2008). In the current set of experiments there were no differences in weight gain between the control group and the HFCS diet group. This is largely because increased caloric intake from HFCS was compensated by decreases in chow consumption in animals. Therefore, overall calorie consumption was similar in both groups. This possibly suggests that repeated consumption of HFCS is sufficient to drive DA dysfunction; an effect that is independent of weight gain. That being said, the accumulation of adipose tissue, and not necessarily weight gain, is a better indicator of whether there will be neural alterations in the brain (Levy et al., 2015); changes in adipose tissue were not measured in our studies.
A dysregulated DA system is similar to that observed following chronic consumption of morphine and cocaine, and is associated with decreases in NAc D2R mRNA in rats (Georges et al., 1999; Turchan et al., 1997). Likewise, in humans, a downregulation of D2R (high affinity receptors) is observed in cocaine (Volkow et al., 1993), methamphetamine (Volkow et al., 2001), and alcohol (Volkow et al., 1996) abusers. Some believe that reduced DA levels lead to an escalation of intake for both DOA and palatable food as an attempt to restore previous DA levels to achieve the same level of pleasure that was previously experienced (Blum et al., 2000; Blum et al., 1995; Volkow et al., 2010).

5.6. Implications for relapse

The risk of relapse during periods of abstinence is a major challenge for the treatment of opiate drug addiction and excessive eating (Peterson and Mitchell, 1999; Unnithan, Gossop, & Strang, 1992; Gossop et al., 2002). The enduring nature of conditioned associations is one of the factors contributing to relapse. During co-self-administration of OXY and HFCS (Chapter 3), both HFCS and OXY cues engendered seeking behaviour. There were also significant increases in the levels of responding for HFCS- and OXY-paired levers on the first day of extinction; in part the paired lever is thought to function as a CS. During Dual SA, both HFCS and OXY cues reinstated responding. This is important, because associative learning plays a role in eating behaviour and is important in the context of current obesity. Obese individuals are particularly hyper-responsive to food cues (measured by increased BOLD responses in reward regions in the brain), indicating that obesity changes food-cue reactivity (Rothemund et al., 2007; Stice et al., 2008; Davids et al., 2010; Martin et al., 2010). Similarly, in the pre-clinical model, Todd, Winterbaur, and Bouton (2012) showed that the presence of excitatory food cues or the removal of inhibitory cues can prompt appetitive behaviour under conditions of satiety. It is believed that
cues associated with highly palatable food can do this by overriding satiety signals (Harb & Almeda, 2014).

This increased motivation for palatable food and drug cues is believed to involve similar DA mechanisms. However, the development of substance dependence involves neurobiological changes that have not been consistently shown for sucrose or other foods (Chen et al, 2008, 2014; Pelloux et al, 2007; Porrino et al, 2004; Vanderschuren and Everitt, 2004; Willuhn et al, 2012). These findings support our interpretation that cues associated with drug reinforcers are more potent in inducing seeking behaviour.

5.7. The influence of a highly palatable diet on opioid abuse liability

It has been suggested that proper nutrition can help reduce the use and effects of illicit opioids (Mohs, Watson, & Leonard-Green, 1990). A lack of macro- and micro-nutrients in the diet is believed to contribute to symptoms of depression (Kohotsu, 2005), anxiety (Smriga et al., 2002), and low energy, which all are factors that may contribute to substance abuse (Neighbours, Kempton, & Forehand, 1992). Furthermore, substance abuse is often associated with malnutrition by way of not eating enough or eating foods devoid of nutrients (Morabia et al. 1989; Grotzky-Giorgi, 2009). Opioid dependent individuals are shown to display a shift in preference from foods that are rich in fat/protein to foods high in sugar (Zador et al., 1996; Nolan & Scagnelli, 2007). This makes sense as sugars have shown to activate both opioid and DA systems in regions of the brain involved in reward (Hietala et al., 1994; Volkow et al., 2012; Gorelick et al., 2005; Heinz et al., 2005). Manipulations of the endogenous opioid system alter the hedonic/reward aspects of feeding in animals (Holtzman, 1979; Mucha & Iversen, 1986; Rudski et al., 1997; Gosnell et al., 2010), and clinical studies report that individuals maintained
on opioids self-report increased consumption and heightened taste preference for sweet food (Morabia et al. 1989).

Consistent with this, the results from the experiments of Chapters 2 and 3 support the interpretation that a diet rich in HFCS can alter responses to opioids. As previously mentioned, in Chapter 2, it was found that continuous (24 hr) access to HFCS resulted in tolerance-like effects to opioids, and in Chapter 3 it was found that intermittent (3 hr) access to HFCS caused sensitization effects, both of which have been linked to continued drug use (Robinson & Berridge, 1993; Koob & LeMoal, 2001). Since the consumption of HFCS can alter behavioural and pharmacological responses to opioids, diet should be a factor that is considered in the treatment of opioid abuse disorders. And so, the reduction of highly refined sugar may help us to reduce the obesity epidemic, but it is also an environmental factor that may mitigate the effects of opioid abuse.

