Behaviour and Neural Indices of the Abuse Liability associated with Intraoral
Self-administration of High Fructose Corn Syrup

by

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ABSTRACT

BEHAVIOURAL AND NEURAL INDICES OF THE ABUSE LIABILITY ASSOCIATED WITH INTRAORAL SELF-ADMINISTRATION OF HIGH FRUCTOSE CORN SYRUP

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Food addiction (FA) is characterized by addictive behaviours including compulsively eating and neurobiological alterations that emerge in response to consuming food. Evidence strongly suggests sucrose is addictive; however, this sweetener has been almost entirely replaced by high fructose corn syrup (HFCS) in food/drink. Interestingly, HFCS impairs metabolic function and its introduction into the food supply is linked to the emergence of a concurrent epidemic of obesity; yet, little is known about its addictive properties. Therefore, we investigated its abuse liability and characterized these effects relative to sucrose.

Using a novel procedure for sugar self-administration (SA), intraoral SA (IOSA) of HFCS was concentration dependent and supported by caloric content. Repeated IOSA led to escalation of intake, bingeing, and enhanced motivation to lever-press, indicating HFCS is reinforcing and possesses abuse liability. Moreover, compared to sucrose, HFCS alters expression of dopamine-2 (D2R) and mu-opioid (MOR) receptor mRNA in a manner putatively associated with exposure to drugs of abuse and the development of addiction. This occurred despite lower levels of HFCS SA, suggesting it may have more potent reinforcing effects and greater abuse liability. Likewise, SA of pellets mimicking HFCS engendered less hypothalamic
activation than sucrose; in line with evidence that high fructose sugars may contribute to poor appetite regulation and possibly excessive intake.

Bupropion (BUP) and naltrexone (NTX) were investigated with respect to their ability to reduce HFCS SA when administered alone, because they diminish the reinforcing properties of abused drugs, and in combination (BUP+NTX), to mimic the pharmacological actions of Contrave®, used to enhance weight-loss. BUP+NTX and NTX reduced IOSA of HFCS and upregulated expression of anorexigenic pro-opiomelanocortin mRNA in the hypothalamus. Moreover, compared to drugs of abuse or IOSA of HFCS alone, the addition of BUP+NTX led to opposing effects on DR2 and MOR mRNA expression, suggesting novel applications for Contrave® in treating FA or substance related abuse disorders.

Together, these studies provide novel evidence that HFCS is addictive and support adopting policies that may limit its availability to reduce the potential harm associated with HFCS consumption as well as curb rising rates of obesity in vulnerable individuals.
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LIST OF ABBREVIATIONS

α-MSH: α-melanocyte stimulating hormone
ACC: Anterior cingulate cortex
AgRP: Agouti related protein
ANOVA: Analysis of variance
ARC: Arcuate nucleus
BDNF: Brain derived neurotrophic factor
BMI: Body mass index
BOLD: Blood oxygen dependent
BUP: Bupropion
CA: Cornu ammonis
CNS: Central nervous system
CS: Conditioned stimulus
DA: Dopamine
DAT: Dopamine reuptake transporter
dG: Dentate gyrus
DIO: Diet induced obesity
DOR: Delta-opioid receptor
dl-PFC: dorsal lateral prefrontal cortex
DR2: Dopamine receptor 2
DSM-V: Diagnostic and Statistical Manual of Mental Health 5th edition
F: Fructose
FA: Food addiction
FDA: Federal Drug Administration
fMRI: functional Magnetic resonance imaging
FR: Fixed ratio
G: Glucose
GHS-R: Growth hormone secretagogue receptors
GL: Glycemic load
HFCS-55: High fructose corn syrup formula 55
ICV: Intracerebral ventricular
IO: Intraoral
IOSA: Intraoral self-administration
IP: Intraperitoneal
IRS: Insulin receptor
IV: Intravenous
KOR: Kappa-opioid receptor
LEPR: Leptin receptor
LH: Lateral hypothalamus
MCH: Melanin-concentrating hormone
MOR: Mu-opioid receptor
n-3 -6: Omega-3 -6
NAc: Nucleus accumbens
NPY: Neuropeptide Y
NTX: Naltrexone
OFC: Orbitofrontal cortex
PET: Positron emission tomography
PFC: Prefrontal cortex
POMC: Pro-opiomelanocortin
PR: Progressive ratio
PVN: Paraventricular hypothalamus
SA: Self-administration
SC: Subcutaneous
SEM: Standard error of the mean
SR: Sustained release
SRADs: Substance related and abuse disorders
SSS: Sensory specific satiety
TR: Taste reactivity
VMH: Ventromedial hypothalamus
YFAS: Yale food addiction scale
VTA: Ventral tegmental area
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CHAPTER 1

Scope of the problem

Exploring the plausibility of food addiction (FA) poses unique challenges. Presently, no commonly agreed upon operational definition exists (Long et al., 2015; Westwater et al., 2016; Ziauddeen & Fletcher, 2013). Consequently, scientists lack a shared theoretical framework from which hypotheses and experimental design can be developed and applied in a replicable and generalizable manner. Such a lack of consensus stems from confusion over whether to define FA solely as a behavioural disorder, as substance dependence, or alternatively, as an eating disorder alongside bulimia nervosa or binge-eating disorders (Albayrak et al., 2015; Davis & Claridge, 1998; Gearhardt et al., 2011; Schulte et al., 2015b; Hebebrand et al., 2014). Presently, it is also not known what specific food(s) or food additives(s) may be addictive and this ambiguity is cited as a primary cause for the confusion and on-going debate regarding how to operationally define FA (Westwater et al., 2016; Ziauddeen & Fletcher, 2013).

It has been suggested that processed foods may possess high abuse potential (Benton, 2010; Hetherington & Macdiarmid, 1993; Ifland et al., 2015; Schulte et al., 2015a). The attributes of food stimuli that warrant classification as “processed” refer specifically to the addition and combination of high concentrations of sugars, fats, salts, and chemicals to food and drink (Monteiro et al., 2010). The primary objective of these manufacturing practices are to increase caloric density and enhance the subjective pleasurable effects ascribed to the sight, smell, and taste of these processed foods (Berridge & Grill, 1983; Drewnowski, 2009; Rozehnalova, 2017). These features distinguish them from raw foods found in nature such as vegetables, fruits, or proteins and those minimally processed for preservation or ease of use including packaged meats, dairy, nuts, or legumes (Fardet, 2016; Monteiro et al., 2010; Weaver et al., 2014).
Processing foods to possess more calories per gram than food found in nature is suggested to be analogous to processing coca leaves into cocaine and or opium from poppy plants into morphine/heroin for the primary purpose of enhancing the drugs potency (Davis & Carter, 2009; Schulte et al., 2015b). Accordingly, it is perhaps not surprising that such stimuli may promote excessive or even compulsive eating as their reinforcing efficacy and subjective effects are likened to those of drugs of abuse (Ifland et al., 2009; Parylak et al., 2011; Schulte et al., 2017a; Spangler et al., 2004). While there is convincing evidence to support this notion, these accounts are often inferential and have yet to adequately address what specific additive (if any), their combination of, or at what concentration when added to food confers its addictive potential (Rippe et al., 2017; Westwater et al., 2016). Therefore, there is a pressing need to explore the hypothesis that processed foods are addictive.

What is clearly understood is that it is possible to eat to excess with maladaptive consequences (Lindberg et al., 2011). Most often this is associated with the development of overweight or obesity, which occurs simply when there is an imbalance between energy expenditure and energy intake (Berthoud et al., 2011; Heymsfield et al., 2014; Kenny, 2011b). When the scale is tipped in favour of the latter, excess accumulation of adipose tissue promotes weight-gain (Horvath, 2005). Overweight and obesity that is associated with excessive eating is often attributed to the development of bingeing behaviour characteristic of eating disorders or compulsive eating characteristic of FA (Engel et al., 2005; Segni et al., 2014; Smith & Robbins, 2013). Yet, these phenotypes account for only a small proportion of overweight or obesity. Less obvious but equally maladaptive eating habits such as “grazing” on small amounts of food in a repetitive or unstructured manner (Conceição et al., 2014; Heriseanu et al., 2017) or “mindlessly eating” while distracted by people or environmental stimuli such as a television are suggested to
be, on average, the cause of a gradual increase in adiposity (Hetherington et al., 2006; Ogden et al., 2013).

Nevertheless, obesity is associated with higher rates of mortality than underweight (unless to excess; World Health Organization, 2015) and is a putative risk factor for the development of non-communicable diseases including metabolic disease, cardiovascular disorders, cancers, osteoarthritis (De Pergola & Silvestris, 2013; Kenny, 2011a), and comorbid substance or mood disorders (de Wit et al., 2010; Faith et al., 2011; Holderness et al., 1994). The prevalence of overweight and obesity has risen three-fold since 1975, alarmingly so among children aged 5-19 (4% in 1975 to over 18% in 2016) and by 2030, approximately 2.16 and 1.12 billion individuals world-wide are projected to be classified as overweight and obese, respectively (Kelly et al., 2008).

Interestingly, there exists considerable overlap between obesity and substance-related abuse disorders (SRADs; American Psychiatric Association, 2013; Volkow et al., 2011). Both disorders are characterized by deficits in learning, motivation, and reward processing (Davidson et al., 2014; Volkow et al., 2013) that develop in response to repeated exposure to substances that reinforce their selection and motivate behaviour through actions on shared neural substrates (Hyman et al., 2006; Wise, 2006b). Obesity and addiction likewise share predisposing environmental (availability of foods/drugs), personality (high trait impulsivity; Michaud et al., 2017), and genetic vulnerabilities (presence of polymorphisms that alter sensitivity to reward; Stice et al., 2008). Moreover, similar neuroadaptations associated with the development of addictive disorders emerge in response to repeated exposure to both drugs of abuse and food (Brown et al., 2015; Johnson & Kenny, 2010; Volkow et al., 2013). Using substance dependence to assess the contribution of addictive-like process to obesity has furthered the understanding of
the condition as well as the notion that particular foods may possess intrinsic properties that enhance their abuse liability (Grosshans et al., 2011; Kenny, 2011a). Unfortunately, it has also mistakenly given the impression that FA is a necessary attribute of or cause of obesity (Meule, 2015; Meule & Kübler, 2012b). Rather, FA likely represents one extreme phenotype for which the addictive-like behaviour it is associated with often leads to overeating; and consequently, it is more prevalent in those overweight and obese.

Moreover, to the lay public, managing weight is often oversimplified to balancing calories in with energy expenditure out to maintain your weight and using caloric deficits to promote weight loss (Meule, 2014). While not entirely misleading, weight status and management is determined by complex interactions between genetic (Hetherington & Cecil, 2010; Stice et al., 2008), metabolic (Narayanan et al., 2010), neurobiological (Volkow et al., 2011), psychosocial (Gearhardt et al., 2012a, 2012; Hetherington, 2007; Wallis & Hetherington, 2004) and environmental factors (Hetherington, 2007; Hetherington et al., 2006; Kirk et al., 2010; Malandrino & Capristo, 2011; Norton et al., 2006). Hence, even though a common appearance is shared, there exists significant individual variability in the aetiology of overweight and obesity that challenges prevention and treatment strategies.

It follows then, that defining obesity with respect only to FA would be an oversimplification of a complex disease and as such this thesis will not equate overweight or obesity with FA. Rather, there exists overlap between the development of the current overweight/obesity pandemic and the increased availability of processed foods in the food supply (Bray et al., 2004; Gearhardt et al., 2011; Monteiro et al., 2013). The putative association between overconsumption of these foods and weight-gain (Lindberg et al., 2011; Louzada et al., 2015; Moubarac et al., 2017) suggests that processed foods may possess addictive qualities that
enhances their ability to promote overeating across a continuum of behaviours from grazing to compulsive overeating (Davis & Carter, 2009; Heriseanu et al., 2017; Thorner, 1970). The presence or absence of FA and or eating disorders may be antecedent and or possibly develop in response to obesity-induced deficits in reward and learning (Brown et al., 2015; Volkow et al., 2011). However, based on the current evidence to be reviewed herein, over-consumption of palatable foods by vulnerable individuals appears to be a common denominator across disorders. In light of the severe eating pathology associated with FA (Gearhardt et al., 2016) and the scope of non-communicable diseases, reduced life affordances, social stigma, and subsequent tax on the economy that the obesity pandemic poses it is important to explore the hypothesis that processed foods are addictive in a manner that identifies what exactly those addictive properties might be (Kelly et al., 2008; Ng et al., 2014).

On food addiction

The concept of FA is not novel despite only a mere decade worth of research investigating its aetiology (Meule, 2015). Perhaps not surprisingly, it was first suggested in 1890 that chocolate may act like a drug of abuse to induce irresistible cravings (Meule, 2015; Weiner & White, 2007). By the early 1930’s, cases of excessive eating were being described as compulsive and likened to drug dependence (Thorner, 1970). The first attempt to operationalize FA was in 1956 by Theron Randolph who described the phenomenon as “a specific adaptation to one or more regularly consumed foods to which a person is highly sensitive (which) produces a common pattern of symptoms descriptively similar to those of other addictive processes” (Randolph, 1956). These early accounts described FA as a combination of maladaptive behaviours including compulsive eating, sensitization of behavioural responses to food, and
inability to abstain from food that emerged in response to exposure to substances with abuse liability (Randolph, 1956).

Presently, FA remains commonly defined from the perspective of a substance use disorder (American Psychiatric Association, 2013; Meule & Gearhardt, 2014; Meule & Kübler, 2012b). It is characterized by intense cravings for particular foods (Ifland et al., 2009; Meule & Kübler, 2012), compulsive overeating (Carlier et al., 2015; Moore et al., 2017; Parylak et al., 2011), eating beyond (and for reasons other than) metabolic need with adverse consequence and an inability to abstain from particular foods (Alba & Williams, 2013; Avena & Gold, 2011; Meye & Adan, 2014). However, it has been alternatively suggested that the evidence for FA to date, only supports classifying the disorder as behavioural dependence and renaming the condition “eating addiction” or “addictive eating” (Albayrak et al., 2015; Hebebranda et al., 2014). If a substance use perspective is employed to establish an operational definition of FA, it will be required that the specific addictive characteristic(s) of food or food-additives be identified.

Both human and animal models that explore aspects of FA provide support for the hypothesis that processed foods may be addictive through evidence of considerable overlap between the behavioural and neurobiological consequences of repeated exposure to processed foods and drugs of abuse (Avena, Rada, & Hoebel, 2006; Ifland et al., 2009; Johnson, 2010; Volkow et al., 2011). Studies of FA that address its definition, how it should be diagnosed, or whether food itself can be addictive tend to rely on the current understanding of substance use disorders to highlight parallel trait vulnerabilities, behaviours, and neuroplasticity associated with dependence on drugs of abuse that also present following repeated consumption of food (Avena et al., 2011; Meule & Kübler, 2012a). Presently, the Diagnostic and Statistical Manual
of Mental Disorders, 5th edition (DSM-V; American Psychiatric Association, 2013) criteria to diagnose SRADs include a range of symptomology that falls within the categories of substance use and substance-induced symptoms, including persistent use despite experiencing substance-related problems such as adverse cognitive, psychological, and physiological symptoms, tolerance, withdrawal, intoxication, or the development of anxiety, sleep disturbances, or neurocognitive disorders (Nathan et al., 2016). Diagnosis is based on a continuum of severity whereby endorsing a maximum of three, five, or six plus symptoms denotes a diagnoses of mild, moderate or severe substance use disorder (American Psychiatric Association, 2013).

The Yale Food Addiction Scale (YFAS) was developed by Gearhardt and colleagues (2009) to qualify and diagnose FA in humans by analysis of responses to self-report questions that reflect adverse behavioural and substance-induced effects of consuming processed food (Gearhardt et al., 2009). These questions are theoretically based on the 11-criteria outlined by the DSM-V for diagnosing SRADs plus one additional criterion that assesses the presence of clinical impairment and distress (Schulte & Gearhardt, 2017). Responses to these 35-items are computed to yield a parallel symptom count from 0-11 that is categorized by the same maximum cuts offs of three, five, or six plus symptoms to fall within a diagnostic range of mild, moderate or severe FA (Gearhardt et al., 2009). The presence of clinically significant impairment or distress is also required for a positive diagnosis of FA (Schulte & Gearhardt, 2017).

The YFAS (and the revised YFAS 2.0 which takes into account DSM-V changes in terminology and diagnostic criteria for SRADs) has been modified into a shortened version for quick diagnostics (modified-YFAS; Schulte & Gearhardt, 2017), for use diagnosing FA in children (YFAS-Children; Gearhardt et al., 2013), and is presently the most widely used scale across cultures and languages (Aloi et al., 2017; Brunault et al., 2014; Chen et al., 2015).
Systematic review of 40-plus studies employing this scale reveals that prevalence rates of FA in the general adult population range from 0.0% – 8.7% and a diagnosis of FA tends to be higher among females than men and in younger children and adults over 33-years of age (Long et al., 2015). Demographics indicate that compared to the general population, prevalence rates are significantly higher among those with a body mass index (BMI) classified as overweight or obese (i.e. BMI ≥ 25; ~ 33%), or those that also present with bulimia nervosa (~ 83.6%) and binge eating disorder (~ 56.8%). This suggests that for a subset of individuals, FA may be related to or a cause of maladaptive eating associated with these conditions (Long et al., 2015).

A diagnosis of FA and its severity is positively associated with trait vulnerabilities for developing SRADs. These include: greater likelihood of engaging in impulsive behaviour to relieve a negative emotional state (negative urgency; Murphy et al., 2014; Wolz et al., 2016), quicker to select immediate gratification over long-term benefits (discounting of delayed reward; VanderBroek-Stice et al., 2017), an inability to persist and or remain on task (lack preservation; Murphy et al., 2014), and higher rates of impulsivity related to motor and attention (Meule et al., 2017). FA is also correlated with more intense food cravings as well as greater reward sensitivity to the subjective pleasurable effects of eating processed foods and tendency to eat to alleviate negative emotional states (Burmeister et al., 2013; Davis et al., 2011). What is more, those diagnosed with FA also score higher on measures of depression and in their early years, are more likely to have been diagnosed with attention hyperactivity disorders or have experienced psychological or sexual abuse (Eichen et al., 2013; Nunes-Neto et al., 2018). Also, like SRADs which rarely occur in and of themselves, FA is higher among those positive for mood disorders (depression, bipolar disorders; Markou et al., 1998; Nunes-Neto et al., 2018), heroin dependence
(Canan et al., 2017), and patients seeking treatment for various psychiatric, gambling, and eating disorders (Albayrak et al., 2017; Gearhardt et al., 2014; Jiménez-Murcia et al., 2017).

FA diagnoses is particularly distressing as it is associated with more severe eating pathology including increased incidence of binge, purge, loss of control, and nocturnal eating (Albayrak et al., 2017; Gearhardt et al., 2014; Ivezaj et al., 2016; Nolan & Geliebter, 2016). These individuals rate their quality of sleep, quality of life, global psychological state and emotional regulation as lower than the general population and are less sensitive to pharmacotherapy aimed at appetite reduction (Davis et al., 2014; Jiménez-Murcia et al., 2017; Wolz et al., 2016). In some instances, it is even possible to associate the severity of their score on the YFAS 2.0 with higher BMI, measures of adiposity, and less adherence and or weight-loss experienced in treatment (de Vries & Meule, 2016; Lent et al., 2014; Pursey et al., 2016).

The YFAS 2.0 identifies a addictive-phenotype that experiences many of the same adverse psychological, behavioural, and physiological effects associated with SRADs but in response to processed food (Gearhardt et al., 2016; Long et al., 2015). Currently under psychometric review are novel scales that measure specific maladaptive eating behaviour that reflect defining features of SRADs, such as compulsivity and motivation including the Addiction-like Eating Scale (Riddick, Christiansen, Halford and Hardman, 2017), Measure of Eating Compulsivity Scale (Schroder, Sellman, & Adamson. 2017), and the Palatable Eating Motives Scale (Burges, Turan, Lokken, & Boggiano, 2014). Collectively, these measures are important diagnostic tools that characterize a shared phenotype between SRADs and FA, inform treatment strategies, and provide support for studying FA from the substance use perspective that assumes food can be addictive. However, attempts to use the YFAS 2.0 (or the like) as proxy for defining FA is met with several empirical limitations including a reliance on self-report data
which is vulnerable to bias (Westwater et al., 2016; Ziauddeen et al., 2012). Arguably, most pressing is the limitation that these scales require participants to respond to questions about eating while considering their experience with and consumption of processed foods such as ice cream, pizza, chips, or soda while omitting those found in nature or minimally processed. Yet to date, what makes processed foods addictive has yet to be adequately addressed.

**On the addictive properties of foods**

*“Nothing would be more tiresome than eating and drinking if God had not made them a pleasure as well as a necessity”* - Voltaire

It is a biological requisite that we eat. By nature then, food has become a revered commodity as evidenced by strict government regulation of its production and manufacturing as well as cultural traditions woven into preparing and consuming food (Rozin, 2005; Westenhoefer & Pudel, 1993). Eating, however, is simply the behavioural response initiated by biological signals that energy in the form of calories derived from food are required to maintain homeostasis (Morton et al., 2006). Yet, there are a variety of factors that contribute to what exactly is eaten. Most obvious, its availability and the costs associated with its acquisition. Coupled with the variety of food options available, these factors inform the development of preferences for specific foods (Norton et al., 2006; Rozin & Vollmecke, 1986; Westenhoefer & Pudel, 1993). Importantly, the pleasurable properties that are ascribed to the sight, smell, and taste of food determine what is selected to eat and critically, influences how much (Fantino, 1984; Flaherty et al., 1994; Macht, 2008; Patel & Schlundt, 2001; Rolls, 2007).

Eating is regulated in part, by biological responses that selectively focus attention and behaviour towards food and learning about environmental cues that predict food availability (Berthoud, 2002). Importantly, these responses guide food-seeking and food-taking behaviour as
well as associative learning processes by fine tuning our ability to sense the energy that food affords based on it nutritional and pleasurable post-ingestional consequences (i.e. its caloric density and palatability; Berthoud, 2002; 2011; Rozin & Zellner, 1985). Such evaluations determine the reinforcing efficacy ascribed to foods; that is, the probability and frequency with which it will be sought and consumed (Berridge & Robinson, 2003). These processes evolved with the goal of maximizing our survival in an environment for which food scarcity was the norm (Davis & Carter, 2009). Consequently, it is argued that there is an inherent tendency to maintain a positive energy balance (Leigh & Morris, 2016) by selecting for energy dense foods as they confer greater survival value. There exists however an obvious mismatch between the environment in which these processes evolved and the one in which we presently operate, and this mismatch is argued to be due to the availability of processed foods (Davis & Carter, 2009; Paul Rozin, 2005).