5.8. Methodological considerations

Differences in procedures and methods in the current set of experiments may have contributed to differences observed in our results and those found in the literature.

Choice of sweetener. It is possible that increased responding for OXY cues over HFCS cues is due to HFCS being the sugar reinforcer instead of sucrose. Our results were inconsistent with those studies that have reported increased choice for sucrose (or saccharin) over psychostimulants (Ahmed et al., 2013; Lenoir & Ahmed, 2008; Lenoir et al., 2007; Cantin et al., 2010); a result also replicated with cues (Madsen & Ahmed, 2015). That being said, it is unlikely that the difference in type of sweetener would have decreased subsequent choice behaviour for HFCS. HFCS is shown to be equally as palatable as sucrose and engenders similar breakpoints on a progressive ratio schedule (Levy et al., 2015). In the same studies HFCS and sucrose were
shown to induce brain mRNA alterations similar to DOA, but these alterations were greater in magnitude for HFCS than sucrose. Furthermore, a comparison of long-term HFCS and sucrose consumption reveals that subjects exposed to HFCS were found to have: increased fat accrual and circulating levels of triglycerides (Levy et al., 2015; Light et al., 2009; Bocarsly et al., 2010), increased proinflammatory cytokines (interleukin 6 and interleukin 1B) in the dorsal hippocampus (Hsu et al., 2015), and increases in neural activation in areas that regulate food intake (Levy et al., 2015). And so, it is unlikely that HFCS decreased effects on learning and reinforcement processes. Instead previous data would lead us to expect a greater increase on reinforcement than sucrose.

Operant intra-oral self-administration. Procedural differences in how food was consumed may have altered the ability of sugars to reinforce behaviour. There were two significant differences in our SA methodology that may have contributed to the lack of effects observed with HFCS during seeking tests. First, the experiments in this dissertation utilized the operant intra-oral (IO) SA procedure. Typically, in studies of food reinforcement, animals are required to voluntarily consume solid foods, drink solution from a spout, or operant respond for solid pellets or drops of fluid delivered in a receptacle. It is possible that the increase in the number of responses required to obtain a food reinforcer led to a strengthening of behaviour in these experiments. However, the IO SA method was developed to allow for immediate reinforcement by allowing animals to self-inject solutions directly into their oral cavity thereby reducing the number of behaviors that are required in traditional food SA models (i.e., approaching the food cup, handling). This type of reinforcement pattern is more similar to DOA than the traditional food reinforcement models. The intravenous consumption of DOA can occur anywhere in the operant chamber; IO SA also allows for this, whereas in the traditional model of
food consumption, consumption occurs beside the predictive lever. Therefore, by using the IO procedure, we are able to better replicate the spatial contiguity associated with IV consumption. In addition, immediate reinforcement has shown to engender stronger conditioning (Dickinson, Watt, & Griffith, 1992).

Another difference in SA methodology was that in the current experiment, animals were trained to self-administer both HFCS and OXY on alternate days, whereas in the studies previously reported, animals were given a choice between concurrently available reinforcers during training (Ahmed et al., 2013; Lenoir & Ahmed, 2008; Lenoir et al., 2007; Cantin et al., 2010). This procedural difference may have affected how animals respond for both HFCS and OXY cues in subsequent tests of relapse.

Microdialysis. Because different subpopulations of neurons in the NAc have different effects on reward related behaviour (Ambroggi et al., 2011), it would have been advantageous to use smaller probes to isolate measurements of DA from different DA neuron subpopulations. The microdialysis probes were 1.5-2 mm in length and spanned hundreds of microns in diameter; hence measurements were conducted within a large span of terminal inputs. Using smaller probes may help us to isolate our effects as the NAc receives inputs from various populations of DA neurons (Ikemoto, 2007); as well there are distinct subpopulations of DA neurons with differing electrophysiological characteristics and projection targets which differentially react to rewards (Ikemoto, 2007; Lammel et al., 2008; Matsumoto and Hikosaka, 2009; Brown et al., 2011).