This notion is supported by evidence that processed foods may impair rudimentary functions for sensing the nutritional value or substrates in food. Brunstrom and colleagues (2018) report that if food stimuli exceed the average caloric content (1.5 calories per gram) of naturally sourced foods such as fruits, vegetables, proteins, participants inaccurately estimate its caloric density or the fullness it should produce (Brunstrom et al., 2018; Monteiro et al., 2010). Not surprisingly, processed foods that possess such high caloric density are also found to impair sensory perceptions associated with taste that confer such nutritional information (van Dongen et al., 2012). That is, it is possible to accurately dissociate the taste of sweetness, saltiness, and savouriness in minimally processed foods such as fruits, vegetables and nuts. Whereas, in processed foods, the combination of these tastes in high concentrations supresses the innate ability to detect such differences (Monteiro et al., 2010; van Dongen et al., 2012). This has led to
the suggestion that processed foods should be labeled as “food-like substances” to denote differences from naturally sourced foods and highlight potential harms (Ifland et al., 2009; 2015).

These findings indicate that processed foods may usurp behavioural and neurobiological processes that evolved to regulate feeding and reward in a way that, like drugs of abuse, may promote addictive-like behaviours (Davis & Carter, 2009; Volkow et al., 2013). If food or food additives are addictive, it is necessary to establish what characteristics confer such abuse potential and importantly, standard and reliable methods for its evaluation. In line with adopting the substance use framework to develop methods for defining and diagnosing FA, it makes sense to also explore the abuse liability of food using indices already in place to classify drugs as addictive (Meule & Kübler, 2012b). Here, abuse liability defined by the Federal Drug Administration (FDA) refers to “the likelihood that a drug with anabolic, psychoactive or central nervous system (CNS) effects will sustain patterns of non-medical self-administration (SA) that result in disruptive or undesirable consequences” (Ator & Griffiths, 2003). Accordingly, the FDA also outlines a set of guidelines that specify what those abuse liabilities are and standard methods for their investigation. These include, but are not limited to a substances acute subjective and physiological effects, pharmacodynamic and pharmacokinetic properties, its reinforcing and discriminative effects, the development of physical dependence, and evidence of misuse by humans (Ator & Griffiths, 2003). Going forward, the ability of processed foods to alter (in a manner different from minimally processed foods) hardwired biological processes that mediate feeding and reinforcement will be reviewed with reference to how these adaptations contribute to the emergence of those behaviours that reflect abuse liability listed here.
Homeostatic and hedonic regulation of food intake

Homeostasis as it relates to appetite requires a network of nutritional, hormonal, and neural signals that continuously monitor energy status (Berthoud, 2002). Based on energy intake and expenditure these signals relay information between the peripheral and CNS to initiate, sustain, and stop food-seeking and -taking (Alba & Williams, 2013; Berthoud & Zheng, 2012; Hussain & Bloom, 2013). From within the CNS, the hypothalamus plays a primary role in this network (Leibowitz & Wortley, 2004). Across the gut-brain axis, the hypothalamus integrates information about the nutritional and chemical properties of ingested food, level of distention or rate of emptying of the gastrointestinal system, and fluctuations in nutrient and hormonal status (blood glucose levels, adipocyte stores, energy expenditure; Lutter & Nestler, 2009; Powley & Laughton, 1981). The neural signals received by the hypothalamus come from both the brain stem receiving afferent inputs from the cranial and vagus nerves as well as the nutrient sensing peptides and hormones from the periphery and gut that cross the blood-brain barrier at the level of the area-postrema and median eminence (Berthoud, 2002; Rogers & Brunstrom, 2015).

The hypothalamus is composed of several sub-regions including the arcuate nucleus (ARC), ventromedial hypothalamus (VMH), paraventricular nucleus (PVN), and lateral hypothalamus (LH; Saper et al., 2002). These sub-regions are composed of neurons that synthesize appetite stimulating (i.e. orexigenic) and supressing (i.e. anorexigenic) substrates that regulate consummatory behaviour as well as facilitate cross-talk between central and peripheral systems to balance energy (Leibowitz & Wortley, 2004). Functionally, optical stimulation of orexigenic or anorexigenic neurons in the ARC stimulates feeding and satiety, respectively (Krashes & Kravitz, 2014). Lesions to either the VMH or PVN induces hyperphagia, while lesions to the LH reduces food intake (Berthoud & Zheng, 2012; Schwartz et al., 2000).
Likewise, food intake is reduced by electrical stimulation of the VMH and animals will readily lever-press to self-stimulate the LH, indicating that in addition to food intake is also mediates some aspects of reinforcement (Delgado & Anand, 1953; Johnson & Kenny, 2010).

Populations of orexigenic and anorexigenic neurons respond to changes in insulin, leptin and ghrelin that are initiated by fluctuations in energy status. In brief, both insulin and leptin are peripherally synthesized by pancreatic beta cells and by white adipose tissue, respectively (Figlewicz et al., 2003; Palmiter, 2007). Peripheral concentrations of insulin and leptin are proportional to adiposity stores and fluctuate in response to changes in cholecystokinin and blood glucose levels to reduce food intake as well as initiate satiety and enhance energy expenditure (Leibowitz & Wortley, 2004; Palmiter, 2007). On the other hand, ghrelin is a powerful appetite stimulant that is synthesized within the stomach from gastric fundus mucosa (Horvath & Abizaid, 2012). Circulating levels of ghrelin are elevated between meals, peak prior to food intake, and fall in response to consumption of food (Horvath & Abizaid, 2012; Naleid et al., 2005).

Receptors for leptin (LEPR), insulin (IRS), and ghrelin (growth hormone secretagogue receptors; GHS-R) are expressed on both orexigenic and anorexigenic neurons within the hypothalamus (Abizaid et al., 2006; Williams & Elmquist, 2012). Within the ARC, ghrelin stimulates orexigenic neuropeptide Y (NPY) and agouti related protein (AgRP) neurons to facilitate food intake (Kohno & Yada, 2012) while the binding of leptin and insulin diminishes food intake by suppressing NPY and AgRP activity (Parker & Bloom, 2012). Conversely, the population of anorexigenic pro-opiomelanocortin (POMC) and cocaine amphetamine regulated transcript neurons within the ARC inhibit feeding via leptin and insulin binding and its anorexigenic effects is suppressed by NPY (Williams & Elmquist, 2012). Additionally, within
the LH, orexin and melanin-concentrating hormone (MCH) expressing neurons stimulate feeding behaviour and their activity is influenced by insulin, leptin, and ghrelin both directly and indirectly through interactions with NPY and AgRP (Parker & Bloom, 2012; Williams & Elmquist, 2012).

Deviation in energy status and expenditure are not the only antecedent to eating. Along with homeostatic needs (Lutter & Nestler, 2009), the reinforcing value and hedonic qualities attributed to the sensory properties of food influence the preparatory, consummatory, and post-prandial responses to food intake (Berthoud, 2011). The combination of a foods’ palatable and energy affording qualities inform its reinforcing value (Berridge & Robinson, 2003; Drewnowski, 2009). The neural responses associated with these reward processes are integrated across a network of mesocorticolimbic structures. These include but are not limited to: the ventral tegmental area (VTA), nucleus accumbens (NAc), dorsal and ventral striatum, prefrontal cortex (PFC), hippocampus, insular cortex, dorsolateral prefrontal cortex (dl-PFC), anterior cingulate cortex (ACC), orbitofrontal cortex (OFC) and amygdala. These regions rely on neurotransmitters including dopamine (DA) and the endogenous opioids to communicate (Berthoud et al., 2011; Blum et al., 2012; Carter et al., 2016; Kelley et al., 2002).

The efficacy of any reinforcer rests, in part, on its ability to elicit a DA response in the mesocorticolimbic system (Wise, 2004). Centrally, this requires the release of DA from DAergic neurons that originate in the VTA and project to the NAc and forebrain structures within the ventral striatum (Volkow et al., 2002). In fact, optical stimulation of VTA DA neurons that enhances DA transients in the NAc sufficiently supports learning of an instrumental response in the absence of any reinforcer (Krashes & Kravitz, 2014). DA neurons in the VTA maintain a steady state of low level tonic DA release, and presentation of salient stimuli elicit short phasic
bursts of DA from these neurons (Schultz, 2016). Importantly, this phasic DAergic response shifts from the primary reinforcer to a range of interoceptive, affective, and environmental cues that through repeated pairings, become associated with the subjective effects and availability of the stimuli (Schultz et al., 1997; 2016). The attribution of incentive salience to these cues imparts a motivational value that allows them to likewise direct behaviour by combining previously learned information about the subjective and reinforcing effects of the stimuli with current physiological and neurobiological needs (Berridge, 2012). The attribution of incentive salience, also described as “wanting”, is largely mediated by DA (Berridge et al., 2009), and allows for more precise direction of behaviour by altering the relative value of a cue in response to changing needs. That is, when hungry, food associated cues are more enticing than when satiated (Berridge, 2012).

The hedonic properties associated with reinforcing stimuli, including those sensory properties related to foods’ palatability, are also mediated by signalling of endogenous opioid peptides. These include the endorphins, enkephalins, dynorphins, and endomorphins that act on mu-, delta- and kappa-opioid receptors (MOR, DOR and KOR; Bakshi & Kelley, 1993; Barbano & Cador, 2007; Kelley et al., 2002; Peciña & Smith, 2010). The experience of pleasure, or in other words, the subjective experience of liking something is critically dependent on MOR signalling (Gosnell & Levine, 2009; Kelley et al., 2002). Objective facial reactions associated with hedonic (“liking”) and aversive (“disliking”) reactions in response to tasting food can be similarly indexed across species (Berridge & Grill, 1983). In both the rostro dorsal quadrant of the NAc shell and the caudal ventral pallidum, microinjection of the MOR agonist DAMGO increases the hedonic response elicited by sucrose in tests of taste reactivity (TR; Peciña et al., 2006; Peciña & Berridge, 2005). Conversely, blocking MOR signalling via naloxone
microinjected into either hedonic “hot spot” suppresses liking responses; indicating that these regions function in tandem to mediate the subjective experience of liking (Peciña & Smith, 2010; Smith & Berridge, 2007). Likewise, in humans, treatment with opioid antagonists decreases subjective ratings of liking for the taste of sucrose (Fantino et al., 1986), pleasantness associated with different foods (Yeomans & Gray, 1997), and preference for high fat/sweet foods (Drewnowski et al., 1992).

These DA and opioid mediated effects on reinforcement and palatability inform their role in mediating different dimensions of appetite that likely contribute to “non-homeostatic” or “hedonic eating”; that is, not eating for energy but rather for pleasure (Murray et al., 2014; Saper et al., 2002). To this end, DA appears to play a primary role in the anticipatory responses that occur in the early phases of feeding (Berthoud, 2002). This facilitates behavioural approach toward food, its associated cues, and the attribution of incentive salience (Berridge, 2012; Schultz et al., 1997). In fact, animals that lack DA develop hyperphagia that leads to death unless rescued by the administration of L-DOPA (Szczyypka et al., 1999). Animals also cease food-seeking and -taking following lesions to the DAergic projections between the VTA and NAc or administration of DA antagonists in the VTA (Palmiter, 2008; Szczyypka et al., 2001). Accordingly, excessive attribution of incentive salience to those processed foods that are highly reinforcing and highly palatable is cited as a cause for the frequency with which they are craved, hard to resist, and often the focus of compulsive food intake (Davis, 2013; Parylak et al., 2011; Robinson & Berridge, 1993).

Opioid signalling has similar effects on consummatory behaviour by mediating palatability and increasing incentive motivation (Kelley et al., 2002). In both humans and rats, MOR agonists increase consumption of food, with specificity for high fat/sweet foods (Baldo et
al., 2010; Zhang et al., 1998), and these effects are suppressed by administration of MOR antagonists (De Tomasi & Juárez, 2011; Yeomans & Gray, 1997). Administration of MOR agonists increases DAergic signalling to the NAc which stimulates consummatory behaviour in sated rats (Zhang et al., 1998). Similarly, within the NAc, MOR agonists enhances sucrose/fat intake as well as motivation for food as indexed by elevations in breakpoint achieved on progressive ratio schedules of reinforcement (Nogueiras et al., 2012; Zhang et al., 2003). Importantly, in the absence of energy deficits, such additional food consumption can be related to the enhancing effect opioids have on palatability suggesting that hedonic signals may override mechanisms that normally initiate satiety (Doyle et al., 1993; Eikemo et al., 2016; Leigh & Morris, 2016; Peciña & Smith, 2010).

Cross-talk between homeostatic and non-homeostatic processes dynamically control appetite (Abizaid & Horvath, 2008; Palmiter, 2007, 2012). Receptors for nutritional signals are expressed and functional within the mesocorticolimbic system where they mediate the reinforcing properties of food and effect consummatory behaviour (Figlewicz & Benoit, 2009). LEPR and IRS are expressed on DA neurons in the VTA and when activated, inhibit DA signalling to reduce sucrose intake (Figlewicz et al., 2003; Figlewicz & Benoit, 2009). Likewise, intracerebral ventricular (ICV) infusion of insulin reduces DA availability by enhancing the expression of the DA re-uptake transporter (DAT; Figlewicz et al., 1994). Moreover, when infused ICV or directly into the VTA of rats, insulin and leptin suppresses feeding for up to 24-hours (Hommel et al., 2006; Sipols et al., 2002). Moreover, in tests of conditioned place preference, administration of ICV leptin or insulin prior to testing reverses place preference for an environment paired with food and blunts motivation to self-administer sucrose on a progressive ratio schedule of reinforcement (Figlewicz, et al., 2004, 2006). GSH-Rs and GSH-R
mRNA is also expressed within DA neurons in the VTA (Abizaid et al., 2006; Zigman et al., 2006). In rats, administration of ICV ghrelin enhances DA overflow into the NAc, and stimulates locomotion as well as food intake (Abizaid, 2009; Abizaid et al., 2006; Horvath & Abizaid, 2012; Jerlhag et al., 2006; Naleid et al., 2005). Moreover, intracranial injections of ghrelin into the VTA enhances the reinforcing and motivational properties of sucrose as measured by self-administration on fixed and progressive ratio schedules of reinforcement and increases palatable food intake (Egecioglu et al., 2010; Skibicka et al., 2011).

Endogenous opioid modulation of nutrient sensing hormones has been well described within the melanocortin system. Here, opioid-induced feeding can be suppressed by stimulation of the melanocortin receptor-3 and -4 which reduce feeding via release of alpha melanocyte stimulating hormone (α-MSH) derived from the cleavage of POMC into α-MSH and β-endorphins (Pandit et al., 2011). Moreover, the anorexigenic effect of α-MSH can be potentiated by naltrexone administration. That is, naltrexone blocks the actions of β-endorphins that bind to MORs expressed on POMC neurons that function as autoreceptors to inhibit the firing rate of POMC (Pennock & Hentges, 2011). Administration of opioid antagonists is also reported to suppress food intake stimulated by AgRP and NPY as well as orexin A via opioid-mediated actions in the NAc (Clegg et al., 2002; Sweet et al., 2004). In addition, tongue protrusions and “liking” of sucrose are enhanced by orexin administration to the ventral palladium hot spot, indicating a role for this nutrient sensing peptide in mediating palatability (Kelley et al., 2002).

Fewer studies have characterized the integration of insulin, leptin and the endogenous opioid systems in relation to its direct or non-homeostatic effects on food intake. Nevertheless, there is evidence that these systems communicate in regions of the brain where their actions are known to influence appetite and reward. With respect to insulin, oral and intravenous
administration of MOR agonists dose-dependently induce hyperglycaemia in rats (Molina & Abumrad, 1994; Sadava et al., 1997) and MOR deficient mice display impairments in insulin secretion likewise leading to hyperglycaemia (Wen et al., 2009). In vitro, MOR agonists reduce the expression of insulin-like growth factors and the expression of IRS in neural stem cells (Salarinasab et al., 2017) as well as produce IRS desensitization and resistance that can be reversed by naloxone in both the hypothalamus and hippocampus of mice (Li et al., 2003). Finally, MOR agonists enhance leptin expression in the NAc, leptin deficiency has been associated with slowing the rate of morphine metabolism in mice, and this peptide is also necessary for the expression of morphine-induced conditioned place preference that is potentiated by analgesia (Dalesio et al., 2016).

**Homeostatic and reward system dysfunction associated with processed foods**

In animals, cafeteria style diets composed of energy-dense palatable foods that characterize the modern western diet are used to model diet-induced obesity (DIO; Rolls et al., 1980). Compared to rats consuming standard laboratory chow, rats on a cafeteria-diet consume up to double the energy intake and accordingly weigh significantly more (Johnson & Kenny, 2010; Martire, Holmes, Westbrook, & Morris, 2013; Robertson & Rasmussen, 2017; Rogers & Blundell, 1984; Rothwell & Stock, 1982). Extended access (40+ days) to cafeteria diets alter patterns of food intake such that animals increase the frequency of eating bouts and a gradual reduction in the day-to-day intake of food has been likened to the development of tolerance to its rewarding effects (Johnson & Kenny, 2010; Martire et al., 2013). Moreover, when switched from cafeteria diets to standard lab chow, these same animals eat significantly less than that of even the chow fed only groups. This has been attributed to differences in not only the palatability of the diets but also the up-regulation of corticotrophin-releasing hormone mRNA in the
hypothesis of cafeteria diet fed rats. This is likewise observed in response to the acute effects of withdrawal from drugs of abuse and consistent with the onset of a negative emotional state (Koob, 2009; Martire et al., 2014; Martire et al., 2015; South et al 2014).

Cafeteria diets also change the expression and function of peripheral and central nutrient sensing hormones and impair energy balance (Erlanson-Albertsson, 2005). Significant increases in the expression of leptin and insulin as well as orexigenic AgRP and NPY from the PVN and VMH have been reported (Lazzarino et al., 2017; Martire et al., 2014). Moreover, processed foods have been linked to epigenetic changes of both POMC and NPY peptides that may alter their ability to respond to perturbations in energy balance and consequently impair critical processes that initiate and importantly stop eating (Lazzarino et al., 2017). In fact, within days of access, processed foods can induce leptin and insulin resistance that renders these signals insensitive to changes in energy balance, as evidenced by hyperphagia that exogenous administration of leptin and insulin fail to alleviate (Martire et al., 2014; Wang et al., 2001).

History of cafeteria diets and DIO is also associated with reward-related behavioural and synaptic deficits observed following repeated access to drugs of abuse. Following maintenance on a cafeteria diet, rats are more vulnerable to stress-induced reinstatement of food-seeking (Chen et al., 2014) and display heightened sensitivity to the effects of the dopamine-2 receptor (DR2) antagonist haloperidol that enhances impulsivity on delayed discounting tasks (Robertson & Rasmussen, 2017). These animals also work harder for palatable foods on progressive ratio schedules of reinforcement as indexed by higher breakpoint, display greater preservative responding during periods of signalled non-availability, and binge on palatable foods when limited to intermittent access schedules (Brown et al., 2015; Murray et al., 2016). Animals fed cafeteria diets also present with up-regulation of AMPA receptors in the NAc which is associated
with increased vulnerability for cue-induced reinstatement of drug-seeking as well as enhanced auto-inhibition of neurons expressing DR2 in the VTA (Cook et al., 2017; Oginsky et al., 2016).

It appears then, having a wide variety of processed foods readily available for consumption (not unlike what characterizes the modern obesogenic environment) predictably leads to overconsumption, a constellation of homeostatic and reward system dysfunction, and in most cases DIO (Caballero, 2007; Hetherington, 2007). This was aptly demonstrated by the association of DR2 reward deficits and compulsive food-seeking in rats maintained on a cafeteria diet for 40-days (Johnson & Kenny, 2010). Compared to chow fed or intermittent access (one-hour processed foods + unlimited chow), rats with unlimited access to processed foods and chow developed DIO as well as elevated threshold for brain reward stimulation of the LH and reduced expression of D2R in the dorsal striatum - all of which progressively worsened with additional weight-gain. Additionally, these same rats develop compulsive food-seeking behaviour as evidenced by their inability to suppress responding for palatable food in the presence of a cue associated with the receipt of aversive foot-shock (Johnson & Kenny, 2010).

That said, it is difficult to determine whether the results of studies employing cafeteria diets are a cause of consuming processed food or the effects of developing DIO. Predisposition for obesity is linked to having the TAq1A allele of the DR2-ANKK1 gene associated with reduced DR2 expression in the dorsal striatum (Stanfill et al., 2015). BMI is also inversely correlated with expression of DR2 in the dorsal striatum (Wang et al., 2001), and in prospective longitudinal studies striatal DA sensitivity decreases as a function of weight-gain and recovers with weight-loss (Stice et al., 2008). The results of imaging studies also indicate that compared to lean participants, obese participants display greater activation of brain regions involved with digestion, sensory processing, and reward evaluation in response to anticipation and
consumption of chocolate milkshakes (gustatory cortex including the insula and frontal operculum, somatosensory cortex, as well as reduced dorsal striatal activity in line with DA hypo-function upon consumption; Gearhardt et al., 2011; Stice et al., 2008). Moreover, obese participants also rate the pleasant taste (liking) of sucrose and saccharine sweetened drinks significantly lower than lean counterparts, yet presentation of food associated cues to these same participants elicits significantly higher reactivity in regions also found to be hyperactivity to drug associated cues by those with SRADs (hippocampus, amygdala, thalamus, anterior insula; Connolly et al., 2013).

Moreover, obese participants also rate the pleasant taste (liking) of sucrose and saccharine sweetened drinks significantly lower than lean counterparts, yet presentation of food associated cues to these same participants elicits significantly higher reactivity in regions also found to be hyperactivity to drug associated cues by those with SRADs (hippocampus, amygdala, thalamus, anterior insula; Connolly et al., 2013).

These data suggest that obesity itself presents with many of the same homeostatic and reward-related deficits that emerge in studies of DIO and explains, in part, why obesity may render some individuals more prone to overeating (Connolly et al., 2013; Stice et al., 2013; Volkow et al., 2013). Cafeteria style diets in animals, like the modern western food environment, lead to obesity and are ecologically valid models that provide additional support for the correlational evidence that a change in the food environment is a likely cause of the current obesity epidemic (Davis, 2013). They do not however, indicate what features of processed foods may be addictive.