Food restriction. The lack of an effect of an intermittent HFCS access diet on CPP may be a result of differences in our restriction methodology. Typically, food restriction paradigms involve the removal of water, chow and palatable food reinforcers for 12 hours (Avena &
Hoebel, 2003; Avena et al., 2004, 2005; Avena, Rada, & Hoebel, 2008). This is believed to induce binge-like behaviour and is believed by some to be necessary in the enhancement of physiological and reward responses (Avena & Bocarsly, 2012). In the current set of experiments, only HFCS was removed, otherwise animals had ad-lib access to chow and water. Even though a “binge-like” phenotype was observed, there were changes in CPP. Many characteristics of abuse liability observed with palatable food are found using food restriction/deprivation paradigms. It may also explain the obtained cross-tolerance effect rather than a cross-sensitization effect in Chapter 2. Food deprivation/restriction is associated with increased SA, lower thresholds for the reinforcing properties of DOA, and increases in motivation to obtain drugs (Carr, 2007; Holsen et al., 2012; Raynor & Epstein, 2003; Stafford et al., 1998); an effect likely driven by increased DA receptor availability following periods of food restriction (Thanos et al., 2008). Although these paradigms provide a lot of information, they do not encapsulate all types of eating behaviour. And so, we maintain that avoidance of food restriction/deprivation provides unique information about the effect of food consumption on behavioural and neural processes.

Conditioned place preference. The lack of an effect by a HFCS rich diet on CPP (Chapter 2) may be a result of the CPP design. The experiments in this dissertation used a 2-chamber design rather than a 3-chamber design. A limitation of the 2-chamber design is that it forces a choice between the drug and vehicle paired compartments, so there is potential of bias for the compartment that the animal was placed in during test session (Prus, Jones, & Roscrans, 2009). That being said, there are also problems with utilizing a 3-chamber design. In the 3-chamber design, animals are placed in a smaller, middle, joining compartment during test session. It is possible that animals have made an association with the central chamber instead of or in
conjunction with the larger chamber, therefore influencing place preference on test day (Lucke-Wold, 2011).

Another procedural choice in our experiment was to utilize a biased design. In a biased design, the reward stimulus is administered to animals in the compartment that is initially preferred by the animal (Prus et al., 2009). Therefore, in a biased design, animals work against a preference, possibly making it more difficult to pick up differences. In an unbiased design the assignment of drug or vehicle pairing with each compartment is made randomly, regardless of the subject’s baseline preference (Prus et al., 2009). A problem with using the unbiased design is that if animals have strong initial preferences for a compartment, there are interpretation issues when a place preference is observed; as preference could be a result of reward effect or initial preference.

5.9. Future directions

The results of the experiments performed in this dissertation offer several avenues that could be further explored: role of different cues, elucidating the involvement of specific brain regions and neurotransmitter systems, sex differences, other measures of addictive liability, and short/long access models.

Cue control. There are potentially differences in how different cues for DOA and palatable foods maintain food reinforced behaviour. Results from Chapter 3 demonstrated that an HFCS light conditioned stimulus (CS) does not support the same level of responding engendered by an OXY associated light CS. It may be possible that the ‘sweet taste’ of HFCS becomes a CS that acquires properties of an incentive stimulus. It would be interesting to further explore the role of different cues in food reinforced behaviour. Since, DA signalling plays a role in ascribing incentive salience to neural representations of reward and cues (Schultz & Dickinson, 2000) it
would be also be worthwhile to investigate whether DA responses in the NAc are altered in response to the sweet taste vs environmental cues; and how they compare to DA release by drug cues.

The involvement of other brain regions. It is possible that different brain regions modulate the effects of drug and sugar seeking behaviour. The basolateral amygdala (BLA) plays an important role in cue associative learning and seeking behaviour (Gallagher & Holland, 1994). BLA neurons develop excitatory responses to a CS that has been paired with reward outcomes (Schoenbaum et al., 1998; Tye & Janak, 2007). Both presentations of drug and food paired stimuli increase activation (cFos expression) in the BLA (Yager et al., 2015; Kufahl, et al., 2009). These effects of the BLA are thought to be mediated by interactions between the BLA and NAc. However, an increase in Fos expression in the BLA does not necessarily lead to an increase in NAc Fos expression (Brown, Robertson, & Fibiger, 1992), suggesting that the BLA may play an independent role in mediating responses to conditioned stimuli. BLA inactivation has been shown to decrease drug-seeking but has no effect on sugar-seeking (McLaughlin & Floresco, 2007; McLaughlin & See, 2003). Moreover, different sub-regions of the BLA have been shown to have different roles in drug and sugar seeking behaviour. Inactivation of the rostral BLA decreases drug- (Kantak et al., 2001), but not sugar- (McLaughlin & Floresco, 2007), seeking behaviour. Conversely, inactivation of the caudal BLA has no effect on drug-seeking (Kantak et al., 2001), but enhances responding for food-paired cues (McLaughlin & Floresco, 2007). However, these studies were also conducted in separate cohorts of animals. The findings would be more convincing if these dissociations in seeking behaviour were achieved in the same animal. This would move us closer to a causal role of the different BLA sub regions in sugar- and drug-seeking behaviour.
Role of dopamine D3 receptors. Some lines of evidence implicate the DA D3 receptors (DRD3) in drug-seeking. In fact, DRD3 antagonism decreases drug-seeking, but has no effects on food-seeking (Khaled et al., 2014). With the advent of more selective drugs, future studies could further elucidate the role of DRD3 on seeking behaviour.