**Processed foods and the emergence of an addicted phenotype in humans**

Self-report studies employing the YFAS 2.0 as well as other studies explicitly implicate processed foods as addictive (Benton, 2010; Hetherington & Macdiarmid, 1993; Ifland et al., 2009; Schulte & Gearhardt, 2017). The consumption of processed foods reportedly leads to cravings, eating beyond metabolic need, and a loss of control over intake while also producing self-reported experiences of euphoria, relief from negative affect, tolerance, and withdrawal symptoms (Bruinsma & Taren, 1999; Hetherington & MacDiarmid, 1993; Ifland et al., 2009).
Recently, Schultz and colleagues (2017) generated food clusters to identify foods with abuse liability by categorizing 30-items of food based on participants’ responses to self-report questions assessing for example, loss of control experienced while eating them. The study revealed that highly processed foods such as cake or pizza reliably clustered together in response to high ratings of pleasure, liking, and craving (Schulte et al., 2017). Moreover, it was evident based on nearly identical clusters of food generated in response to high loss of control that those foods that are highly craved and liked may be more likely to promote excessive intake (Schulte et al., 2017). Even with the subjective nature of these accounts, and the still unanswered question of what exactly the addictive properties of these foods are, these data consistently call attention to the fact that not all foods are equally addictive (Schulte et al., 2017). This underscores the importance of not limiting the classification of FA to solely a behavioural disorder, whereby those addictive behaviours would presumably apply to any food substrate (Schulte et al., 2015a; Wiss et al., 2017).

In humans, few studies have quantitatively explored what foods or food substrates have addictive potential when consumed by vulnerable people. Presently, the glycemic index or glycemic load (GL) of a specific food is the only quantitative measure tested (Schulte et al., 2015a). Glycemic index reflects the magnitude of the post-prandial spike in blood glucose a food elicits and GL accounts for both the foods’ glycemic index as well as carbohydrate content (Thornley et al., 2008). Diets composed of foods that promote hyperglycaemia are associated with diabetes mellitus, cardiovascular disease, cognitive impairment, and even FA (Parrot & Greenwood, 2007; Thornley et al., 2008; Zeevi et al., 2015). Perhaps not surprisingly, compared to unrefined foods, processed foods tend to possess significantly higher GLs. The post-prandial consequences of consuming foods that promote such hyperglycaemia suggest that they may
promote overeating; they include greater activation of the striatum and NAc as well as lower blood sugar coupled with greater subjective reports of hunger (Lennerz & Lennerz, 2017; Lennerz et al., 2013). To associate GL with abuse liability, 35-items of food (18 of which qualified as processed) were rank ordered according to participants ratings of that foods’ abuse liability using YFAS 2.0 criteria (Schulte et al., 2015a). Seventeen of the 18 processed foods rounded out the top of the list as most addictive and the magnitude of the foods GL was used to predict whether the food was classified as addictive to establish a positive relationship between high GL and the abuse liability of food (Schulte et al., 2015a).

The rationale for using GL to evaluate the addictive potential of food is compelling. It is hypothesized that processed foods are manufactured through methods that alter their pharmacokinetic properties to increase their potency and rate of absorption. This occurs through the addition of high concentrations of refined sugars, fat, salts, flours, and chemicals and by removal of natural fibre, proteins, and water, respectively (Mithieux, 2014; Schulte et al., 2015a). Such properties contribute to the subjective experience of reward and reinforcement associated with a substance and are used to evaluate the addictive potential of drugs in both humans and animals (Carter & Griffiths, 2009; Schulte et al., 2017). Yet, there is considerable individual variability in humans’ post-prandial response to food as measured by GL that are determined by individual differences in insulin sensitivity, genetics, and even gut microbiota (Zeevi et al., 2015). Consequently, GL may have poor utility as a generalizable measure of a foods abuse liability. Nevertheless, these data as well as self-report studies suggest that processed foods and drugs of abuse engender similar behavioural and neurobiological effects associated with SRADs (Benton, 2010; Hetherington & Macdiarmid, 1993; Ifland et al., 2009).
Lifetime prevalence rates of drug use and dependence also suggest that although many people try or recreationally use drugs of abuse, only a minority develop dependence (Anthony et al., 1994). Likewise, despite the ubiquity and frequency in consumption of processed food, only a small proportion of the general population presents with FA (~ 8 – 15% ; Long et al., 2015; Schulte & Gearhardt, 2017). Sensitivity to reward is biologically adaptive as it allows salient stimuli to motivate and guide appetitive behaviour. Like most traits though, reward sensitivity can be assessed on a continuum and heightened sensitivity to reward may render some more responsive to the potent reinforcing effects of drugs of abuse and processed foods (Bardo et al., 2013; Davis et al., 2007; Loxton & Tipman, 2017; Piazza et al., 2000). In fact, there is significant comorbidity between FA, eating disorders and SRADs (Becker & Grilo, 2015; Beitscher-Campbell et al., 2016; Holderness et al., 1994). Moreover, a shared preference for highly palatable solutions and drug-intake in both humans and animals is also reported (Gosnell & Krahn, 1998; Garbutt, & Janowsky, 1999; Kampov-Polevoy et al., 1997, 1999; Levy et al., 2013; Nolan & Scagnelli, 2007). Together, these data imply that there is likely a shared vulnerability to acquire behaviours motivated by strong incentive stimuli.

FA that presents with high sensitivity to reward is associated with more severe eating pathology, cravings, and incidence of emotional eating (Loxton & Tipman, 2017). In fact, this trait vulnerability as measured by the presence of a genetic marker for increased DAergic signalling in the ventral striatum was used post hoc to accurately distinguish those who simply self-identify as food addicted from those who formally received a FA diagnosis once all participants were assessed by the YFAS 2.0 (Davis et al., 2004). Reward sensitivity is also associated with greater reactivity of the mesocorticolimbic system in response to processed food and drug cues (Beaver, 2006; Dawe, Gullo, & Loxton, 2004), as well as activation of the dorsal
striatum, amygdala and OFC in response to tasting calories (Roberts et al., 2016). It may also be reasonable to use individual differences in GL response as a proxy for reward sensitivity to explain in part, why many individuals can regularly consume processed foods in the absence of weight-gain or dependence while a subset of those who experience significantly higher GL responses may be more sensitive to its reinforcing properties and at higher risk for overeating or FA (Ludwig et al., 1999; Thornley et al., 2008).

Another key feature of prolonged drug use is that wanting or motivation for drug-seeking and -taking drug remains high, increasing over time; yet, subjective “liking” of the drug is paradoxically low (Robinson & Berridge, 1993). The appetitive approach behaviours that reflect “wanting” such as conditioned approach or subjective reports of wanting/desire (Pool, et al., 2016) can be dissociated from appetitive consummatory “liking” responses including orofacial responses indexed by TR tests or subjective reports of liking/pleasure (Berridge & Robinson, 2003; Grill & Norgren, 1978). This is also reflected by distinct DAergic and opioid mediated responses that underlie reinforcing and pleasurable experiences, respectively (Berridge, 2009; Nogueiras et al., 2012; Pool et al., 2016).

The shift in balance from liking to wanting has been attributed to the chronic overstimulation of DAergic signalling in the NAc by repeated drug use which lends to the attribution of excessive incentive salience to the drug (Wise, 2006). Then, as the DAergic response shifts from the drug to being elicited by antecedent cues (Di Chiara, 1999), there is a strengthening of associative learning processes that critically enhance the motivational properties of those stimuli (Berridge, 2007; Schultz, 2016). Consequently, drug associated cues easily trigger intense craving that narrows attention and behaviour towards drug-seeking and -taking. Moreover, chronic overstimulation of the DAergic pathways by drugs is well known to down-
regulate D2R availability in the dorsal striatum leading to a hypo-dopaminergic response and an anhedonic state characterized by the inability to experience pleasure (Wise, 2008). In other words, cues associated with strong incentive stimuli such as drugs of abuse acquire excessive motivational properties yet also produce a deficit in perceived reward. These behavioural and neural alterations underlie the incentive sensitization theory posited by Robinson and Berridge (1993) and are well described in the human and animal literature to explain the shift from voluntary to habitual/compulsive drug use (Berridge & Robinson, 2016; Tibboel et al., 2015).

If processed foods have drug-like addictive qualities then it is possible that similar behavioural and neural alterations characterize the intense cravings and compulsive eating pathology in FA. Accordingly, subjective ratings of liking do not differ across a range of minimally to highly processed foods (Polk, et al., 2017). However, processed foods are always associated with significantly higher levels of craving and desire, that is, elevated measures of wanting (Polk et al., 2017). The positive association between processed foods and craving is also stronger in participants diagnosed with FA and as a function of the severity of their symptom count (Joyner et al., 2015; Polk et al., 2017; Schulte et al., 2016). It is not surprising then, that despite similar liking reported for a range of low to high calorie food images, the latter stimuli elicit greater responding on progressive ratio schedules of reinforcement (Giesen et al., 2009). Moreover, cravings are reported to be most often elicited by the combination of high carbohydrates and sugars that is typical of processed foods (White et al., 2002).

Frequent and intense cravings are a source of motivation for the loss of control and compulsive behaviour characteristic of addiction. There also appears to be considerable overlap in the reactivity of brain regions that mediate these subjective cravings across food- and drug-associated cues (Noori et al., 2016; Schreiber et al., 2013; Volkow et al., 2013). Position
emission tomography (PET) studies reveal that increased DAergic signalling in the dorsal striatum is positively correlated with subjective reports of increased hunger and desire at the time of tasting a food in line with the involvement of these regions in food anticipation and reward (Volkow et al., 2002a; Berthoud, 2002). These responses are also predictably linked to energy status at the time of testing (Siep et al., 2009; Small et al., 2001). Crucially, it has been reported that exposure to high versus low caloric foods elicits greater reactivity in brain regions associated with reward, motivation, and emotional tone (dorsal striatum, dLPFC, hypothalamus, cerebellum; Killgore et al., 2003). Such differences are also sensitive to changes in energy status and more pronounced by obesity (Charbonnier et al., 2015; Dimitropoulos et al., 2012; Goldstone et al., 2009; Rothemund et al., 2007; Stoeckel et al., 2008). While differences in cue reactivity for high and low caloric foods has yet to be explored in those diagnosed with FA, higher FA scores are associated with enhanced activation of the anterior cingulate cortex, OFC, and amygdala in response to food cues as well as heightened reactivity in the dLPFC and dorsal striatum in response to food consumption (Gearhardt et al., 2011).

Alternative explanations for the transition from voluntary to habitual/compulsive use in addictive disorders hinge on the idea that compulsive behaviour stems from a desire to relieve the negative emotional state that occurs during withdrawal from the primary reinforcer (Koob & Le Moal, 2008). Here, drug addiction is argued to reflect three stages; firstly, the binge/intoxication phase characterized by initial drug use. Secondly, by the experience of acute withdrawal from the drug, physical withdrawal, and the emergence of negative affect in the form of stress or anxiety, and thirdly, anticipation of subsequent drug use (Koob & Volkow, 2010). The transition between these stages is also mediated in part by a drug induced hypodopaminergic state that lessens the subjective reward associated with the drug as well as the
excessive attribution of incentive salience to its cues (Koob & Volkow, 2016). However, this model asserts that desire to avoid negative affect associated with absence of the drug and by its associated cues motivates a shift to the “dark side” of addiction and compulsive drug-seeking and -taking (Koob & Le Moal, 2008). This dark side is characterized by a sensitization of the brains’ stress response system which is highly integrated within processes that mediate reinforcement, learning, and reward (Koob, 2009; Koob & Le Moal, 2005).

FA has been similarly conceived with emphasis on the role of pathological eating to excess for the purpose of relieving a negative affective state (Macht, 2008; Meye & Adan, 2014; Parylak et al., 2011). This is likened to self-medication and primarily leads to overeating of processed foods (Gearhardt et al., 2009, 2016; Wise, 2008). Emotional eating is prevalent among those with mood and eating disorders as well as FA and associated with significant weight-gain (Bourdier et al., 2018; Burmeister et al., 2013; Frayn et al., 2016; Gianini et al., 2013; Hemmingsson, 2014; Konttinen et al., 2010; Pivarunas & Conner, 2015; Zeeck et al., 2011). Over-eating in response to negative emotional states is believed to be caused, in part, by the ability of processed foods to cue hunger as well as enhance activity in regions that direct appetitive behaviour to motivate eating in the absence of any energy deficit (Killgore & Yurgelun-Todd, 2006; Parylak et al., 2011). For example, negative affect while viewing high (but not low) caloric food images has been associated with heightened activation of brain regions associated with hunger while positive affect elicits responses associated with satiety (Killgore & Yurgelun-Todd, 2006).

Taken together, operational definitions and methods for measuring the abuse liability associated with specific food or features of food have yet to be realised within the human literature. In theory, GL reflects abuse liability by assigning pharmacokinetic value to food based
on the foods’ intrinsic properties and represents one possible approach to compare the abuse potential of different foods with a consistent value – yet, individual variability in post-prandial responses to the same foods diminishes its utility (Zeevi et al., 2015). Additionally, individual differences in hereditary predispositions, lifestyle, weight-status, reward sensitivity, and compliance with experimental diets are noted limitations in the ability to isolate the short- and long-term behavioural and neurobiological consequences of addictive foods in many of the clinical studies described here. These challenges highlight a critical role for using animal models that may afford greater control of these factors as well as the option to employ methodologies that better address what the addictive properties of food and food additives are, if any.

**Processed foods and the emergence of an addicted phenotype in animals**

In animal models of FA, methods to study the development of addictive-like behaviours motivated by food have been designed on the basis of experiments that study the addictive potential of drugs of abuse (De Jong et al., 2012; Lynch et al., 2010). Across all definitions of addiction, loss of control/compulsive drug-seeking emerges as a defining feature (Koob, 2009). In animals, this is assessed after prolonged periods of access to drugs of abuse and measured by responding for conditioned incentive stimuli despite punishment or by the lack of conditioned suppression in the presence of cues that signal punishment (Ahmed & Koob, 1998; Deroche-Gamonet et al., 2004; Jentsch et al., 2014). In these studies, adverse consequences fail to deter animals from drug-seeking and this compulsive behaviour is associated with individual trait and neurobiological vulnerabilities that enhance risk for addiction (Jentsch & Taylor, 1999; Jentsch et al., 2014; Mitchell & Potenza, 2014; Vanderschuren & Everitt, 2004).

Compulsive behaviour tends to selectively emerge in response to access to highly palatable or processed food and is often observed only in a subset of animals (Di Segni et al.,
2014). Studies reveal that DIO, a history or propensity to binge, food-restriction, and withdrawal from palatable food leads to consumption of a range of processed foods despite adverse consequences (Dore et al., 2014; Johnson & Kenny, 2010; Lataglia et al., 2010; Oswald et al., 2011; Teegarden & Bale, 2007). Alternative paradigms also index compulsivity by measuring lack of flexibility in feeding behaviour (Heyne et al., 2009). In these studies, animals refuse to consume a less palatable food following the removal of a concurrent palatable option, and will persist consuming the palatable diet even when paired with bitter tastes (Heyne et al., 2009). The emergence of compulsive eating in animals is also associated with disruption of DAergic signalling and reduced DR2 availability across regions of the dorsal and ventral striatum (Johnson & Kenny, 2010; Moore et al., 2017; Patrono et al., 2015). Moreover, strategies to reduce compulsive drug-seeking, including suppressing noradrenergic signalling in the PFC have been effectively used to diminish compulsive food-seeking in rats (Di Segni et al., 2014; Lataglia et al., 2010; Moore et al., 2017).

However, the form of pathological eating that is most often studied in animal models of FA is that of binge eating. In SRADs, binge is described as a “bout of intake” in a short period of time that is usually precipitated by a period of abstinence from the drug and is characteristic of the initial binge/intoxication phase (American Psychiatric Association, 2013; Le Moal & Koob, 2007). A similar operational definition exists for bingeing on food, that is, in a small defined period of time eating significantly more than what would typically be consumed (Corwin et al., 2011). Episodes of bingeing are often focused on processed foods, and the behaviour frequently presents among those diagnosed with FA, some forms of overweight or obesity, and is a core feature of most eating disorders (Avena, 2010; Ivezaj et al., 2016). Three prominent models that
vary in the manipulation of energy status and pattern of access to food have been developed to elicit and study bingeing in animals (Corwin et al., 2011).

In the intermittent access model, rats are provided with *ad libitum* access to chow and concurrent palatable food (vegetable shortening or liquid sucrose) that is made available for approximately two-hours per day either daily or three-times per week (Corwin & Wojnicki, 2006; Wojnicki et al., 2006; Wojnicki et al., 2007). Over the course of four to seven-weeks, compared to daily access, limited access promotes escalation of intake and bingeing on the palatable food and is associated with enhanced motivation as indexed by higher breakpoints when responding on a progressive ratio schedule (Wojnicki et al., 2006).

Bingeing can likewise be induced by exposing animals to a combination of manipulations that are purported to reflect predisposing factors for eating disorders, that is, cycling between periods of unrestricted and restricted access to processed foods and stress (Boggiano et al., 2005). To this end, animals are maintained on 12-day cycles that consist of five-days of food-restriction (66 % of their average daily chow intake), two-days of *ad libitum* access to chow or chow plus Oreo cookies, followed by four-days of *ad libitum* access to chow alone (Boggiano & Chandler, 2006). On day 12, animals are given four-hours of *ad libitum* access to chow or chow plus Oreo cookies in their home cage, but, just prior to test, are stressed by a three sec 0.6 mA foot shock. By the third iteration of the cycle, only the combination of access to Oreo cookies and stress elicits bingeing, and it is selectively on Oreo cookies (Boggiano et al., 2005). In this model, bingeing is associated with hedonic eating as animals are not food-restricted at test. Moreover, because it is precipitated by stress it is also equated with emotional eating and stress relief (Corwin et al., 2011; Parylak et al., 2011).
The most widely cited evidence for FA in animal models comes from the laboratory of Bart Hoebel, whereby they studied how intermittent access to sucrose influences the development of criteria set by the DSM-V for diagnosing SRADs (American Psychiatric Association, 2013; Corwin et al., 2011). The “sugar dependence” model highlights a specific macronutrient of interest as having abuse potential (Avena et al., 2008a). Here, rats receive *ad libitum* access to chow or chow plus concurrent access to 10% sucrose solutions from a bottle, under conditions of either unlimited (24 hour) or intermittent (12 hours) access. The latter group is cycled between 12-hours of access to food/12-hours of food deprivation (Avena et al., 2006). Over a period of 21 days, rats with intermittent access to chow plus sucrose begin to binge selectively on the sucrose solution in the first hour of availability and escalate daily intake of sucrose (Avena, et al., 2008a). Subsequently, these same animals display greater motivation to self-administer glucose in operant SA during extinction conditions (Avena et al., 2005) and symptoms of opioid withdrawal including head shakes and teeth chattering emerge within 24-hours of abstinence (Avena et al., 2008b). In fact, when tested 36-hours into abstinence, animals also display enhanced anxiety on the elevated plus-maze (Avena et al., 2008b; Colantuoni et al., 2002).

Several behavioural and neuroadaptive responses associated with bingeing on sucrose support the notion that these animals may indeed be “sugar dependent” and indicates sucrose has abuse liability. For example, repeated overstimulation of extracellular DAergic release in the NAc is considered a feature mostly unique to drugs of abuse that underlies their potent reinforcing effects and abuse liability (Wise, 2006a). This DAergic response typically habituates in response to repeated exposure to food, but, bingeing on sucrose elicits concurrent DA release in the NAc shell across repeated test sessions (Bassareo & Di Chiara, 1999; Luca, 2014; Rada et
al., 2005). This effect is also enhanced in both underweight rats and by food restriction, suggesting that some forms of restraint eating may in fact enhance sensitivity to the reinforcing effects of palatable foods (Avena et al., 2008a). Moreover, noted behavioural similarities between withdrawal from opiates and the effects of sucrose abstinence can be likewise precipitated by opiate antagonists (Avena et al., 2008b). Bingeing on sugar also leads to attenuated DA and enhanced acetylcholine release in the NAc shell during withdrawal that are similarly measured during withdrawal from opiates and nicotine (Avena et al., 2008b; Colantuoni et al., 2002) as well as gene expression changes in the NAc associated with repeated exposure to opiates (Spangler et al., 2003, 2004). These sucrose dependent rats also drink more ethanol (Avena et al., 2004) and display cross-sensitization to the locomotor stimulatory effects of amphetamines (Avena & Hoebel, 2003). Repeated amphetamine exposure likewise sensitizes responses to sucrose and these reciprocal relationships are commonly observed between classes of drugs considered to have similar properties and reflective of their shared abuse liability (Lynch et al., 2010; Robinson, 2010; Stewart & Badiani, 1993; Robinson & Berridge, 2008).

Preclinical animal models of abuse liability are used primarily to assess the reinforcing and discriminative effects of a drug, as well as its ability to induce dependence (Ator & Griffiths, 2003; Lynch et al., 2010). The “gold standard” for assessing a drugs abuse liability is operant SA (Carter & Griffiths, 2009; Collins et al., 1983). In this context, reinforcement is assessed by a drugs ability to support SA and abuse liability by SA to excess, or in other words, for non-therapeutic purposes (Balster & Bigelow, 2003). This procedure is useful because it allows for testing of diverse parameters (route of administration, rate and speed of delivery, pharmacological interactions) and can be assessed across any dose range (Carter & Griffiths, 2009). Additionally, features of the testing environment within the operant chamber
(lights/sounds paired with delivery of the drug, or that signal the availability or non-availability of the drug), the drug itself (tests of substitution and discrimination), and testing parameters (history of drug exposure, pharmacological pre-treatments) can be manipulated to design studies that assess the spectrum of behavioural sequela associated with developing SRADs.

With respect to sucrose SA, animals are placed into testing chambers where they are trained to emit an operant response such as a lever press and subsequently, in a separate location also within the operant chamber, animals retrieve and consume a sucrose pellet from a feeding trough or drink a solution of sucrose from a sipper spout (Figlewicz et al., 2011; Gosnell et al., 2010). Different schedules of reinforcement can be imposed whereby fixed ratio 1 schedules (FR1; White & Milner, 1992) reinforce each lever press, while the response requirement to receive successive sucrose delivery can be increased exponentially on a progressive ratio schedule (PR; Richardson & Roberts, 1996). Both schedules measure reinforcement but FR1 is suggested to reflect incentive consummatory behaviour while PR is considered to reflect incentive approach (motivation; Arnold & Roberts, 1997; Spealman & Goldberg, 1978).

Any reinforcing stimulus with abuse liability should increase the rate of SA and in this respect operant SA of sucrose has been well characterized (Ator & Griffiths, 2003; Sharma et al., 2012). In rodents, sucrose supports robust operant responding across a range of concentrations of solutions and pellets on both FR1 and PR schedules of reinforcement (Alsiö et al., 2009; Figlewicz et al., 2006; Sharma et al., 2012). Both schedules are sensitive to changes in concentration and lever pressing is purportedly maintained by the sugars caloric content and not sweet taste (Han et al., 2016; Sclafani & Ackroff, 2003). Moreover, following acquisition of sucrose SA, resistance to extinction and reinstatement of sucrose-seeking by cues as well as by
stress (Madsen et al, 2012; Riga et al., 2015) elicits similar activation of neural substrates involved in vulnerability to relapse to drugs of abuse (Bobadilla et al., 2017; Grimm et al., 2011).

Sucrose SA also shares with SRADs, many of the trait vulnerabilities that predict risk for addiction. That is, high trait impulsivity predicts escalation of sucrose-seeking and enhanced reactivity to sucrose associated cue (Diergaarde et al., 2009). Moreover, rats bred for high saccharin preferences respond at a higher rate for sucrose on both FR1 and PR schedules (Gosnell et al., 2010), and repeated exposure to stress enhances PR responding and motivation for sucrose on measures of resistance to extinction and cue-induced reinstatement of sucrose-seeking (Riga et al., 2015). Most notably, resurgence and reinstatement of sucrose-seeking has been reliably induced by a range of cues and stressors (Calu et al., 2014; Harkness et al., 2016; Shahan et al., 2015).

Sucrose SA also elicits neural activation (measured by Fos) across both the hypothalamus and mesocorticolimbic system and there is evidence for functional integration between both systems (Figlewicz et al., 2003, 2008, 2011). That is, operant SA of sucrose is suppressed by ICV administration of leptin and insulin (Figlewicz et al., 2006b) as well as intra-VTA administration of α-MSH (Shanmugarajah et al., 2017) and orexin R1 antagonists (Cason & Aston-Jones, 2014). Conversely SA is enhanced by administration of orexigenic peptides including ICV-ghrelin and intra-VTA AgRP (Overduin et al., 2012; Shanmugarajah et al., 2017). Moreover, SA of sucrose is sensitive to manipulation by systemic pre-treatment with MOR antagonist naltrexone as well as monoamine enhancing drugs including bupropion (Bruijnzeel & Arkou, 2003; Gosnell et al., 2010; Randall et al., 2015). These same drugs likewise influence the SA of drugs of abuse and are prescribed as adjunct therapy for the treatment of SRADs (Giuliano et al., 2013).
Along with evidence for the abuse liability of sucrose, there is also concern over the use of high fructose corn syrup (HFCS; Bray et al., 2004). Nearly 60 years ago, HFCS was introduced into the food industry and rapidly replaced sucrose as the primary sweetener in beverage and food (Bray et al., 2004; Ma et al., 2017). HFCS is a corn-based sweetener synthesized by the enzymatic isomerization of dextrose to fructose (Hanover & White, 1993). In the commonly used formula 55 (HFCS-55), it is composed of 55% fructose and 42% glucose unbound as monosaccharides as well as 3% glucose polymers (i.e. polycose; Collino, 2011). There are important differences in the digestion, absorption, and metabolism of fructose and glucose (Melanson et al., 2008). Most notably, compared to glucose, fructose may have limited effects on cessation of food intake as it elicits weak post-prandial insulin and ghrelin responses and does not stimulate insulin secretion from pancreatic beta cells (Curry, 1989; Teff et al., 2004). These findings, along with the observation that the introduction of HFCS-55 to the food industry positively correlates with the emergence of the obesity epidemic has been used to support the notion that fructose may be more “hazardous” than glucose (Bray, 2010; Bray et al., 2004; Brunstrom et al., 2018; Naleid et al., 2008; Stanhope & Havel, 2010).

The small but increased ratio of fructose to glucose between HFCS-55 and sucrose (55%:42% and 50:50%, respectively) is believed to be a primary cause for differences noted following short- and long-term exposure to these sugars. Rats gain more weight when diets are supplemented by HFCS-55 sweetened solutions despite consuming on average fewer calories from sugar than the sucrose drinking group (Bocarsly et al., 2010). Similarly, following 7-months of access, HFCS-55 induces greater weight-gain and elevated circulating levels of triglycerides and adiposity (Bocarsly et al., 2010). Compared to sucrose, HFCS-55 also enhances lipid synthesis in the liver (Mock et al., 2017), increases risk for cardiovascular disease
(Sadowska & Bruszkowska, 2017), and disrupts the expression and function of nutrient sensing hormones in both the peripheral and CNS, as evidenced by insulin resistance and down-regulation of POMC expression in the hypothalamus (Ma et al., 2013, 2017; Soto et al., 2017).

The notion that HFCS-55 may be more hazardous than sucrose is under debate however, as the effects of fructose, glucose or their combination do not significantly differ in most clinical studies (Chung et al., 2014). That is, no post-prandial differences are observed in circulating plasma levels of insulin, ghrelin, leptin, glucose, or triglycerides following consumption of beverages sweetened with isocaloric concentrations of sucrose or HFCS-55 (Melanson et al., 2008; Soenen & Westerterp-Plantenga, 2007; Yu et al., 2013). Nor do the subjective ratings of satiety or fullness reported by participants (Akhavan & Anderson, 2007). Yet, these data are at odds with findings that consumption of glucose, but not fructose, suppresses activity in brain regions responsible for stimulating appetite and enhancing food reward including the hypothalamus, insula, and striatum. These findings imply that fructose fails to elicit appropriate satiety responses (Luo et al., 2015; Page et al., 2013). Moreover, significant concentration-dependent increases in risk for cardiovascular disease have been reported within two-weeks of maintenance on a diet supplemented by drinks sweetened with 10%, 17.5% or 25% energy requirement from HFCS-55 (Stanhope et al., 2011, 2015).

**Summary of the issues**

Processed foods and food additives such as sugar appear to engender behavioural and neural responses that support the notion that they have the potential for abuse (Avena et al., 2008a; Ifland et al., 2015; Schulte et al., 2015a, 2017). Firstly, these foods alter peripheral and central responses to eating that impair energy sensing abilities (Brunstrom et al., 2018; van Dongen et al., 2012) in ways that favour overconsumption and the dulling of satiety signals...
(Erlanson-Albertsson, 2005; Fardet, 2016; Page et al., 2013; Saper et al., 2002). Unlike minimally processed or naturally sourced foods, processed foods also elicit neural responses and adaptation to regions of the brain responsible for reinforcement, motivation, decision making, inhibitory control, and emotional affect that are likewise observed following repeated use of drugs of abuse (Dimitropoulos et al., 2012; Kelley et al., 2005; Killgore & Yurgelun-Todd, 2006, 2007; Tang et al., 2012; Wang et al., 2004). Furthermore, similar effects of these food stimuli and drugs of abuse on well-defined appetitive and motivational features of SRADs support the hypothesis that they may be addictive (Berridge & Robinson, 2016; Davis & Carter, 2009; Parylak et al., 2011). Yet to date, such clinical studies have yet to determine what food or food additives(s) are addictive.

Animal models of FA afford the experimental conditions under which features of addictive-like behaviour and abuse liability can be more rigorously assessed (Gold & Avena, 2013). Addictive-like behaviours emerge in response to consuming a range of processed foods or food additives such as sugar (Avena, Rada, & Hoebel, 2008a; Dore et al., 2014; Johnson & Kenny, 2010; Oswald et al., 2011). Some of these procedures may be quite sensitive to the incentive food stimuli selected which limits their utility for assessing the abuse liability of a range of stimuli. To date, other than sucrose and glucose, the “sugar dependence” model which arguably has received the most attention for its ability to model aspects of FA has not been used to assess the addictive potential of other reinforcers. In fact, in our laboratory, we could not elicit likewise bingeing on Oreo cookies using the model described by Avena and colleagues (2008a).

There are also issues related to the generalizability of results obtained from studies of FA entirely reliant on binge behaviour. In these experiments, not only is the emergence of binge behaviour necessary to reveal behavioural and neurobiological indices of addiction, but it is also
essential to employ food-restriction in all but one model to elicit bingeing (Corwin & Wojnicki, 2006). While these methods model clinically relevant forms of pathological eating such as binge/food restriction or compulsive eating; food-restriction in and of itself enhances reward sensitivity and motivation for both food and drugs of abuse (Cabeza de Vaca & Carr, 1998; Carr, 2007; Moscarello et al., 2009). Also limiting the interpretation and generalizability of these data is the reliance on binge behaviour as it is not a necessary antecedent or sufficient criteria for developing or diagnosing addictive disorders (American Psychiatric Association, 2013).

Most importantly, the experimental methods often employed to model aspects of FA in animals do not directly measure abuse liability of food. Instead, there is a reliance on the observation that a range of stimuli capable of producing an addictive-like phenotype share common features of being highly palatable and calorically dense. These data reflect an inferential relationship between these foods and abuse liability. This is often the primary critique of human and animal studies of FA and arguably the most pressing question to address in the field of FA research (Hebebrand et al., 2014; Westwater et al., 2016).

Alternatively, the addictive potential of foods may be best assessed by using methods and criteria already in place for determining and classifying the abuse potential of drugs of abuse, that is, operant SA (Carter & Griffiths, 2009). To date, operant SA has been performed with solutions sweetened with sucrose, chocolate syrups, as well as flavoured pellets composed of different ratios of additives including sugars and fats (Alsiö et al., 2009; de Jong et al., 2013; Figlewicz et al., 2006). These data provide support for the notion that high concentrations of added sugars in processed foods and sweetened drinks may confer abuse liability. In fact, when given the choice, rats display a strong preference for drinking solutions sweetened with sucrose or the non-caloric sweetener saccharin over self-administering IV infusions of cocaine (Lenoir et
al., 2007). There is however a lack of knowledge regarding the abuse liability of HFCS-55 despite having almost entirely replaced sucrose as the primary added sugar in food and drink (Bray et al., 2004). The overlap between the introduction of HFCS-55 into the food supply and the emerging obesity epidemic along with controversy over its ability to disrupt metabolic function to promote excessive food intake and weight-gain suggest that it may have addictive potential that has yet to be explored.

**Operant intraoral SA procedures**

For the present thesis, we developed and evaluated the utility of using operant intraoral (IO) SA (IOSA) to measure abuse liability and assess the hypothesis that HFCS-55 is addictive. The rationale for modifying the route of administration for sugar SA rested on the observation that current forms of sugar SA do not always allow for accurate or necessary manipulations of key parameters of operant SA designed to assess abuse liability. In chapter 2, task specific issues are reviewed in detail along with how the use of IOSA may better address those limitations.

Most notably, operant IOSA SA allows for the delivery of passive IO infusions of controlled quantities of any concentration and any volume of any water-soluble food additive. This feature allows for sensitive measures of concentration-response curves, substitution and discrimination procedures, and eliminates the delay between operant response and delivery of the primary reinforcer, a factor that plays an important role in the acquisition and maintenance of operant behaviour (Killeen, 1994).

Importantly, it also allows for manipulation of parameters for testing aspects of abuse liability that are either not possible and or when available lack accuracy under standard food SA procedures. For example, in studies of IV drug SA, following periods of extinction whereby responding is not reinforced, drug primes (i.e., administration of a small quantity of the drug;
Bossert et al., 2013) reinstate responding. Therefore, the ability to deliver passive IO infusions can be used to study “food” primed reinstatement and likewise cross-reinstatement between different sugars and drugs of abuse to compare their relative reinforcing efficacy and ability to promote relapse (Grimm et al., 2012). Moreover, the ability to use diverse schedules of reinforcement is critical to study different aspects of abuse liability such as motivation assessed by PR schedules (Richardson & Roberts, 1996). On this schedule, whereby each lever response is not reinforced, the delivery of passive IO infusions ensures the reward is delivered upon making the appropriate number of responses set by the schedules requirements to assess motivation. This may be inaccurately measured by animals required to move from the lever to either the sipper tube or receptacle to retrieve the reward when it is delivered and once there, it is likewise difficult to guarantee rewards were not missed or dropped.

**Studying the abuse potential of sugar**

The studies presented in this thesis were designed to assess the abuse liability associated with HFCS-55 by measuring for the first time in rats, operant IOSA reinforced by this sweetener. To this end, in Chapter two (Levy et al., 2014), the reinforcing efficacy of a range of concentrations of HFCS-55 (8%, 25%, and 50%) were measured according to its ability to promote the acquisition and maintenance of operant SA behaviour. We also assessed SA behaviour in response to incentive contrasts in the concentration of HFCS-55 (Crespi, 1942), as well as in response to different schedules of reinforcement to evaluate aspects of abuse liability related to its reinforcing and motivational effects. These experiments revealed that IOSA can be employed to study the acquisition and maintenance of operant behaviour reinforced by sweet solutions similarly to operant behaviour elicited by drugs of abuse and sucrose. Importantly,
these data suggested that HFCS-55 may have abuse potential based on criteria used to assess the addictive potential of potent reinforcers like drugs of abuse.

A comparison of the behavioural, physiological and neurobiological consequences of consumption of HFCS-55 and sucrose were explored in Chapter 3 (Levy et al., 2015). Such comparisons are necessary criteria to evaluate the unknown abuse potential of a substance and the results of these studies may also address relevant issues in the on-going debate and controversy over the hazards of sweetening food and drink with either sugar (Carter & Griffiths, 2009; Rippe & Angelopoulos, 2013). Using IOSA, animals self-administered isocaloric solutions of 25% HFCS, 20% sucrose or non-caloric but sweet saccharin 0.1% w/v solutions under different schedules of reinforcement. Following IOSA, mRNA for D2R and MOR receptor genes were quantified in striatal regions involved in addictive behaviours and measures of metabolic health, including hepatic fatty acids and general adiposity were assessed. These experiments suggested that the abuse liability of HFCS-55 may exceed that of sucrose by virtue of its reinforcing efficacy in operant IOSA and its subsequent association with addictive-like alterations to the expression of D2R- and MOR- mRNA. HFCS-55 consumption was also accompanied by disturbances in hepatic function related to the development of metabolic disorders.

The results of experiments performed in Chapters 2 and 3 suggested HFCS-55 has abuse liability that may exceed that of sucrose. Even though animals consumed almost twice as much sugar when they were self-administering sucrose, exposure to HFCS-55 led to behavioural indices and neural adaptations that more closely reflect those engendered by repeated access to drugs of abuse. Therefore, in Chapter 4 (Levy et al., 2018), the pharmacological actions of a novel drug approved for its anti-obesity effects, Contrave® (combination of bupropion (BUP);
DA reuptake/partial noradrenergic agonist) and naltrexone (NTX; non-selective competitive MOR antagonist) were investigated with respect to its ability to reduce consummatory behaviours associated with HFCS-55 (Greenway et al., 2010). The combination of BUP and NTX, and the drugs alone were administered either continuously (via subcutaneous osmotic mini-pump) or acutely (via subcutaneous injection prior to IOSA sessions) to animals self-administering 25% HFCS-55 in IOSA or 50% HFCS-55 from bottles in home cages. Subsequently, mRNA for genes associated with its purported therapeutic effects on enhancing weight-loss, mood, as well as with reward processing were quantified (Apovian, 2016; Greenway et al., 2009a; McElroy et al., 2013). The results of these experiments suggested that Contrave® may aid with weight-loss by reducing caloric intake as evidenced by reduced consumption of HFCS-55 in operant IOSA and in-home cages. That said, it was also revealed through behavioural as well as gene expression analysis that its anorexigenic effects on appetite and purported ability to relieve depressive symptoms may be attributable primarily to the effects of NTX alone and BUP alone, and not a cause of their synergetic effects (Greenway et al., 2009a). Most interestingly, the combination of BUP+NTX appeared to alter gene expression involved in reward processing in a manner that may reduce vulnerability for addictions suggesting interesting applications for this drug in also reducing compulsive food- or drug-intake in addictive disorders.
Chapter 2

Journal of Visualized Experiments (JoVE)

A novel procedure for evaluating the reinforcing properties of tastants in laboratory rats: Operant intraoral self-administration

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*In the preparation of Chapter 2, additional experimental data was added to the published manuscript.
Abstract

This paper describes a novel method for studying the bio-behavioural basis of addiction to food. This method combines the surgical component of taste reactivity (TR) with the behavioural aspects of operant self-administration (SA) of drugs. Under brief general anaesthesia, rats are implanted with an intraoral (IO) cannula that allows delivery of test solutions directly in the oral cavity. Animals are then tested in operant SA chambers whereby they can press a lever to receive IO infusions of test solutions.

The food addiction (FA) hypothesis predicts that some foods may share with drugs of abuse the ability to reinforce behaviours leading to their overconsumption. It is necessary then, to identify what food or features of food such as added sugar, may be addictive. Therefore, the current experiments in male Sprague-Dawley rats employed IOSA procedures to assess the reinforcing efficacy and possible abuse liability associated with high fructose corn syrup formula 55 (HFCS-55) using measures and criteria typically employed to evaluate the addictive potential of drugs.

Naïve, food-restricted rats implanted with IO cannulas were trained to lever press for IO infusions of sweet solutions. TR tests were also performed. It was found that animals acquired and maintained IOSA of 8%, 25% and 50% HFCS-55, but not IOSA of the non-caloric but sweet 0.1% w/v saccharin solution used for comparison, even though both sweeteners produced similar hedonic reactions in TR. Additionally, manipulation of the schedule of reinforcement and concentration of HFCS-55 available to self-administer during IOSA significantly influenced lever responding for HFCS-55 and operant behaviour was not abolished by ad libitum feeding prior to testing. Notably, within three weeks of lever responding for 25% HFCS-55, escalation of intake was observed coupled with the emergence of binge-like behaviour in rats.
Taken together, these data indicated that IOSA can be used to study acquisition and maintenance of operant responding for sweet solutions. HFCS-55 reinforcement appears related to its caloric content and the behavioural profile of operant responding for HFCS-55 is comparable to that observed when rats self-administer other sugars with purported abuse potential (i.e. sucrose) as well as drugs of abuse. Future studies should investigate the possibility that HFCS-55 engenders additional “addictive-like” behaviours (i.e. withdrawal, resistance to punishment) as well as explore whether likewise neuroadaptations of brain reward circuitry emerge.

**Keywords**

Introduction

Overconsumption of food has contributed to the modern epidemic of overweight and obesity (Lindberg et al., 2011; World Health Organization, 2015) and it is important then to understand why patterns of excessive food intake develop and persist despite negative health consequences. That is, why eating may become addictive (Avena & Gold, 2011; Randolph, 1956). The study of the neurobiological and behavioural basis of food addiction (FA; Gearhardt et al., 2011; Parylak et al., 2011) reveals that consumption of processed foods may promote behavioural dependence (Ifland et al., 2015; Pursey, Stanwell, Gearhardt, Collins, & Burrows, 2014; Schulte et al., 2015b) as well as neuroadaptations of brain reward circuits in both humans (Gearhardt, Yokum, et al., 2011; Wang, 2002) and laboratory animals (Avena, Rada, & Hoebel, 2008b; Johnson & Kenny, 2010). As common neurobiological substrates regulate the hedonic and reinforcing properties of food and addictive drugs, it is possible, that like drugs of abuse, some foods may be addictive (Koob et al., 2004; Morganstern, Barson, & Leibowitz, 2011; Schulte, et al., 2015a; Volkow, Wang, Tomasi, & Baler, 2013).

In laboratory rats, there are diverse procedures for studying the addictive properties of drugs of abuse. To this end, operant intravenous (IV) drug self-administration (SA) is considered the “gold standard” for assessing a substances addictive potential and several reviews outline standard procedures and criteria that can be readily applied to food (Ator & Griffiths, 2003; Balster, 1991; Carter & Griffiths, 2009; Henningfield, Cohen, & Heishman, 1991). However, adapting parallel methods and criteria to assess these features in food presents methodological challenges. To address some of these limitations, the current experiments applied principles for studying operant IV drug SA to the investigation of operant SA of sweet solutions delivered by intraoral (IO) infusion.
Traditionally, IO infusions are delivered to study the palatability of tastants in taste reactivity (TR; Grill & Norgren, 1978). Briefly, an IO cannula is surgically implanted into the cheek of rats and IO infusions of solutions are passively delivered (Grill & Norgren, 1978). In our application, rats can lever press in the operant chamber to actively self-infuse a test solution directly into the oral cavity (Panksepp & Trowill, 1967a); hence the term IOSA. The disadvantage of requiring minor surgery is offset by advantages over traditional food SA procedures that involve (a) measuring quantities of a solution drank from a bottle, (b) operant responding for solid pellets, or (c) operant responding for drops of fluid delivered through a spout. Most notably, similar to the delivery of passive IV infusions of drugs, the delivery of passive IO infusions in IOSA shortens the delay between the operant response (i.e. lever pressing) and the delivery of the primary reinforcer; a factor that plays a critical role in the acquisition and maintenance of operant behaviour (Killeen, 1994).

In comparison to (a), IOSA involves an active and measurable operant response (i.e., pressing a lever) and therefore it is possible to modify the schedule of reinforcement which establishes the relationship between response requirements and delivery of IO infusions. For example, by employing a progressive ratio (PR) schedule, whereby responses required for successive infusions increase exponentially within a session (Richardson & Roberts, 1996), it is possible to assess how much an animal “wants” the next infusion (Berridge & Robinson, 2003). This important aspect of “seeking” behaviour cannot be assessed when measuring the volume of a solution drank from a spout. Moreover, when such schedules can be employed using operant SA procedures described by (b) and (c), it requires the animal move from the location where the operant response is made (i.e. lever) to a different location within the operant chamber to obtain the reinforcer (i.e. receptacle or bottle). This imposes a delay between the response and obtaining
the primary reinforcer (Killeen, 1994), and relies on the assumption that animals move and retrieve the reinforcer when it is delivered according to the precise schedule of reinforcement being assessed. Even when employing bottles with the ability to deliver fluid through a spout according to licking rate and different schedules of reinforcement (Sclafani & Ackroff, 2003), it is still not possible to guarantee rewards are always obtained (i.e., missed pellets/drops of fluid).

In comparison to (b), IOSA allows testing of any concentration and any volume of any water-soluble food additive. The ability to control and manipulate concentration/volume ratios is mandatory in experiments where intake can be modulated by both the caloric value of a solution (leading to nutrient-specific satiety; Smeets et al., 2006; van Dongen, van den Berg, Vink, Kok, & de Graaf, 2012) and by how much of that solution can be consumed within a given period (i.e., fullness). The delivery of passive IO infusions of controlled quantities of the test solution also makes it possible to measure orofacial responses of “liking” (measured by tongue protrusions; Berridge & Robinson, 2003) and assess whether the subjective palatability of a particular tastant changes over time in response to IOSA.