Sex differences. Only male rats were utilized in the experiments performed. There are reported sex differences in the homeostatic and hedonic responses to sugar and DOA that may influence vulnerability to develop “addictive” behaviours. For example, sex-dependent differences exist in drug absorption and bioavailability (Soldin et al., 2011), in responses elicited in the NAc to drug stimulation (Cummings et al., 2014; Cosgrove et al., 2014; Perry et al., 2016), as well in homeostatic regulation (Mauvais-Jarvis, 2015). These factors may converge to influence rates of escalation and abuse liability. And so, exploration of sugar and drug reinforced behaviours in females may provide us more meaningful information about sex differences

Other measures of addictive-like behaviors in animals. Various aspects of “addictive” behaviours were not addressed in the current studies such as insensitivity to punishment, reinforcing efficacy, and impulsivity. Differences have been noted in the ability of an aversive stimulus to promote drug-taking or procurement of food (Vanderschueren & Everitt, 2004); whereas long-term access to cocaine decreases conditioned suppression induced by an aversive stimulus, long-term access to sucrose does not produce these effects (Vanderschueren & Everitt, 2004; Pelloux, Everitt, & Dickinson, 2007).

The assessment of reinforcing strength (or potency) of a substance is often done using progressive ratio (PR) schedules of reinforcement. These schedules typically reflect the maximum effort a rat will exert to obtain an infusion of the drug (Richardson & Robert, 1996).
While, continuous access schedules offer information on whether a drug will support SA, and whether the drug can act as a reinforcing stimulus (Arnold & Roberts, 1997), these schedules are often unable to detect changes in the animal’s motivation (Cummins & Leri, 2008).

It is believed that addiction involves cycles of impulsivity and compulsivity (Koob & Le Moal, 2001), and that the transition from occasional drug use to addiction is a result of relative levels of impulsivity and compulsivity. And so, an examination of whether sugar and drug reinforcers produce differences in whether or how this transition comes about would further inform treatment outcomes. An examination of whether DOA and sugars have comparable effects on these measures of addiction liability will help us to further clarify the parallels and points of divergence among these reinforcers.

*Short-access vs long-term access in self-administration.* There was a lack of an interaction between HFCS and OXY during Dual SA (Chapter 3). It may be a result of limited access to both reinforcers. In animals, long access (6h) models produce characteristics of abuse that are not observed with short access (1 hr) models (Ahmed & Koob, 1998). It is possible that using longer access models may have changed the abuse phenotype observed with HFCS. An escalation of drug-intake after long-access training is a highly replicable phenomenon that has been documented to occur for several DOA, including cocaine (Ahmed et al., 1998), methamphetamine (Kitamura et al., 2006) and heroin (Lenoir et al., 2008). Various studies have shown that there are changes in neural and behavioral plasticity associated with long access models that are not seen with short access models (Ferrario et al., 2005; Shahar et al., 2007; Briand et al., 2008).
5.10. Conclusions and Implications

The experiments in this thesis contribute to the literature that DOA and sugars act on similar systems of learning and reinforcement, but there are differences in the extent to which these systems are engaged. More specifically, DOA result in a greater persistence of responding than sugars. Elucidating the differences between primary reinforcers can take us a step closer in developing targeted behavioural or pharmacological treatments. Identifying situations where drug and non-drug reinforcers differ might also help us to understand why drug reinforcers are unique in their ability to produce drug-seeking. The exact mechanisms underlying the differential efficacy of opioid- and sugar-related stimuli are not known, and future investigations should explore what specifically causes DOA to be such powerful reinforcers.

Recognizing the behavioural and neurobiological changes that drive consumption of hyper palatable foods can allow for the development of treatments for obesity that are more targeted. This may allow us to identify humans with impaired reward processing using neurobiological markers. Efforts can then be directed at correcting these deficits to restore proper functioning and perhaps decrease risk of obesity. This then has implications for the reduction of intake of high sugar foods by affecting policies such as: (1) increases in taxation on sugar products; (2) subsidizing whole foods; (3) regulation of availability and advertising; (4) better regulation on labelling and product formulation and restrictions on where they can be sold; (5) education programs that highlight the harmful qualities of added processed sugar products and their potential in changing responses to other rewards.

Lastly, prescription opioids are important therapeutic agents in the treatment of pain; however, their over prescription in combination with illegal sharing and their high potential for abuse, has led to a public health crisis. Therefore, it is important to identify factors that may
contribute to increased vulnerability to opioid abuse and addiction. The results of this
dissertation suggest that a diet rich in HFCS could have effects on abuse liability, therefore a
closer attention to diet may help to mitigate the effects of opioid abuse.
Chapter 6
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