Importantly, IOSA allows for manipulation of parameters for testing aspects of abuse liability that are not readily available using procedures described by (a), (b) and (c). The ability to administer passive IO infusions has significant applications for the study of relapse to food-seeking (Calu et al., 2014; Nair, Adams-Deutsch, Epstein, & Shaham, 2009). That is, in studies of IV drug SA, following periods of extinction whereby operant responding is not reinforced, drug primes (i.e., administration of a small dose of the drug; Zhou, Leri, Cummins, Hoeschele, & Kreek, 2008) can “reinstate” responding (Leri & Stewart, 2001; Panlilio & Goldberg, 2007). Passive IO infusions may be likewise employed to study food-seeking behaviour elicited by “food” primes, as well as important attributes of abuse liability (Lynch et al., 2010) assessed by
the development of cross-sensitization or cross-reinstatement between stimuli and potent reinforcers such as drugs of abuse. This feature of IOSA also provides a means to compare parallel operant behaviours (i.e. lever pressing) motivated by different reinforcing stimuli (i.e. food versus drug), delivered using passive administration allowing for easy comparison of behaviour in “substitution” or “discrimination” studies employed to determine the abuse liability of a substance (Ator & Griffiths, 2003).

IOSA of tastants is also preferable to IV and intra-gastric SA. In fact, although IV infusions of sugars in both humans and laboratory animals produce physiological consequences similar to those observed following oral consumption (Dunnigan & Ford, 1975; Lê & Tappy, 2006), this is a poor model of how sweets are normally consumed (i.e., orally). Moreover, gustatory signals produced by mastication confer important information about the palatability of food and when this stage of the digestive process is omitted, the development of maladaptive behaviours such as overeating is reduced (Fernstrom et al., 2012; Scheggi, Secci, Marchese, De Montis, & Gambarana, 2013).

An important assumption of the FA hypothesis is that particular foods should be addictive (Meule, 2015; Schulte et al., 2015a) and there exists much debate (Meule, 2014; Schulte, Joyner, Potenza, Grilo, & Gearhardt, 2015; Westwater, et al., 2016) as to whether any one food or food additive can be labeled as such. Evidence for sucrose dependence (Avena et al., 2008a) and operant SA of sucrose in rats (Figlewicz et al., 2011; Gosnell et al., 2010; Harkness et al., 2016; Shahan, Craig, & Sweeney., 2015) suggests that the addition of high concentrations of sugar to food and drink may underlie the purported addictive potential of some processed foods (Ifland et al., 2015). Interestingly, there is a lack of knowledge regarding the abuse liability of high fructose corn syrup formula 55 (HFCS-55; 55 % fructose + 42 % glucose + 3% polycose)
despite having almost entirely replaced sucrose as the primary added sugar in food and drink over the past ~ 60 years (Bray et al., 2004).

It has been suggested that, compared to sucrose, a higher ratio of fructose to glucose in HFCS-55 may lend to more hazardous effects on health (Bray et al., 2004; Chung et al., 2014; Rippe & Angelopoulos, 2016; Sadowska & Bruszkowska, 2017); however, this hypothesis has been challenged by inconsistent findings (White, 2008, 2013). For example, in humans, consumption of glucose (but not fructose) suppresses activity in brain regions responsible for stimulating appetite and enhancing food reward (Page et al., 2013). Also in human, compared to sucrose, HFCS-55 is associated with adverse metabolic disturbances (Le et al., 2012; Stanhope et al., 2008) that are likewise reported in rats (Hsu et al., 2015; Mock et al., 2017) along with greater weight-gain (Bocarsly et al., 2010). Yet, clinical studies report that the metabolic consequences and subjective ratings of satiety do not differ following intake of isocaloric beverages of sucrose and HFCS-55 (Akhavan & Anderson, 2007; Sadowska & Bruszkowska, 2017; Yu et al., 2013). Hence, greater clarity of its effects on behavioural and neurobiological processes implicated in addictive-like behaviours is required given the ubiquity with which HFCS-55 is added to food and drink (Bray et al., 2004).

The current experiments were designed to characterize operant SA of HFCS-55 using the IOSA procedures described above (Grill & Norgren, 1978; Panksepp & Trowill, 1967a). Rats were surgically implanted with IO cannulas and trained in standard operant chambers to press a lever for passive IO infusions of HFCS-55. To assess the reinforcing properties of HFCS-55, in Experiment 1, animals self-administered different concentrations of HFCS-55 (8%, 25% or 50%) on a fixed ratio 1 (i.e., 1 press = 1 infusion; FR1) schedule of reinforcement (Spealman & Goldberg, 1978). Once animals acquired and maintained SA behaviour, the FR1 was substituted
for a PR schedule of reinforcement, concentrations of HFCS-55 were manipulated to create incentive contrasts (Flaherty, 1982), and the influence of food-restriction versus *ad libitum* feeding prior to IOSA was assessed. The results of Experiment 1 suggested that HFCS-55, particularly when self-administered at 25%, supported robust operant SA. To assess whether infusions of HFCS-55 was reinforced by primarily the sweet taste or caloric value of the sugar, Experiments 2 and 3 compared the pattern of IOSA of 25% HFCS-55 to that of 0.1% w/v saccharin, a non-caloric sweetener. Finally, prior to IOSA, TR tests were performed to assess the relative palatability of the different concentrations of HFCS-55 and saccharin to relate hedonic responses to SA behaviour.

**Materials and Methods**

**Subjects**

Adult male Sprague-Dawley rats (Charles River, QC), weighing 200-225 grams (g) at the beginning of the experiments, were singly housed and maintained on a reverse light/dark cycle (7:00 am OFF - 7:00 pm ON) with *ad libitum* access to food and water except when otherwise indicated, or during behavioral testing which occurred during the 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.

**Food**

Rats were fed a standard laboratory chow (14% protein and 4% fat; Teklad Global Diets, Harlan Laboratories). The test solutions were stored at room temperature for the duration of the experiments and made by diluting HFCS-55 (serving of 4 g = 0 g (0%) fat, 0 g (0%) protein and 3.08 g (77%) carbohydrate; Natures Flavors, California) and saccharin (sodium salt hydrate 99+ %, ACROS Organics, New Jersey) with reverse osmosis water. Given the experimental
apparatus employed (i.e., diameter of syringes and tubing), the viscosity of different concentrations of HFCS-55 tested did not influence the consistent delivery of IO infusions (80 µl/infusion, over 2.5-seconds) between groups.

Surgery

Intraoral cannulation and head cap

Rats were surgically implanted with intraoral (IO) cannulas under general anesthesia with 5% isoflurane for induction and maintenance. Rats were administered Depocillin (0.33 mg/kg SC, Pen Aqueous, Guelph, ON) and Carprofen (0.1 mg/kg IP, Pfizer, Kirkland, QC) 30-minutes prior to surgery. A thin walled 15-gauge stainless steel needle was inserted at the base of the neck, directed SC around the ear and brought out behind the first molar inside of the oral cavity. Intra Medic polyethylene plastic tubing (inner diameter of 0.86 mm and outer diameter of 1.27 mm; Becton Dickinson, VWR) was run through the needle to exteriorize the cannula. One mesh disc (7 mm in diameter) and three-square elastic discs (8 x 8 mm) were threaded and drawn to the exposed skin at the back of the neck to stabilize the cannula. The portion of the cannula in the oral cavity was secured by a flagged-end of the tubing over surgical mesh (Peektex Mesh 200 micros; Small Parts Inc., Miramar, FL) that rested flush against the inner cheek. Prior to the start of surgery, a nylon bolt (28 mm length; 4 mm point; 8 mm head) was modified by a 2-mm groove carved along its length. The bolt was mounted to the skull of the rat in the center of four jeweller screws and secured with dental cement. Rats received an additional injection of Carprofen 24 hours following surgery, and the IO cannula was flushed daily with chlorhexidine (Ayerset, Fort Dodge, Iowa).

Apparatus

Taste Reactivity (TR)
The TR apparatus included a clear Plexiglas chamber (22.5 cm x 26.0 cm x 20.0 cm) with an opaque lid placed over a transparent glass surface. A digital video camera (Sony, DCR-HC48) was pointed at mirror placed at a 45° angle under the glass surface to visualize the ventral surface of the rat and record orofacial reactions (Neath, Limebeer, Reilly, & Parker, 2010).

**Operant intraoral self-administration (IOSA)**

Twenty-six Plexiglas operant conditioning chambers (model ENV-008CT, Med Associates, Lafayette, IN) were used, and each chamber was enclosed in a larger sound-attenuating plywood cabinet (model ENV-018M, Med Associates). Each operant chamber had a house light (28 V) and two levers, one retractable and one stationary, located 10 cm apart and 8 cm above the floor of the box. The retractable lever (active lever) was connected to an infusion pump (Razel Scientific Instruments, Stamford, CT) positioned outside the sound-insulating cabinet. The stationary lever (inactive lever) served to control for baseline, non-reinforced operant behaviour; pressing this lever had no consequence, but all presses were recorded. A white light (28 Watts) located 3 cm above the active lever served as a stimulus light paired with the delivery of IO infusions.

**General Procedures**

**Taste reactivity**

Rats were individually placed in the test chambers for a 3-minute habituation period. During this period, the IO cannula was attached to an infusion pump and water was infused at a rate of 0.5 ml/minute. Twenty-four hours later, rats were returned to the chamber, attached to the infusion pump, and infused with the test solution at a rate of 0.5 ml/minute. Orofacial reactions were recorded for the duration of the 3-minute test, and behavior was subsequently scored using The Observer (Noldus Information technology, Sterling, VA). The reactions scored were:
number of forward tongue protrusions (extensions of the tongue out of the front of the mouth) and lateral tongue protrusions (extensions of the tongue out of the sides of the mouth, sweeping along the lips. Because the results of these measures were comparable, frequencies of forward and lateral tongue protrusions were combined to obtain a single index of palatability.

*Operant intraoral self-administration (IOSA)*

Rats self-administered solutions of HFCS-55 or saccharin, for 3-hours a day, 6-hours into the dark cycle. Each session began with the activation of a house light, entry of the active lever, and illumination of a light stimulus above the active lever for 30-seconds. Subsequently, presses on the active lever resulted in the delivery of an 80 µl infusion of the test solution over 2.5-seconds on a FR1 schedule of reinforcement. To allow sufficient time for ingestion, a time-out period (Carter & Griffiths, 2009) of 27.5-seconds was imposed during which the active lever was retracted and the stimulus light was activated. No limit was imposed on the number of infusions obtainable within each session. Acquisition of stable intake was defined by 1) a significant difference in responding between active and inactive levers for at least two consecutive sessions; and 2) less than 20% variation in the number of infusions earned per session for two consecutive sessions. In some experiments, a PR schedule of reinforcement was also employed. On this schedule, the number of responses required to obtain each successive infusion increased exponentially within session according to the formula: \( 5^{e^{(\text{injection number} \times 0.2)}} \) (Richardson & Roberts, 1996). The breakpoint was indexed by the last infusion received prior to cessation of responding on the active lever for at least 1-hour.

*Experiment 1*

Experiment 1 investigated the utility of using IOSA procedures for studying operant SA of sweet solutions. To this end, IOSA was employed to assess the reinforcing properties of
HFCS-55. This was measured first, by the acquisition and maintenance of operant SA (Lynch, 2017; Lynch et al., 2010) and secondly, by changes in SA behaviour in response to manipulating the schedule of reinforcement and concentration of HFCS-55 employed. To facilitate operant SA, rats (n = 22; naïve) were food-restricted for 18-hours prior to each session and then fed ad libitum chow for 3-hours immediately following IOSA (Carroll, France, & Meisch, 1979). Energy status can influence appetitive behaviour (Raynor & Epstein, 2003; Shalev, 2012) such that food-restriction enhances while satiety diminishes the incentive value of food reinforcers (Corwin, Avena, & Boggiano, 2011; Scheggi, Secci, Marchese, De Montis, & Gambarana, 2013). Therefore, it was reasoned that animals should be food-restricted to reduce possible variability in satiety caused by individual differences in consumption of chow prior to IOSA testing and free-fed following each session.

To assess whether a concentration–response curve for HFCS-55 could be established, rats were trained to self-administer 8% (0.026 kcal/infusion; n = 6), 25% (0.08 kcal/infusion; n = 8), or 50% (0.17 kcal/infusion; n = 8) HFCS-55. Just prior to the start and again following the initial 15-days of training on the FR1 schedule, animals were tested on a PR schedule of reinforcement (PR Test I (experiment day 1) and PR test II (experiment day 17), respectively; Richardson & Roberts, 1996). These PR tests were employed to measure whether animals would work for IO infusions of HFCS-55 and whether motivation was affected by concentration (Reilly, 1999; Roberts, Loh, & Vickers, 1989). Then, following PR test II; the highest and lowest concentrations were reversed: that is, animals trained on 8% were switched to 50%, and those trained on 50% were switched to 8% to measure changes in responding to positive and negative incentive contrasts (Crespi, 1942). At the new concentrations, animals received 15-additional IOSA sessions, and as a constant, rats in the 25% HFCS-55 group self-administered the same
concentration of HFCS-55 across all IOSA sessions and schedules of reinforcement tested.

Finally, to assess the effects of *ad libitum* feeding and possible satiety on IOSA of HFCS-55, animals self-administered HFCS-55 following *ad libitum* access to chow for 24-hours.

*Experiment 2*

Experiment 1 revealed that 25% HFCS-55 supported robust operant SA behaviour, and so, in Experiment 2, pattern of IOSA of 25% HFCS-55 was assessed for possible changes in operant SA behaviour that reflect abuse liability (Ator & Griffiths, 2003). Therefore, rats (n = 22; naïve, food-restricted) were trained to self-administer 25% HFCS-55 for 21-consecutive days (one 3-hour session/day). The second objective was to determine whether IOSA acquired and maintained by 25% HFCS-55 would persist if it was substituted by the non-caloric sweetener, saccharin. The glucose in HFCS-55, and possibly fructose, stimulates dopamine (DA) signaling in mesolimbic regions of the brain that mediate the development of addictive behaviors (i.e. ventral tegmental area (VTA) and nucleus accumbens (NAc); Wise, 2006; Levine, Kotz, & Gosnell, 2003). Saccharin, however, has no caloric value (Miller & Frattali, 1989), and although it is voluntarily consumed from a bottle (Carroll, Morgan, Anker, Perry, & Dess, 2008), and reinforces operant behavior in rats (Gosnell et al., 2010; Mierzejewski et al., 2009), it only has weak effects on DA release in the NAc (Blackburn, Phillips, Jakubovic, & Fibiger, 1986; Weiss, Lorang, Bloom, & Koob, 1993). Therefore, following 21-days of 0.1% w/v saccharin self-administration it was substituted for 25% HFCS-55. This concentration was selected because it elicits hedonic reactions in TR tests (Neath et al., 2010), rats voluntarily drink it from a bottle (Carroll et al., 2008), and because it reinforces operant behavior measured by drinking the sweet solution from a spout in operant chambers (Gosnell et al., 2010; Mierzejewski et al., 2009). Rats
self-administered 0.1% w/v saccharin for one IOSA session, followed by a final test whereby 25% HFCS-55 was reintroduced.

Experiment 1 also suggested that *ad libitum* feeding reduced but did not abolish IOSA of HFCS-55. However, this manipulation was only assessed over the duration of a single IOSA session. Hence, the final objective of Experiment 2 was to explore the effects of energy status on the acquisition and maintenance of operant SA of 25% HFCS-55. To this end, an additional group of rats (n = 9, naïve, non-food-restricted) were trained to self-administer 25% HFCS-55 for 15-consecutive days (one 3-hour session/day) as described above.

**Experiment 3**

The results of substituting 25% HFCS-55 for 0.1% w/v saccharin in Experiment 2 indicated that rats self-administered saccharin at a much lower rate than HFCS-55. But, it was possible that this resulted from testing SA of saccharin in rats that had previous experience in consuming HFCS-55 (i.e. negative contrast effects; Crespi, 1942; Flaherty, 1982), or from having selected a concentration of saccharin that when using IOSA procedures does not support operant behaviour. The goal of Experiment 3 then, was to assess IOSA of different concentrations of saccharin. To this end, animals (n = 22; naïve, food-restricted) were trained to self-administer 0.1% w/v saccharin for 16-consecutive days (one 3-hour session/day). Then, a concentration-response substitution analysis was performed. To this end, over 4-consecutive days (one 3-hour session/day), rats self-administered a range of saccharin concentrations (0.01%, 0.1%, 1% and 10% w/v saccharin), and the order of concentrations tested was counterbalanced across animals and days. Finally, on the last IOSA session (experiment day 21), saccharin was substituted for 25% HFCS-55. These tests were performed in a subset of 14-rats only because 8-
subjects from the initial sample were sacrificed immediately following session 21 for analysis of epidydimal and visceral adipose tissue not reported in the current manuscript.

Finally, in Experiments 1 and 3, hedonic orofacial reactions were assessed using TR tests in a subset of HFCS-55 and saccharin-naïve rats (i.e. prior to IOSA) in response to passive IO infusions of either 8% (n = 8), 25% (n = 8) 50% (n = 8) HFCS-55 as well as 25% HFCS-55 (n = 5) and 0.1% w/v saccharin (n = 6), respectively.

Statistical analyses

One- and two-factor repeated measures Analyses of Variance (ANOVA) and t-tests were employed as required and significant interactions or main effects were explored using the Student-Newman-Keuls method for multiple comparisons (α = 0.05). The exact values of non-significant results were not reported, and the analyses were performed using SigmaStat (3.5 for Windows, Systat Software, Inc).

Results

Experiment 1

Figure 1 Panel A represents the initial 15-sessions of lever pressing on a FR1 schedule of reinforcement for infusions of different concentrations of HFCS-55 and in Panel B, the 15-IOSA sessions following the switch in concentration that increased 8% to 50%, and decreased 50% to 8%. The concentration of 25% HFCS-55 was not altered. Animals acquired IOSA of HFCS-55 (Panel A). The ANOVA revealed significant main effects of Session [F(14,266) = 5.7, p < 0.0001] and of Concentration [F(2,19) = 7.4, p < 0.05] that were primarily caused by a difference between 25% and 8% (p = 0.044), and 25% and 50% (p = 0.003). Although lever pressing for 8% was higher than for 50%, the variability in the 8% group appeared too large to reach statistical significance. A separate ANOVA on infusions following the switch likewise revealed
significant main effects of Session \([F(14, 266) = 2.4, p < 0.01]\) and of Concentration \([F(2,19) = 14.8, p < 0.001]\). Again, responding for 8% and for 50% did not differ statistically; however, multiple comparisons revealed that responding for 8% decreased across sessions, while responding for 50% remained stable. Also notable, as soon as 50% was substituted for 8%, variability in responding increased reflecting similar differences in the stability of operant SA maintained by these concentrations of HFCS-55 prior to the switch. Responding on the inactive lever was low across concentrations and did not differ significantly between groups prior to or following the concentration switch.

On PR Tests I and II, the breakpoints achieved responding for 8%, 25% or 50% HFCS-55 are depicted in Figure 2. The ANOVA revealed a significant Test by Concentration interaction \([F(2,19) = 3.5, p < 0.05]\) and significant main effect of Test \([F(1,19) = 27.7, p < 0.001]\). Concentration dependent effects were not observed on PR Test I. However, following 15-days of lever pressing on a FR1 schedule of reinforcement, the breakpoints achieved when responding for 25% and 50%, but not 8% HFCS-55 on PR Test II were significantly higher than those observed on Test I. Within PR Test II, breakpoints achieved when responding for the two highest concentrations were significantly higher than those observed in animals’ lever pressing for 8% HFCS-55. Finally, breakpoint did not differ on PR Test II between groups responding for 25% and 50% HFCS-55.

Finally, the effect of energy status (i.e. food-restricted versus ad libitum fed) on lever pressing for infusions of 8%, 25% and 50% HFCS-55 are represented in Table 1. The ANOVA revealed a significant main effects of Energy Status \([F(1, 19) = 20.02, p < 0.001]\) and of Concentration \([F(2, 19) = 5.8, p < 0.01]\), indicating that lever pressing for HFCS-55 on the FR1 schedule of reinforcement was significantly reduced (but not abolished) by ad libitum feeding.
Experiment 2

Figure 3 Panel A represents operant responding for 25% HFCS-55 on the active and inactive levers. The ANOVA assessing the 21-sessions of IOSA revealed a significant Session by Lever interaction \[F(20, 420) = 30.35, p < 0.001\] and significant main effects of Session \[F(20, 420) = 50.24, p < 0.001\] and of Lever \[F(1, 420) = 408.49, p < 0.001\]. Multiple comparisons indicated that within session-1, rats developed a significant bias of responding on the active lever that persisted across 3-weeks of testing. By session-4, animals were responding significantly higher on the active lever relative to sessions-1 through -3. Subsequently, active lever pressing escalated across SA sessions and multiple comparisons indicated that by session-9, responding on the active lever remained stable.

The patterns of active lever responding within three IOSA sessions (i.e., 1, 5 and 21) are depicted over time in Figure 4 (i.e. 10-minute bins across 180-minutes). The ANOVA revealed a significant Session by Time interaction \[F(34, 714) = 8.93, p < 0.001\] as well as significant main effects of Session \[F(2, 42) = 11.70, p < 0.001\] and of Time \[F(17, 714) = 4.66, p < 0.001\]. As IOSA progressed a change in the pattern of 25% HFCS-55 intake overtime was observed. That is, lever pressing for infusions of 25% HFCS-55 was significantly higher during the initial 90-minutes of the IOSA sessions and this peak in operant responding gradually escalated across test sessions (i.e. increased from sessions 1 through 21).

Figure 3 Panel B depicts the results of substituting 0.1% w/v saccharin (S) on session-22 and 25% HFCS-55 on session-23. The ANOVA revealed a significant Test by Lever interaction \[F(1, 13) = 93.41, p < 0.001\] as well as significant main effects of Test \[F(1, 13) = 79.33, p < 0.001\] and of Lever \[F(1, 13) = 134.42, p < 0.001\]. Compared to lever pressing for IO infusions of 0.1% w/v saccharin, rats responded significantly more on the active level for 25% HFCS-55
and multiple comparisons revealed that despite this difference, 0.1% w/v saccharin still elicited greater responding on the active than inactive lever.

The SA behaviour displayed in Figure 5 was collected from animals self-administering 25% HFCS-55 under identical experimental parameters for IOSA but conditions of either food-restriction (n = 22, also depicted in Figure 3 Panel A) or ad libitum feeding (n = 9). Operant responding for IO infusions of 25% HFCS-55 in ad libitum fed rats was assessed by a separate ANOVA and revealed a significant main effect of Session \( F(14, 112) = 5.2, p < 0.001 \). Like food-restricted animals, ad libitum fed rats acquired and maintained stable operant SA characterized by an increase in responding across initial sessions that was significantly higher (relative to sessions-1 and -2) by session-13.

**Experiment 3**

Figure 6 Panel A represents IOSA of 0.1% w/v saccharin as well as changes in SA behaviour in response to the substitution of different concentrations of saccharin and 25% HFCS-55 (Panel B; 0.01%, 0.1%, 1%, 10% w/v saccharin). The ANOVA revealed only a significant main effect of Lever \( F(1, 20) = 13.71, p < 0.01 \), suggesting that rats displayed an overall bias of responding on the active lever but no change in responding developed as IOSA of 0.1% w/v saccharin progressed from sessions-1 through -16. When different concentrations of saccharin and 25% HFCS-55 were tested, the ANOVA revealed a significant Substitution by Lever interaction \( F(4, 80) = 30.77, p < 0.001 \) as well as significant main effects of Substitution \( F(1, 80) = 47.47, p < 0.001 \) and of Lever \( F(4, 80) = 35.02, p < 0.001 \). Active lever responding was significantly higher for 25% HFCS-55 over any concentration of saccharin tested. This effect was more clearly observed in Figure 7 displaying the results of lever pressing for saccharin or HFCS-55 within these same test sessions over time (i.e. 10-minute bins across 180-minutes).
The ANOVA revealed a significant Substitution by Time interaction \( F(68, 1428) = 2.84, p < 0.001 \) and significant main effects of Substitution \( F(4, 84) = 36.08, p < 0.001 \) and of Time \( F(17, 1428) = 7.06, p < 0.001 \). Across the 180-minute session, active lever responding was significantly higher for 25% HFCS-55 compared to any concentration of saccharin tested and differences in responding over time between saccharin concentrations was not observed.

Finally, the ANOVA on frequency of tongue protrusions measured during TR tests following IO infusions of 8%, 25% and 50% HFCS-55 (Figure 8 Panel A) revealed a significant main effect of Concentration \( F(2, 20) = 4.5, p < 0.05 \). Tongue protrusions were significantly higher in response to 50% compared to the lower (25% and 8% HFCS-55) concentrations tested. However, observed frequency of tongue protrusions following IO infusions of 25% HFCS-55 and 0.1% w/v saccharin did not differ statistically (Figure 8 Panel B).

**Discussion**

Experiments were designed to assess the hypothesis that HFCS-55 may be addictive using IOSA procedures which combine methods traditionally employed to study behaviour motivated by drugs of abuse (operant IV SA; Carter & Griffiths, 2009) with those used to assess the palatability of tastants (TR; Grill & Norgren, 1978). To this end, rats were implanted with an IO cannula that allowed for the delivery of test solutions directly into their mouth (Panksepp & Trowill, 1967a). Following recovery from surgery, animals were tested in operant SA chambers whereby they pressed a lever to receive IO infusions of test solutions. The utility of this procedure was assessed through a series of experiments designed to characterize the reinforcing effects and possible abuse liability associated with operant IOSA of HFCS-55 in rats.

Consistent with reports that lever pressing for intraoral self-injection of chocolate milk in rats supports acquisition, extinction, and spontaneous recovery of operant responding (Panksepp
& Trowill, 1967a, 1967b), the results of Experiment 1 suggest that the IOSA procedures described here can be employed to study the acquisition and maintenance of operant SA of sweet solutions. Moreover, operant responding for different concentrations of HFCS-55 on FR1 and PR ratio schedules of reinforcement is similar to the dose-response relationships observed when rats self-administer drugs of abuse (Carroll & Lac, 1997; Deroche-Gamonet et al., 2004) and sucrose (Sclafani & Ackroff, 2003).

Experiment 1 revealed that HFCS-55 reinforced operant IOSA behaviour in a concentration-response dependent manner. That is, on a FR1 schedule (i.e., 1 press = 1 infusion), animals self-administer fewer infusions of HFCS-55 when the concentration is higher (see Figure 1 to compare 25% and 50% HFCS-55) and low concentrations of HFCS-55 (i.e. 8%) support only variable SA behaviour. On the PR Tests, whereby the required lever presses to receive subsequent infusions of HFCS-55 increased exponentially, animals displayed greater motivation for higher concentrations of HFCS-55 as indexed by higher breakpoints achieved when responding for infusions of 25% or 50% HFCS-55 (see Figure 2 PR Test II). Concentration-dependent increases in breakpoint are similarly observed in rats operant responding for sucrose (Reilly, 1999; Sclafani & Ackroff, 2003). Moreover, feeding animals ad libitum chow prior to IOSA reduced, but did not abolish lever pressing for IO infusions of HFCS-55 or alter the concentration-dependent differences described above.

Not surprisingly, a switch from 50% to 8% resulted in a reduction of HFCS-55 intake and increased variability in responding, while a switch from 8% to 50% led to a stabilization of responding as evidenced by reduced variability. Perhaps unexpectedly though, a decrease in lever pressing was also observed in this group (see Figure 1 Panels A and B for comparison). The observed adjustments in operant responding may have reflected the change in caloric value
of HFCS-55 infused; in other words, in response to a change in the perceived magnitude of the reinforcer (Flaherty, 1982). This is likely, as the shift from 50% to 8% engendered changes in operant responding for HFCS-55 consistent with negative contrast effects (Crespi, 1942) that are typically observed following a switch from repeated access to preferred reinforcers (Flaherty & Rowan, 1986; Flaherty, Turovsky, Krauss, Turovsky, & Krauss, 1994; Lombardi & Flaherty, 1978) or palatable cafeteria diets (Rogers, 1985) to less calorically dense or palatable options. It may also reflect a change in the perceived palatability as hedonic responses were higher in response to IO infusions of 50% than 8% HFCS-55 (see Figure 7 Panel A). However, in tests of anticipatory contrast, it has been found that consummatory behaviour is sensitive to not only changes in the perceived caloric and or hedonic value of the solutions but also to its satiating post-ingestional effects (Moss, Clarke, & Kehoe, 2002). In view of that, when switching from 8% to 50% HFCS-55, the satiating effects that a higher caloric infusion had on lever pressing for HFCS-55 may have influenced the ability to measure positive contrast effects (Moss et al., 2002). Rather, it may have been reflected by increased stability and less variability in lever pressing.

In line with the hypothesis that sugar may be addictive (Avena et al., 2008a), the ability of HFCS-55 to reinforce operant SA suggests it may possess abuse liability similar to that of sucrose (Avena et al., 2006; Figlewicz et al., 2011; Sclafani & Ackroff, 2003). Hence, Experiment 2 explored possible changes in patterns of operant responding for 25% HFCS-55 over repeated SA sessions that may reflect the development of addictive-like behaviour. It was found that SA of 25% HFCS-55 peaked at the start of IOSA sessions (see Figure 3), remained elevated across the initial 90-minutes (see Figure 4), and this “loading” increased in magnitude over the three-weeks of training. These effects are reminiscent of escalation of drug-taking and -
loading (Ahmed, 2011; Edwards & Koob, 2013; Mandt, Copenhagen, Zahniser, & Allen, 2015) observed when rats have repeated access to drugs of abuse (Ahmed & Koob, 1998; Cummins & Leri, 2008) or bingeing on sucrose (Avena et al., 2009). This behaviour was likely dependent on having experience self-administering HFCS-55 as animals in Experiment 3 that received only one session of HFCS-55 (following 16-consecutive IOSA sessions self-administering 0.1% w/v saccharin) displayed a pattern and level of HFCS-55 intake that was similar to that displayed on day-5 in rats tested in Experiment 2. While not assessed by the current experiments, it is possible that repeated access to HFCS-55 may engender similar neuroadaptations that facilitate the emergence of escalation and binge-like behaviour towards drugs of abuse and sugar in rats (Koob & Volkow, 2010; Mantsch, Yuferov, Mathieu-Kia, Ho, & Kreek, 2004; Nathan & Bullmore, 2009; Rada, Avena, & Hoebel, 2005).

Similar to the results of Experiment 1, IOSA was reduced by ad libitum feeding (by ~ 50%), but did not abolish lever pressing for IO infusions of 25% HFCS-55. In fact, comparable patterns of acquisition and maintenance of operant SA were emitted by food-restricted and ad libitum fed rats (see Figure 5). Although food-restriction maintains a higher level of operant responding, it is not necessary to facilitate the acquisition of IOSA. As the interpretation of SA behaviour, especially on schedules of reinforcement purported to measure motivation (i.e. PR schedules), may be limited by the use of food-restriction (Corwin, Avena, & Boggiano, 2011; Scheggi, Secci, Marchese, De Montis, & Gambarana, 2013), it may be advantageous to employ IOSA in its absence.

HFCS-55 likely reinforces operant behaviour based on its post-ingestional consequences, that is, its caloric content and not just sweet taste. In fact, a wide range of sweet but non-caloric concentrations of saccharin tested in Experiments 2 and 3 could not maintain IOSA at the same
level of 25% HFCS-55. Additionally, lever pressing decreased when 0.1% w/v saccharin was substituted for 25% HFCS-55 in Experiment 2 and conversely, increased when 25% HFCS-55 was substituted for saccharin in Experiment 3 (see Figures 3 and 6 for comparison). Minimal operant responding for saccharin in IOSA or when substituted for HFCS-55 cannot be fully explained by differences in the sugars palatability (Dess, 1993) because hedonic orofacial reactions to 25% HFCS and 0.1% w/v saccharin were not statistically different (see Figure 8 Panel B). It is feasible then, that the lack of post-ingestional consequences associated with saccharin (Miller & Frattali, 1989) may underlie differences in reinforcement of operant behaviour in IOSA consistent with the observation that saccharin, even if sweet, has weak reinforcing properties in rats (Beeler et al., 2012; Messier & White, 1984; Scheggi et al., 2013).

Metabolic disturbance and weight-gain are associated with voluntary drinking of HFCS-55 solutions in rats (Bocarsly et al., 2010; Sadowska & Bruszkowska, 2017). Group differences in weight-gain or chow intake were not observed in response to IOSA of 8%, 25%, or 50% HFCS-55 (Experiment 1). This was surprising, as 8% HFCS-55 induces significant weight-gain over a length of comparable time (i.e. 3 weeks; Bocarsly et al., 2010), and in those experiments, animals consumed approximately 21-kcal from 8% HFCS-55 over 12-hours of access which is likewise comparable to the average consumption of 18-kcal of 8% HFCS-55 from IO infusions obtained within 3-hours of IOSA. The brand of HFCS-55 employed between studies was consistent and so it is possible that, the cycle of food-restriction employed in Experiment 1 and or differences in the animals consumption of laboratory chow between studies (not reported for comparison; Bocarsly et al., 2010) may have contributed to the lack of weight-gain observed.

To the authors’ knowledge, the results of the experiments reported here provide the first evidence for the abuse liability associated with consumption of HFCS-55 as measured by
methods and criteria traditionally employed to assess the addictive properties of drugs of abuse. Similar to other sugars with purported abuse potential (Avena et al., 2008a; Bobadilla et al., 2017; Spangler et al., 2004) HFCS-55 is reinforcing (Ator & Griffiths, 2003; Carter & Griffiths, 2009). It supports robust operant SA for a range of concentrations of HFCS-55 over extended periods of time, that is likely maintained by the caloric value of HFCS-55, and not abolished by possible satiety prior to testing. Notably, following repeated access to self-administer HFCS-55, escalation of intake was observed and rats developed a tendency to “binge” on HFCS-55 at the start of IOSA sessions.

Yet, it is important to highlight that these findings address only a subset of criteria (Ator & Griffiths, 2003) necessary to label a substance as addictive. Therefore, based on these data it would be premature to label HFCS-55 as such. It is imperative that future studies assess additional criteria including whether following IOSA of HFCS-55 animals experience withdrawal (Avena, Bocarsly, et al., 2008), develop insensitivity to punishment (Vanderschuren & Everitt, 2004), resistance to extinction or vulnerability to relapse (Deroche-Gamonet et al., 2004), and importantly, whether the development of neuroadaptations associated with these addictive phenotypes emerge.

The results of the experiments performed here also suggest that IOSA procedures can be successfully employed to study the reinforcing properties of tastants in rats. IOSA may be a useful method of operant SA, that like other similar methods (Panksepp & Trowill, 1967b), accounts for limitations (Panksepp & Trowill, 1967a) that may allow for testing of more diverse parameters of a substances abuse liability. It is however necessary, to take into consideration the following procedural points when employing IOSA that may limit the interpretation of data. When the animal is placed into the operant chamber and their IO cannula is attached to the
Tygon tubing, there is the possibility of disconnection during the session. To prevent and correct this issue, it is essential to frequently observe the animals for leaks (i.e., check for fluid build-up that is easily apparent on the head cap). In addition, approximately 50-70 mm of the IO cannula exits from the back of the animals, and consequently, the IO cannula can be chewed when the rats are in their home cage. To correct this issue, a connector can be constructed from a 20-gauge needle which replaces the missing portion of the IO cannula. In extreme cases, the entire cannula can be replaced by repeating the surgery.

Taken together, important questions not yet fully understood regarding the behavioural and neurobiological basis of FA may be addressed using the novel IOSA approach (Pi-Sunyer et al., 2010; Westwater et al., 2016; Ziauddeen & Fletcher, 2013). First, it is clear that a contributing factor to obesity is excessive consumption of processed foods (Lindberg et al., 2011) refined by high concentrations of additives including sugar (Avena et al., 2008a; Bray et al., 2004; Schulte et al., 2015). There is much controversy over differences in the ratio of monosaccharide’s present in sugars such as sucrose and HFCS-55 that may affect their reinforcing efficacy in a way that renders them more or less likely to promote excessive consumption (Bray, 2013; Rippe, 2014; White, 2013; White, Foreyt, Melanson, & Angelopoulos, 2010) and this can be systematically studied using IOSA. Secondly, IOSA can be maintained for long periods of time. This would allow for the investigation of individual differences (Deroche-Gamonet et al., 2004; Flagel, Akil, & Robinson, 2009) and neurobiological correlates (Avena, Gold, Kroll, & Gold, 2012; Kaye et al., 2013) of voluntary intake of purported addictive foods and its contribution to the development of behaviours associated with FA or diet-induced obesity. Third, IV and IOSA studies can be performed in parallel to identify shared neurobiological factors that are involved in the regulation of intake and relapse to compulsive
reward-seeking (Bobadilla et al., 2017; Everitt et al., 2008; Nieh et al., 2015). Importantly, IOSA may also be employed to test the efficacy of pharmacological therapies to reduce compulsive food-taking and -seeking behaviour that characterizes and challenges treatment of FA and some forms of eating disorders and obesity (Maksimov et al., 2015; Yeomans & Gray, 1997).

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Author contributions

AML and FL were responsible for study concept and design. AML, CL, JF, and US contributed to the acquisition of data. LP assisted with analysis and interpretation of findings. AML and FL drafted the manuscript. All authors critically reviewed content and approved final version for publication.
Table 1: Mean (sem) active lever responses made by rats responding for 8% (n = 6), 25% (n = 8), or 50% (n = 8) HFCS-55 following 18-hours of food-restriction (food-restricted) and again following 24-hours of ad libitum access to laboratory chow (ad libitum fed). Animals responded significantly less (p = 0.001) for IO infusions of HFCS-55 following ad libitum access to chow.
Figure 1: Mean (sem) infusions obtained on a fixed ratio 1 schedule of reinforcement made by rats responding for 8% (n = 6), 25% (n = 8), or 50% (n = 8) HFCS-55 for 15-consecutive IOSA sessions (one 3-hour session/day) prior to (Panel A) and following (Panel B) the switch in concentration (i.e., 8% to 50% open white symbols, and 50% to 8% solid black symbols).
Figure 2: Mean (sem) breakpoints achieved by rats responding on a progressive ratio schedule of reinforcement for 8%, 25% or 50% HFCS-55 prior to (PR Test 1) and following (PR Test II) the initial 15-sessions of IOSA on the fixed ratio schedule of reinforcement. The * indicates a significant difference between PR Tests I and II and the ** indicates a significant difference from 8% HFCS-55.
Figure 3: Panel A: Mean (sem) active and inactive lever responses made by rats (n = 22) responding on a fixed ratio 1 schedule of reinforcement for 25% HFCS. Panel B: Mean (sem) number of active and inactive lever responses made during the substitution tests with 0.1% w/v saccharin (S) and 25% HFCS-55. The * indicate a significant difference between levers, and the ** indicates a significant difference between active lever responses made for saccharin and HFCS-55.
Figure 4: Mean (sem) active lever responses on a fixed ratio 1 schedule of reinforcement made over time (10-minute bins across 180-minutes) by rats (n = 22) responding for 25% HFCS-55 on IOSA sessions 1, 5, and 21. The portion of the graph shaded gray indicates a significant difference between active lever responding between sessions 1, 5, and 21 observed across session time (0- through 90-minutes).
Figure 5: Comparison of mean (sem) active lever responses for infusions of 25% HFCS-55 on a fixed ratio 1 schedule of reinforcement made by food-restricted (n = 22) and ad libitum fed (n = 9) rats.
Figure 6: Panel A: Mean (sem) active and inactive lever responses on a fixed ratio 1 schedule of reinforcement made by rats (n = 22) responding for 0.1% w/v saccharin. Panel B: Mean (sem) number of active and inactive lever responses made during the substitution tests with 0.01%, 0.1%, 1%, 10% w/v saccharin (S) and 25% HFCS-55. The * indicates a significant difference between levers and the ** indicates a significant difference in active lever responses made for 25% HFCS-55 from both 0.1% and 1% w/v saccharin.
Figure 7: Mean (sem) active lever responses on a fixed ratio 1 schedule of reinforcement made over time (10-minute bins across 180-minutes) by rats (n = 22) responding during the substitution tests with 0.01%, 0.1%, 1%, 10% w/v saccharin (S) and 25% HFCS-55. The * indicates a significant difference between responding for 25% HFCS-55 and all concentrations of saccharin tested.
Figure 8: Panel A: Mean (sem) frequency of tongue protrusions observed in taste reactivity tests by groups of naïve rats in response to passive intraoral infusions of 8% (n = 8), 25% (n = 8) or 50% (n = 8) HFCS-55. The * indicates a significant difference between 50% HFCS-55 and the two lower concentrations. Panel B: Mean (sem) frequency of tongue protrusions made by groups of naïve rats in response to passive intraoral infusions of 0.1% w/v saccharin (n = 6) or 25% HFCS -55 (n = 5).
Fructose: glucose ratios - a study of sugar self-administration and associated neural and physiological responses in the rat

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Abstract

This study explored whether different ratios of fructose (F) and glucose (G) in sugar can engender significant differences in self-administration and associated neurobiological and physiological responses in male Sprague-Dawley rats. In Experiment 1, animals’ self-administered pellets containing 55% F + 45% G or 30% F + 70% G, and Fos immunoreactivity was assessed in hypothalamic regions regulating food intake and reward. In Experiment 2, rats self-administered solutions of 55% F + 42% G + 3% polycose (high fructose corn syrup formula 55; HFCS-55), 50% F + 50% G (sucrose) or saccharin, and mRNA for dopamine 2 (D2R) and mu-opioid (MOR) receptor genes were assessed in striatal regions involved in addictive behaviors. Finally, in Experiment 3, rats self-administered HFCS-55 and sucrose from bottles in home cages, and the composition of polyunsaturated fatty acids in the liver were quantified.

It was found that higher fructose ratios engendered lower self-administration, lower Fos expression in the lateral hypothalamus/arcuate nucleus, reduced D2R and increased MOR mRNA in the dorsal striatum and nucleus accumbens core, respectively, as well as elevated omega-6 polyunsaturated fatty acids in the liver. These data indicate that a higher ratio of fructose may enhance the reinforcing effects of sugar and possibly lead to neurobiological and physiological alterations associated with addictive and metabolic disorders.

Keywords: Fructose; Glucose; Self-Administration; Hypothalamus; Nucleus Accumbens; Dopamine 2 Receptor; Mu Opioid Receptor; Fatty Acid; Hepatic; Rat
Introduction

The World Health Organization estimated that between 1980 and 2008, the rate of obesity (BMI > 30 kg/m$^2$) doubled worldwide (World Health Organization, 2012). In Canada alone, the prevalence of obesity in adults has tripled between 1985 and 2011 (Twells, Gregory, Reddigan, & Midodzi, 2014). These trends are of serious concern, because obesity is causally linked to cardiovascular disease, type 2 diabetes, stroke, liver disease, and cancer (Manson, Skerrett, Greenland, & VanItallie, 2004; National Task Force on the Prevention and Treatment of Obesity, 2000; van Baak, 2013).

There are several factors that promote the development and maintenance of obesity, including interactions between genetic (Alfredo Martínez et al., 2007; Stice et al., 2008), metabolic (Narayanan et al., 2010), neurobiological (Volkow, Wang, Fowler, Tomasi, & Baler, 2012), psychosocial (Christakis & Fowler, 2007; Kirk et al., 2010), and economic (Fortuna, 2012) factors. Nevertheless, it is clear that one important component is nutrition, with particular reference to excessive consumption of foods containing high levels of sugars (Bocarsly et al., 2010; Ifland et al., 2009; Johnson et al., 2013) and fats (Bray & Popkin, 1998).

Within this context, there is the interesting issue of whether different sugars play a differential role in contributing to overeating and obesity. More specifically, it is proposed that fructose may be more “hazardous” than glucose because of its limited effects on cessation of food intake and on activation of brain regions that regulate this homeostatic response (Cha, Wolfgang, Tokutake, Chohnan, & Lane, 2008; Moran & McHugh, 1981; Page et al., 2013). These observations have led to the hypothesis that sweeteners, such as high fructose corn syrup formula 55 (HFCS-55), which contain a higher fructose to glucose ratio than sucrose, may be
“addictive” and, thus, critically involved in hedonic overeating leading to obesity (Bray et al., 2004; Ferder, Ferder, & Inserra, 2010; Khitan & Kim, 2013; Murray et al., 2016).

This hypothesis, however, has been debated, because it is unclear whether the behavioral and neural effects of pure fructose and pure glucose can accurately predict the effects of combinations of these same monosaccharaides at different ratios (White, 2008, 2013). More specifically, is it possible that a 55% fructose-42% glucose ratio (typical of HFCS-55) can engender bio-behavioral effects that are significantly different from those of a 50% fructose-50% glucose ratio (typical of sucrose)? On the one hand, it has been reported that HFCS-55 produces a smaller insulin response than sucrose in younger adults (Stanhope & Havel, 2008a) and that it has more pronounced effects on weight-gain and abdominal adiposity in rats compared to sucrose (Bocarsly et al., 2010). On the other hand, HFCS-55 and sucrose consumption do not appear to elicit differences in short-term measures of satiety (Akhavan & Anderson, 2007; Soenen & Westerterp-Plantenga, 2007) or metabolic responses (i.e. plasma levels of leptin and ghrelin; Melanson et al., 2007). Therefore, three complementary experiments were designed to address the question of whether differences in the ratio of fructose (F): glucose (G) in sugar impact reward-related behaviors as well as associated neural and physiological responses.

Material and Methods

Animals

Adult male Sprague-Dawley rats (Charles River, St-Constant, QC, Canada) weighing 225–250 g were used for all experiments. Rats were single-housed, maintained on a reverse light/dark cycle (7:00 a.m. lights off, 7:00 p.m. lights on) and given ad libitum access to rat chow and water in the home cage. Where indicated in the subsequent procedures, rats were food restricted for 18 h prior to each self-administration session and then fed chow ad libitum for three
hours immediately following the session. Behavioral testing occurred during the active (dark) phase. 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.

_Surgery_

**Intraoral Surgery**

Rats were surgically implanted with intraoral (IO) cannulas and head posts under general anesthesia with 5% isoflurane for induction and maintenance (for detailed descriptions of surgical procedures, please see: Levy et al., 2014). Following surgery, IO cannulas were flushed once per day for three days with a solution of chlorhexidine (Ayerset, Fort Dodge, IA, USA).

_Apparatus_

**Operant Self-Administration of Pellets**

Self-administration of pellets was conducted in an automated apparatus (Med Associates, St. Albans, VT, USA) comprised of 8-arms radiating from a central octagonal hub. The central hub was equipped with 8-automatically operated guillotine doors. A pellet receptacle was located at the end of each arm, and a photo beam situated inside detected nose pokes. A pellet dispenser was positioned behind each receptacle, located outside the arm (for a detailed description of the apparatus please see (Rkieh, Cloke, Gallagher, Winters, & Leri, 2014) and (Leri et al., 2013) for a schematic diagram).

**Operant Self-Administration of Solutions**

Twenty-six Plexiglas operant conditioning chambers (Model ENV-008CT, Med Associates, Lafayette, IN, USA), each enclosed in a larger sound-attenuating plywood cabinet (model ENV-018M, Med Associates), were employed. Each operant chamber had a house light
(28 W), a cue light (28 W) and three levers. The “active” lever was retractable, was positioned 8 cm above the floor and 10 cm from the “inactive” lever. A second “inactive” lever was positioned 8 cm above the floor, on the opposite side of the chamber. The active lever was connected to an infusion pump (Razel Scientific Instruments, Stamford, CT) positioned outside of the sound-insulating cabinet. The cue light was located 3 cm above this lever and served as a conditioned stimulus (CS) paired with the delivery of intraoral infusions. The inactive levers served to control for baseline, non-reinforced operant behavior: pressing on either had no consequences, but all presses were recorded.

*Home Cage Self-Administration*

To measure HFCS-55 and sucrose drinking in home cages, 100 mL no drip water bottles (Thermo Scientific, Waltham, MA, USA) with metal spouts (Ancare, Bellemore, NY, USA) secured within rubber stoppers (Fisher Scientific, Ottawa, ON, Canada) were placed in the home cage of rats alongside standard water bottles.

*Taste Reactivity*

The taste reactivity apparatus included a clear Plexiglas chamber (22.5 cm × 26.0 cm × 20.0 cm) with an opaque lid placed over a transparent glass surface. A digital video camera (Sony, DCR-HC48) was pointed at a mirror placed at a 45° angle under the glass surface to visualize the ventral surface of the rat and record orofacial reactions (Limebeer, Litt, & Parker, 2009).

*Food and Sugars*

For all experiments, rats were fed a standard laboratory chow (18% protein and 4% fat; Teklad Global Diets, Harlan Laboratories). In Experiment 1, the solid pellets were purchased from Bio-Serv (45 mg Dustless Precision Pellets, Frenchtown, NJ, USA). Pellets contained:
dextrose, fructose, cellulose and magnesium stearate. The nutritional profile of the pellets was: 0% fat, 0% protein, 89.5% carbohydrate. Pellets differed in percent of monosaccharaides used: high ratio fructose = 55% F-45% G; low ratio fructose = 30% F-70% G. At these ratios, the caloric value of the pellets was nearly identical: high ratio fructose = 3.66 kcal/gram (0.165 kcal/pellet); low ratio fructose = 3.58 kcal/gram (0.161 kcal/pellet).

For Experiments 2 and 3, solutions were made by diluting HFCS-55 (Natures Flavors, CA, USA), sucrose (RedPath, ON, Canada) or saccharin (sodium salt hydrate 99+ %, ACROS Organics, NJ, USA) in reverse osmosis water. These solutions were stored at room temperature for the duration of the experiments. The nutritional profiles were: HFCS-55 (serving of 4 g) = 0 g (0%) fat, 0 g (0%) protein and 3.08 g (77%) carbohydrate; sucrose (serving of 4 g) = 0 g (0%) fat, 0 g (0%) protein and 4 g (100 %) carbohydrate. In Experiments 2 and 3, a solution of 25% HFCS-55 was selected because this concentration has been found to maintain robust operant IOSA (Levy et al., 2014). The 20% sucrose solution was selected to match the caloric value of the 25% HFCS-55. More specifically, both 25% HFCS-55 and 20% sucrose contained 0.75 kcal/mL. The non-caloric sweetener saccharin (Miller & Frattali, 1989) was initially tested at 0.1%, because at this concentration, it elicits hedonic orofacial reactions in taste reactivity (Neath et al., 2010) and because rats voluntarily drink it from a bottle (Carroll, Morgan, Anker, Perry, & Dess, 2008).

Behavioral Testing

Operant Self-Administration of Pellets

In the three days prior to beginning self-administration in Experiment 1, 36 rats (not food-restricted) were handled for 5 min/day and were randomly assigned to 55% F + 45% G or 30% F + 70% G pellets. During these three days, the groups (n = 18) received 20 pellets/day in
their home cages. Each self-administration session began by placing the rat in the central hub of the apparatus for a 1-min adaptation period. Following adaptation, all guillotine doors were raised, and animals were tested for 10-min. During this period, rats obtained a pellet by entering any of the 8-arms and nose poking at the receptacle. Following this nose poke, the pellet dispenser became inactive; the animal was required to exit that arm, enter the central hub and then enter either the same or a different arm to obtain another pellet. In other words, animals were required to move from arm, to central hub, to arm to obtain successive rewards. At the end of each 10-min test, the receptacles were inspected, and the number of unconsumed sugar pellets was recorded. Testing was conducted once/day for 14-consecutive days, and rats were not food restricted for the duration of this experiment. Daily weight-gain and chow consumption was measured and recorded approximately 2-hr following each test session.

Ninety-minutes after the conclusion of the last self-administration session, brains were collected by euthanizing animals with an overdose of pentobarbital (Somnotol: 54.7 mg/kg, McGill University, Montreal, QC, Canada) and by intracardiac infusion with 0.9% saline and 4% paraformaldehyde. In order to interpret the Fos immunoreactivity data, a control group was also included (n = 11). These animals were transported and kept in the testing room according to the time line described above, but were not exposed to the maze or to the sugar pellets. Their brains were collected 90-min following the 14th day of exposure to the testing room. Finally, a separate group of rats (n = 8; not food-restricted) were tested for taste reactivity to measure hedonic orofacial reactions in response to infusions of solutions sweetened with the pellets tested above.

Operant Intraoral Self-Administration

In the five days prior to beginning self-administration in Experiment 2, rats (n = 32; food-restricted) were handled for 5-min/day and were randomly assigned to either 25% HFCS-55 or
20% sucrose solutions. Prior to the beginning of self-administration, rats received a single taste reactivity test to measure hedonic orofacial reactions in response to the sugar solutions. Isocaloric solutions of HFCS-55 or sucrose were self-administered daily during sessions lasting 3 hr each. Sessions began with the activation of a house light, entry of the active lever and illumination of the conditioned stimulus (CS) light above it for 30-sec. Subsequently, presses on the active lever resulted in a 2.5-sec intraoral infusion of 80 µL of HFCS-55 or sucrose (caloric content = 0.08 kcal/infusion). A time-out period of 27.5-sec was imposed to allow sufficient time for ingestion of the intraoral infusion. During this period, the active lever was retracted, and the CS light was activated. No limit was imposed on the number of infusions obtainable within each session.

Animals were trained to lever press on both continuous (i.e. fixed ratio 1 (FR1)) and progressive ratio (PR) schedules of reinforcement. On the PR schedule, the number of responses required to obtain each successive infusion increased exponentially within the session according to the formula: 
$$5^{e_{\text{injection number} \times 0.2}}$$ (Richardson & Roberts, 1996). The breakpoint was indexed by the last infusion received prior to cessation of responding on the active lever for at least 1-hr (Richardson & Roberts, 1996). Rats self-administered 25% HFCS-55 or 20% sucrose for a total of 47 sessions, and two PR tests were performed on Days 11 and 21 of testing.

On Day 48, animals were euthanized, and brains were collected for mRNA quantification. To interpret the results of the mRNA analyses, an additional group of rats (n = 22) was trained to lever press for intraoral infusions of 0.1% w/v saccharin, a non-caloric, but sweet solution, on an FR1 schedule of reinforcement under identical conditions. These animals were tested for 17 days, but because self-administration was minimal, alternative concentrations
were tested over 3 additional days. The number of infusions obtained did not increase; therefore, behavioral testing was terminated after 20 days of testing, and brains were collected on Day 21.

**Home Cage Self-Administration**

In the 3-days prior to beginning home cage self-administration in Experiment 3, rats \( n = 16 \); *ad libitum* fed) were handled for 5-min/day and were randomly assigned to drink either 25% HFCS-55 or 20% sucrose. These animals were provided with *ad libitum* access to rat chow, water and either HFCS-55 or sucrose solutions in their home cage for 45 consecutive days. At the same time every day, body weight, chow, water and sugar consumption were measured and recorded. On Day 46, animals were euthanized, adipose and liver tissues weighed and hepatic fatty acid composition measured by gas chromatography.

**Taste Reactivity**

Taste reactivity (TR) testing (Grill & Norgren, 1978; Limebeer et al., 2009) occurred over 2-days prior to beginning self-administration in Experiments 1 and 2. In Experiment 1, the pellets were dissolved in water (0.5 mL/pellet) using a magnetic stir bar and hot plate. Rats were individually placed in the test chambers for a 3-min habituation period. During this period, the intraoral cannula was attached to an infusion pump, and water was infused at a rate of 0.5 mL/min. Twenty-four hours later, rats were returned to the chamber, attached to the infusion pump and infused with the test solution at a rate of 0.5 mL/min. Orofacial reactions were recorded for the duration of the 3-min test, and behavior was subsequently scored using The Observer (Noldus Information Technology, Sterling, VA, USA).

The frequency of forward (extensions of the tongue out the front of the mouth) and lateral (extensions of the tongue out the sides of the mouth, sweeping along the lips) tongue protrusions were scored. Because the results of these measures were comparable, frequencies of forward and
lateral tongue protrusions were combined to obtain a single index of palatability (Neath et al., 2010). In Experiment 2, identical procedures were employed to test the different sugar solutions self-administered (i.e., 25% HFCS-55, 20% sucrose and 0.1% w/v saccharin).

Fos Immunoreactivity

In Experiment 1, brains were processed for Fos immunoreactivity (please see (Honsberger & Leri, 2008) for detailed description of procedures). For each image captured (Leica software V3.6, Leica Microsystems Inc., Concord, ON, Canada), Fos immunoreactive cells were counted, divided by the area, and a mean density value ((\text{count/um}^2) \times 10^6) per animal was calculated from 4–6 images from multiple bilateral slices using ImageJ (National Institutes of Health, Bethesda, MD, USA). Fos density was quantified by researchers blind to treatment groups.

The Paxinos and Watson (2004) brain atlas was used to identify locations of interest in reference to bregma, as well as changes in shape or size of areas analyzed for Fos immunoreactivity. Specifically, to determine where slices were in reference to bregma (ranging between −2.52–−3.12) for the hypothalamus, the size and shape of the hippocampus and lateral ventral were used, as well as the size and location of the optic tract (Watson & Paxinos, 2004). Once bregma was determined, the periforinal areas of the lateral hypothalamus were localized with reference to the fornix, the dorsomedial hypothalamus was located around the crest of the third ventricle and the arcuate nucleus was drawn around its base.

Solution Hybridization RNase Protection-TCA Precipitation

Following collection, brains were stored at −80 °C. The caudate-putamen and core/shell subdivisions of the nucleus accumbens were dissected on ice for subsequent mRNA quantification. For detailed descriptions of the solution hybridization RNase protection-TCA
precipitation protocol, please see (Zhou et al., 2006). The mRNA levels for D2R and MOR were quantified (in attomoles/µg) in specific regions on the basis of known levels of constitutive expression, as well as previous results showing gene expression changes as a result of exposure to cocaine (Leri et al., 2009).

Gas Chromatography

Following collection, livers were stored at −80 °C. The frozen tissue (0.05 g of tissue from each lobe of the liver) was thawed on ice prior to fatty acid extraction (for detailed descriptions of the procedures, please see (Folch, Lees, & Stanley, Sloane, 1957). Fatty acid peaks were identified by comparison to retention times of fatty acid methyl ester standards, and fatty acid levels were determined quantitatively from the internal standard and expressed as µg/liver.

Statistical Analyses

Data reported in this manuscript were analyzed by mixed analysis of variance (ANOVA) and t-tests. Significant interactions and main effects were analyzed by multiple comparisons using the Student–Newman–Keuls method (α = 0.05). The exact values of non-significant results were not reported, and all analyses were performed using SigmaStat (v. 3.5 for Windows, SPSS Inc., NY, USA).

Results

Experiment 1

Taste Reactivity

The mean frequency of tongue protrusions in response to infusions of solutions made by dissolving 55% F + 45% G or 30% F + 70% G pellets did not differ significantly (mean ± SEM = 72.6 ± 10.1 and 70.8 ± 14.1, respectively).
Operant Self-Administration of Pellets

The mean total number of nose pokes emitted during 14 self-administration sessions was not statistically different between groups (Figure 1A). Although rats that self-administered 55% F + 45% G pellets nose poked less over the testing period (Figure 1B), the ANOVA revealed only a significant main effect of Session \[F(13, 442) = 10.97, p < 0.001\]. Moreover, during the 14 days of testing, no significant differences in daily consumption of chow or weight-gain were observed between groups (data not depicted).

Representative microphotographs of hypothalamic regions selected for immunohistochemistry are included in Figure 2A–F. Mean Fos density in control, 55% F + 45% G and 30% F + 70% G groups is represented in Figure 3A–C. In the perifornical area of the lateral hypothalamus (Figure 3A), the ANOVA was significant \[F(2, 33) = 8.08, p < 0.001\], and multiple comparisons indicated that Fos density was significantly lower in rats that self-administered 55% F + 45% G. Within the arcuate nucleus (Figure 3B), Fos density was not significantly different between groups. However, *a priori* planned comparisons revealed that, compared to the control group, Fos density was significantly lower in rats that self-administered 55% F + 45% G \[t(19) = 2.2, p < 0.05\]. Finally, in the dorsomedial hypothalamus (Figure 3C), no significant differences in Fos density were observed.

Experiment 2

Taste Reactivity

The mean frequency of tongue protrusions measured in response to infusions of 25% HFCS-55, 20% sucrose or 0.1% w/v saccharin did not differ significantly (mean ± SEM = 130.6 ± 10.1, 121.8 ± 14.1, 118.8 ± 14.1, respectively).

Operant Intraoral Self-Administration
The mean number of infusions obtained by lever pressing on an FR1 schedule for 25% HFCS-55 or 20% sucrose across the self-administration period is represented in Figure 4A. The ANOVA revealed significant main effects of Group \( F(1, 240) = 16.78, p < 0.001 \) and of Session \( F(8, 240) = 46.5, p < 0.001 \). Multiple comparisons indicated that self-administration of HFCS-55 was significantly lower than sucrose across IOSA sessions 5 through 40. To understand this difference, the patterns of lever pressing for HFCS-55 and sucrose were compared within a IOSA session in the middle of the experiment (i.e. Session 20; Figure 4B). The ANOVA revealed a significant Group by Session interaction \( F(17, 510) = 3.8, p < 0.001 \), as well as significant main effects of Group \( F(1, 510) = 12.9, p < 0.001 \) and of Session \( F(17, 510) = 14.3, p < 0.001 \). Multiple comparisons indicated that IOSA of HFCS-55 and sucrose differed primarily in the latter half of each session. When these same animals were tested on the PR schedule, the ANOVA revealed no significant group differences in breakpoints achieved on the first (mean ± SEM; HFCS-55 = 10.9 ± 0.4; sucrose = 10.3 ± 0.6) or the second (HFCS-55 = 9.0 ± 0.5; sucrose = 10.4 ± 0.6) PR tests. Finally, over the duration of the self-administration period, no significant group differences emerged in the daily consumption of chow, weight gain, or total caloric intake (chow and sugar combined; data not depicted).

In a separate group of rats, self-administration of 0.1% w/v saccharin was minimal (Table 1), and no significant differences in number of infusions across sessions were observed. Likewise, no significant differences in patterns of self-administration over time (10-min bins) were noted (data not depicted). Finally, self-administration did not change when the concentration of saccharin was varied

Figure 5 represents D2R mRNA expression in the dorsal striatum (Figure 5A) and MOR mRNA expression in the nucleus accumbens core (Figure 5B) and shell (Figure 5C) following
self-administration of 25% HFCS-55, 20% sucrose, and 0.1% w/v saccharin. In the dorsal striatum, the ANOVA on D2R mRNA expression was significant \( [F(2,22) = 13.3, p < 0.001] \), and multiple comparisons indicated that expression was significantly lower in animals that self-administered HFCS-55, in comparison to both sucrose and saccharin. In the nucleus accumbens core, the ANOVA was also significant \( [F(2,20) = 13.0, p < 0.001] \), and multiple comparisons indicated that MOR mRNA was significantly higher in rats that self-administered HFCS-55, in comparison to both sucrose and saccharin. The ANOVA on MOR mRNA expression in the nucleus accumbens shell was significant \( [F(2,20) = 5.5, p < 0.01] \), and multiple comparisons revealed that expression differed between the HFCS-55 and the sucrose groups only.

Finally, MOR mRNA expression was also quantified in the dorsal striatum, but no significant differences were found (mean ± SEM mRNA in attomole/µg: HFCS-55 = 0.17 ± 0.01; sucrose = 0.17 ± 0.01; saccharin = 0.16 ± 0.01).

Experiment 3

Home Cage Self-Administration

The mean volume of 25% HFCS-55 or 20% sucrose self-administered by drinking from a bottle in the home cage is represented in Figure 6A. The ANOVA revealed significant main effects of Group \( [F(1, 8) = 24.6, p < 0.001] \) and of Session \( [F(8, 112) = 13.7, p < 0.001] \). Similar to Experiments 1 and 2, self-administration of HFCS-55 was significantly lower than that of sucrose. Figure 6B represents total caloric intake over the entire self-administration period, which was significantly lower in the HFCS-55 group \( [t(14) = 3.1, p < 0.01] \). Additionally, Figure 6B represents the proportion of total caloric intake accounted for by chow or by sugar consumption, and the ANOVA revealed a significant Group by Food interaction \( [F(1, 14) = 13.8, p < 0.01] \), as well as significant main effects of Group \( [F(8, 14) = 9.5, p < 0.01] \) and of
food \[F(1, 14) = 29.9, p < 0.001\]. Multiple comparisons indicated that the overall group
difference in total caloric intake was the result of differences in sugar, but not chow
consumption. That is, the proportion of calories derived from sugar was significantly lower in
rats drinking HFCS-55, while no significant group differences in calories derived from the
consumption of chow were observed.

Figure 8A represents the mean total fatty acids measured in livers. Overall, the fatty acid
concentration was significantly higher in the livers of rats that self-administered HFCS-55 \[t(14)
= 2.4, p < 0.05\]. More specifically, when the PUFA \(n\)-6 and \(n\)-3 were measured (Figure 8B), the
ANOVA revealed a significant Group by PUFA interaction \[F(1, 14) = 6.5, p < 0.05\], as well as
significant main effects of Group \[F(1, 14) = 5.6, p < 0.05\] and of PUFA \[F(1, 14) = 228.4, p <
0.001\]. Multiple comparisons indicated that the level of hepatic \(n\)-6 was significantly higher in
rats that self-administered HFCS-55, while no group difference in the level of \(n\)-3 was observed.

**Discussion**

Three experiments were performed in rats to investigate whether sugars composed of
different ratios of fructose (F) :glucose (G) engender differences in self-administration, as well as
associated neurobiological and physiological responses. Across all experiments, it was found that
a higher ratio of fructose was associated with reduced sugar self-administration. This behavioral
difference could not be attributed to factors inherent to the procedures employed (i.e., length of
self-administration sessions, mode of delivery or quantity of sugar consumed), nor to the
nutritional status of the animals at the time of testing (i.e., food-restricted versus *ad libitum* fed).
Interestingly, exposure to the high fructose ratio sugar was also associated with lower Fos
expression in the perifornical area of the lateral hypothalamus and arcuate nucleus, reduced
expression of D2R mRNA in the dorsal striatum and elevated MOR mRNA in the core of the
nucleus accumbens. Similar neural alterations have been observed following exposure of drugs of abuse (Besson et al., 2013; Georges, Stinus, Bloch, & Le Moine, 1999; Leri et al., 2009). Finally, in these same animals, hepatic n-6 PUFAs were elevated, and this has been associated with chronic inflammatory states characteristic of metabolic diseases and obesity (Simopoulos, 2002; Simopoulos, 2013).

In Experiment 1, non food-restricted rats self-administered pellets containing 30% F + 70% G or 55% F + 45% G, and 90-min after the last self-administration session, their brains were processed for Fos immunoreactivity in hypothalamic regions involved in the regulation of food intake and reward. In self-administration, rats tended to emit fewer nose pokes for high ratio fructose pellets. Although not statistically different, this observation was in the same direction of the significant behavioral effects reported in Experiments 2 and 3, following HFCS-55 and sucrose self-administration. In the analysis of Fos immunoreactivity, compared to the control group, the intake of the high ratio fructose pellets was associated with a significant reduction in Fos density in the perifornical area of the lateral hypothalamus and arcuate nucleus (see Figure 3A, B). These hypothalamic regions contain cells that synthesize and release peptides regulating food reward and food intake, including neuropeptide Y (Brown, Coscina, & Fletcher, 2000), melanocortins (Pandit, la Fleur, & Adan, 2013), cocaine- and amphetamine-regulated transcript (Philpot & Smith, 2006), and orexins (Cason et al., 2010). While the phenotype of the Fos-expressing cells was not identified (Hoffman & Lyo, 2002), these data are in line with the observations that fructose is generally less effective than glucose in suppressing activity in the hypothalamus and other brain regions (Page et al., 2013). Interestingly, the taste reactivity test indicated that palatability or “liking” (Robinson & Berridge, 1993) of the pellets was not
different, suggesting that the difference in Fos activity was not related to differences in the taste of the pellets.

In Experiment 2, food-restricted rats self-administered isocaloric solutions of 25% HFCS-55 or 20% sucrose on FR1 and PR ratio schedules of reinforcement, and brains were processed for mRNA expression of genes associated with addictive behaviors. Self-administration of HFCS-55 was significantly lower than sucrose on the FR1 schedule (see Figure 4A). The pattern of self-administration behavior within Session 20 indicated that initial (90-min; Figure 4B) lever pressing was indistinguishable between groups. However, HFCS-55 self-administration declined, while sucrose self-administration remained elevated in the latter half of each session (180-min in total). In contrast, the breakpoint achieved (Richardson & Roberts, 1996) on the PR schedule did not differ between groups or across PR tests (Sessions 11 and 21). It is unlikely that the observed group difference on the FR1 schedule was due to differences in satiety levels. In fact, food restriction controlled the quantity of food consumed in the hours prior to self-administration. Likewise, following each session, no significant differences in daily chow consumption or growth emerged. The results of the TR test also indicated that the group difference was not due to differences in palatability, as orofacial reactions did not differ in response to solutions made with either 25% HFCS-55 or 20% sucrose.

Compared to HFCS-55 and sucrose, self-administration of the non-caloric 0.1% w/v saccharin solution (Miller & Frattali, 1989) did not support self-administration or engender differences in the pattern of self-administration within sessions. As a matter of fact, a wide range of saccharin concentrations (see Table 1) could not maintain self-administration at the same level of HFCS-55 or sucrose, suggesting that these sugars are reinforcing because of their caloric content. The saccharin findings were not particularly surprising in light of data suggesting that
this sweetener has weak reinforcing properties in rats (Messier & White, 1984) and weak effects on dopamine release in the nucleus accumbens (Blackburn et al., 1986; Weiss et al., 1993). Furthermore, the minimal responding observed during saccharin self-administration could not be attributed to differences in TR, as orofacial reactions in response to infusions of saccharin were statistically indistinguishable from those made in response to HFCS-55 and sucrose.

Therefore, given that the sugar solutions were isocaloric, perceived as equally palatable, and the nutritional status of animals at the time of testing was controlled, these data suggest that HFCS-55 may be a more potent reinforcer than sucrose. This is consistent with the observation that increasing the concentration of reinforcing stimuli, such as drugs of abuse (Carroll & Lac, 1997; Deroche-Gamonet et al., 2004), reduces self-administration on FR1 schedules. Although breakpoint achieved on PR tests is also employed as an index of the reinforcing efficacy of stimuli (Richardson & Roberts, 1996), this schedule may not have been sensitive enough to detect differences in the reinforcing properties of the sugars employed in the current experiment given the limited number of reinforcers obtained. This is a likely interpretation, as group differences were only revealed in the latter half of the self-administration sessions on FR1 schedules. Moreover, caloric content appears to play a principal role in determining the breakpoint achieved. That is, our laboratory has previously reported that as the caloric content and concentration of HFCS-55 increases, the breakpoint achieved on PR schedules also increases (Levy et al., 2014). Likewise, increasing the caloric content and concentration of sucrose engenders similar increases in breakpoints (Sclafani & Ackroff, 2003). Therefore, it is possible that in the current experiment, group differences in breakpoints achieved were not observed because isocaloric solutions of HFCS-55 and sucrose were employed.
The results of the mRNA analysis indicated that even though less HFCS-55 was self-administered compared to sucrose, alterations in the expression of reward-related genes in two regions of the brain implicated in addictive behaviors were specific to HFCS-55 intake. HFCS-55 self-administration reduced the expression of D2R mRNA in the dorsal striatum compared to both sucrose and saccharin controls (see Figure 5A). Sucrose self-administration also reduced the expression of D2R mRNA in the dorsal striatum, but this was only different from rats that self-administered saccharin. In the dorsal striatum, expression of D2R mRNA, as well as D2R availability is reduced by repeated consumption of cafeteria diets (Johnson & Kenny, 2010), sucrose (Spangler et al., 2004), and exposure to drugs of abuse (Besson et al., 2013; Georges et al., 1999; Leri et al., 2009). These neural adaptations are associated with deficits in reward sensitivity, as well as enhanced impulsive (Besson et al., 2013) and compulsive behaviors (Johnson & Kenny, 2010). Sucrose self-administration has been found to reduce the expression of D2R mRNA in the dorsal striatum, not only in the current experiment, but also other studies (Spangler et al., 2004). However, to our knowledge, this is the first report of similar effects following HFCS-55 self-administration and of significant differences between HFCS-55 and sucrose.

Elevated MOR mRNA in the nucleus accumbens core was also observed following HFCS-55, but not sucrose or saccharin self-administration. This region is critically involved in the attribution of incentive salience to stimuli during instrumental and Pavlovian learning (Bassareo, De Luca, & Di Chiara, 2007; Hall, Parkinson, Connor, Dickinson, & Everitt, 2001; Kelley, 1999), and similar neural adaptations are reported following repeated administration of drugs of abuse, such as cocaine (Leri et al., 2009; Leri, Zhou, Goddard, Cummins, & Kreek,
2006). However, it should be noted that the interpretation of these results is limited to protein biosynthesis and/or release, because the protein levels were not measured.

In Experiment 3, bottles of either 25% HFCS-55 or 20% sucrose were placed alongside water and chow in the home cages of rats, and following self-administration, adipose and liver tissue were weighed and the composition of fatty acids in the liver measured by gas chromatography. Once again, even if the solutions were isocaloric and animals were not food-restricted, it was found that HFCS-55 engendered lower self-administration than sucrose (see Figure 6A). Over the duration of the experiment, total caloric intake was lower in rats that self-administered HFCS-55 (see Figure 6B). This difference was attributable to group differences in sugar intake. That is, the proportion of calories derived from chow consumption did not differ between HFCS-55 and sucrose groups; however, the proportion of calories derived from sugar consumption was significantly lower in rats self-administering HFCS-55 (see Figure 6B). This caloric discrepancy was associated with differences in the accumulation of adipose tissue: epidydimal (but not inguinal) fat pads of rats self-administering sucrose were significantly larger (see Figure 7). Similar changes in epidydimal fat pads have been reported in rats maintained on high caloric cafeteria diets (López et al., 2003).

Interestingly, self-administration of HFCS-55 increased the level of hepatic n-6 fatty acids. The level of n-3 was not altered (Figure 8). n-3 (commonly derived from fish oils) is anti-inflammatory and protects against, as well as helps recover, loss of functions associated with insulin resistance, non-alcoholic liver and cardiovascular diseases, as well as low-grade inflammatory states characteristic of obesity (Browning et al., 2007; Browning, 2003; Dangardt et al., 2010; Din, Newby, & Flapan, 2004). In contrast, diets rich in n-6 (commonly derived from corn oils or soybeans; Browning et al., 2007) contribute to the development of these pathologies,
firstly, because they are utilized in the synthesis of pro-inflammatory molecules (for detailed review, please see: Simopoulos, 2002; Simopoulos, 2013) and, secondly, by suppressing anti-inflammatory responses. Therefore, the results of Experiment 3, and other studies (Hsu et al., 2015), lend support to the hypothesis that frequent consumption of HFCS-55 may be a risk factor for developing diseases that are characterized by chronic inflammatory states (Simopoulos, 2013).

Conclusions

These self-administration experiments in rats provide converging evidence that differences in fructose: glucose ratios can have a significant impact on self-administration behaviors. It is possible that sugars containing a higher ratio of fructose engendered lower self-administration because this combination has more potent reinforcing effects. The subsequent brain and liver tissue analysis supported this interpretation. Altered neural activity in regions of the hypothalamus responsible for food reward, gene expression changes putatively associated with addiction, and accumulation of fatty acids in the liver associated with inflammatory responses were specific to HFCS-55 self-administration and occurred despite reduced consumption in comparison to sucrose. In other words, it took less exposure to sugars with a higher ratio of fructose to glucose to engender both neural and physiological changes associated with addictive and metabolic diseases. Future intraoral self-administration studies employing isocaloric solutions of pure glucose and pure fructose will be necessary to elucidate the relative contribution of these monosaccharides to reinforcement (it is not possible to manufacture pure glucose or pure fructose sugar pellets). Furthermore, in addition to 55% fructose and 42% glucose, HFCS-55 contains 3% glucose polymers (i.e. polyose; Ackroff & Sclafani, 2011).
Because polycose contributes to the reinforcing efficacy of HFCS-55 (Ackroff & Sclafani, 2011), it should likewise be tested on its own in future studies of sugar self-administration.

Acknowledgments

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Author Contributions

Different authors designed different experiments in the study. AM.L, P.M. and F.L. analyzed the data and drafted the manuscript. AM. L, P.M., Y.Z. K.K., S.D., T.D., A.S. and M.F.F. executed the different experiments. All authors contributed to the final version of the manuscript.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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Table 1: Mean (SEM) infusions obtained on a fixed ratio one schedule of reinforcement by rats that self-administered infusions of 0.1% (self-administration Sessions 1–15), 0.01%, 1% and 10% saccharin.

<table>
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<th>0.01%</th>
<th>1%</th>
<th>10%</th>
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<td>Infusions</td>
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<td>47.4 ± 11.7</td>
<td>39.5 ± 8.7</td>
<td>31.2 ± 7.5</td>
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Figure 1: (A) Mean (SEM) total number of nose pokes emitted for 55% F (fructose) + 45% G (glucose) or 30% F + 70% G pellets. (B) Mean (SEM) daily number of nose pokes emitted over 14 sessions of self-administration for 55% F + 45% G or 30% F + 70% G pellets.
Figure 2: Representative microphotographs of c-Fos (calibration line = 200 µm) in the arcuate nucleus hypothalamus of rats that self-administered 55% F + 45% G (A) or 30% F + 70% G (B), perifornical area of the lateral hypothalamus of rats that self-administered 55% F + 45% G (C) or 30% F + 70% G (D), and dorsomedial hypothalamus of rats that self-administered 55% F + 45% G (E) or 30% F + 70% G (F). The black arrows indicate the location of the third ventricle (A,B,E,F) and fornix (C,D). Between each panel, diagrams from Paxinos and Watson (2004) with selected structures are blackened.
Figure 3: Mean (SEM) Fos density measured in the perifornical area of the hypothalamus (A), arcuate nucleus (B) and dorsomedial hypothalamus (C) of rats that self-administered 55% F + 45% G or 30% F + 70% G pellets. * Significant difference from the control group.
Figure 4: (A) Mean (SEM) infusions obtained on a fixed ratio one schedule of reinforcement by rats that self-administered isocaloric infusions of 25% high fructose corn syrup (HFCS-55) or 20% sucrose. * Significant difference between groups. (B) Mean (SEM) infusions of 25% HFCS-55 and 20% sucrose obtained over time (ten-minute intervals) within self-administration Session 20. * Significant difference between groups.
Figure 5: Mean (SEM) mRNA (attomole/µg) measured in the dorsal striatum (A), nucleus accumbens core (B) and nucleus accumbens shell (C) of rats that self-administered 25% HFCS-55, 20% sucrose or 0.1% saccharin. * Significant difference from 0.1% saccharin groups; ** significant difference from the 0.1% saccharin and 20% sucrose groups.
Figure 6: (A) Mean (SEM) sugar (mL) intake by rats that self-administered 25% HFCS-55 or 20% sucrose in their home cages. * Significant difference between groups. (B) Mean (SEM) total calories (kcal) consumed by rats. The proportions of calories derived from chow and sugar consumption are represented by the black and white stacked bars, respectively. * Significant difference in total caloric intake between the 25% HFCS-55 and 20% sucrose groups.
Figure 7: Mean (SEM) percent organ to body weight ratio of epididymal fat pads, inguinal fat pads, as well as whole livers in rats that self-administered 25% HFCS-55 or 20% sucrose in home cages. * Significant difference between groups.
Figure 8: (A) Mean (SEM) total fatty acids (µg) measured in the livers of rats that self-administered 25% HFCS-55 or 20% sucrose in home cages. (B) Mean (SEM) polyunsaturated fatty acids n-6 and n-3 measured in the livers of rats that self-administered 25% HFCS-55 or 20% sucrose in home cages. * Significant difference between groups.
Chapter 4

Neuropharmacology

Bupropion and naltrexone combination alters high fructose corn syrup
Self-administration and gene expression in rats

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Abstract

Non-communicable diseases and mortality associated with overweight and obesity are rising and the development of adjunctive pharmacotherapies for reducing caloric intake is a promising strategy to support sustained weight-loss. Contrave®, a combination of bupropion (BUP) and naltrexone (NTX) in sustained release formula was recently approved for this purpose. The experiments described here were designed to explore its pharmacological actions by assessing its effects on consumption of sugar solutions in rats and in the same animals, mRNA expression of genes associated with its purported therapeutic effects on appetite, depression and reward were quantified.

To this end, male Sprague-Dawley rats received 12-days of continuous exposure (subcutaneous osmotic mini-pumps) to either BUP (40 mg/kg/day), NTX (4 mg/kg/day) or the combination of BUP+NTX (40+4 mg/kg/day), and were tested during this period, on operant intraoral self-administration (IOSA) of high fructose corn syrup formula 55 (HFCS-55) using fixed ratio 1 (FR1) and progressive ratio (PR) schedules of reinforcement, on voluntarily drinking HFCS-55 from bottles in home cages, and on tests of taste reactivity and locomotion. A separate experiment was also performed to explore the effects of acute administration (subcutaneous injection just prior to IOSA sessions) of BUP (30 mg/kg), NTX (3 mg/kg), or the combination of BUP+NTX (30+3 mg/kg), on IOSA of HFCS-55 using FR1 and PR schedules of reinforcement.

Continuous BUP+NTX and NTX reduced IOSA of HFCS-55 on FR1 (but not PR) schedules of reinforcement and likewise decreased drinking of HFCS-55 from bottles in home cages. These effects on consummatory behaviour were not associated with drug induced changes in the perceived palatability of HFCS-55 or locomotor behaviour. Similarly, acute BUP+NTX
and NTX reduced IOSA of HFCS-55 on FR1 schedules; however, on the PR schedule, BUP+NTX and BUP induced large elevations in breakpoint revealing possible differences in the neurochemical substrates mediating satiety and reward processing in operant SA. Notably, the gene expression analysis revealed that the appetite suppressing and mood enhancing effects associated with Contrave® may be attributable to the pharmacological actions of NTX or BUP alone, respectively; and not their purported synergistic effects. Most interestingly, continuous administration of BUP+NTX led to alterations in the expression of mRNA for genes involved in processing reward in a manner that may reduce vulnerability for addictions; hence, the potential for prescribing Contrave® as adjunctive therapy for reducing compulsive food- or drug-intake in models of addictive behaviour should be explored in future studies.

**Keywords**

Intraoral Operant Self-Administration, Contrave®, Bupropion, Naltrexone, High Fructose Corn Syrup, Taste Reactivity, Progressive Ratio, Food Addiction, Obesity.
Introduction

Overweight and obesity are putative risk factors for non-communicable diseases including metabolic and cardiovascular disorders, comorbid substance and mood disorders (Gearhardt, Harrison, et al., 2012; Singh, 2014), and some cancers (World Health Organization, 2015). In vulnerable individuals (Beaver et al., 2006; Yokum, Gearhardt, Harris, Brownell, & Stice, 2014) highly processed foods (i.e. food/drink manufactured to contain high concentrations of sugars, fats, salts, and or chemicals to increase caloric density and enhance palatability; Malandrino & Capristo, 2011) may promote overconsumption (Ifland et al., 2009; Schulte, Smeal, & Gearhardt, 2017). In fact, the ubiquity with which these foods are available and readily consumed is argued to be a primary contributing factor (Lindberg et al., 2011) to the worldwide epidemic of overweight and obesity (World Health Organization, 2015).

In addition to eating beyond metabolic need (i.e. hedonic eating; Rozehnalova, 2017) there also exists significant individual variability in genetic (Blum et al., 2006; Farooqi & O’Rahilly, 2007), environmental (Hetherington et al., 2006; Ifland et al., 2015), and trait vulnerabilities (Davis et al., 2007; Davis, Strachan, & Berkson, 2004; Davis & Carter, 2009) for developing obesity that challenge prevention and treatment. In fact, weight-loss strategies focused solely on lifestyle change (i.e. reducing caloric intake and increasing exercise) are often unsuccessful as evidenced by frequent relapse of weight-gain.

Consequently, adjunctive pharmacotherapies to support sustained weight-loss (Colon-Gonzalez, Kim, Lin, Valentino, & Waldman, 2013) have been developed including Contrave®; a combination of bupropion (BUP; dopamine (DA) and norepinephrine reuptake inhibitor) and naltrexone (NTX; non-selective competitive mu-opioid receptor (MOR) antagonist) delivered in sustained release oral formula (Greenway et al., 2009a). Clinical trials indicate that chronic
administration of Contrave® (~ 56-weeks) enhances weight-loss (Anderson et al., 2002; Greenway, et al., 2010; Greenway et al., 2009b) by reportedly curbing excessive eating/binge eating behaviour and cravings for highly palatable foods (Guerdjikova et al., 2017). In addition to weight-loss, participants also experience improvements in cardiovascular and metabolic function (Apovian, 2016; Apovian et al., 2013; Hollander et al., 2013), depressive symptoms (McElroy et al., 2013), and quality of life (Kolotkin, Chen, Klassen, Gilder, & Greenway, 2015).

Alone, BUP is widely prescribed for its antidepressant effects (Dong & Blier, 2001; Kitamura et al., 2010; Tomarken, Dichter, Freid, Addington, & Shelton, 2004) and NTX for its ability to reduce binge-eating (Alger, Schwalberg, Bigaouette, Michalek, & Howard, 1991), and both are employed as adjunctive pharmacotherapy in the treatment of substance related abuse disorders (SRADs; Sherman, Ungureanu, & Rey, 2016). When combined, the ability of these drugs to enhance weight-loss is purportedly due to the synergistic effect BUP and NTX have on enhancing the release of pro-opiomelanocortin (POMC); an appetite suppressing peptide released from anorexigenic neurons in the arcuate nucleus of the hypothalamus (Reece, 2011; Greig and Keating, 2015). In humans, Contrave® has been associated with not only enhanced weight-loss but also blunting hypothalamic reactivity to food cues (Wang et al., 2014), and in rats, the combination of systemic administration of BUP+NTX reduces consumption of food beyond that of their anorexigenic effects when administered alone (Clapper et al., 2013; Greenway et al., 2009; Wright & Rodgers, 2013).

To offer further insight as to how the pharmacological effects of Contrave® might aid with weight-loss, experiments were designed to explore the hypothesis that Contrave® may reduce appetitive behaviour and motivation for food. High fructose corn syrup formula 55 (HFCS-55; Parker, Salas, & Nwosu, 2010) has been associated with overeating as well as the
development of obesity (Bray et al., 2004; Parker et al., 2010) and purported to possess abuse liability in studies of operant SA (Levy et al., 2014, 2015). Relative to sucrose, the small increase in the ratio of fructose to glucose in HFCS-55 (50%:50% versus 55%:42%, respectively) is linked to metabolic disturbance and or weight-gain in humans (Bray et al., 2004; Sadowska & Bruszkowska, 2017; Stanhope & Havel, 2008; Rippe & Angelopoulos, 2016) and animals (Bocarsly et al., 2010; Hsu et al., 2015; Mock et al., 2017). In fact, even though animals voluntarily self-administer significantly less HFCS-55 than sucrose, exposure to HFCS-55 (but not sucrose; Levy et al., 2015) produces neural alterations in rats associated with enhanced vulnerability for SRADs (Volkow et al., 2001; Volkow et al., 1993; Wise, 2004).

To the authors’ knowledge, administration of the combination of BUP and NTX that more accurately reflects chronic levels of drug exposure achieved in clinical populations has yet to be tested in animals. To this end, the effects of continuous (subcutaneous osmotic mini-pump for 12-days; Theeuwes & Yum, 1976; Turner, Brabb, Pekow, & Vasbinder, 2011) and acute (subcutaneous injection just prior to IOSA sessions) administration of BUP, NTX, or their combination BUP+NTX on operant intraoral self-administration (IOSA; Levy et al., 2014) of HFCS-55 was assessed. Continuous drug exposure procedures maintain steady levels of drug in blood and in animals, is purported to produce physiological and neurobiological responses over time that better reflect changes induced by regimens of chronic exposure (Billes & Cowley, 2007; Davidson, Lee, & Ellinwood, 2005). Animals drinking HFCS-55 from bottles in home cages were also tested under identical procedures for continuous drug administration to validate the behavioural and neurochemical results of IOSA experiments.

The pharmacological effects of these drugs have been associated with nausea (i.e. BUP+NTX; Greenway, et al., 2010; Greenway et al., 2009), dysphoria (i.e. NTX; Miotto,
McCann, Basch, Rawson, & Ling, 2002), and effects on locomotor behaviour (i.e. BUP; Nielsen, Shannon, Bero, & Moore, 1986). Therefore, taste reactivity (Grill & Norgren, 1978) and locomotion tests were performed on the first and last day of continuous drug administration procedures to consider whether any observed change in HFCS-55 consumption was attributable to drug-induced changes in palatability or locomotion, respectively.

The expression of mRNA for genes associated with the purported therapeutic effects of Contrave® were also measured in animals following continuous exposure to BUP, NTX or their combination while self-administering HFCS-55 in IOSA or from bottles. Firstly, in the hypothalamus, POMC mRNA expression was evaluated to verify the purported synergistic effect of BUP+NTX on its activity (Greenway et al., 2009a). Secondly, in the hippocampus, brain derived neurotrophic factor (BDNF) mRNA expression was assessed because of its status as a biomarker for depression (Lee & Kim, 2010; Yu & Chen, 2011) and Contrave® reportedly relieves symptoms of depression in obese individuals (McElroy et al., 2013). Finally, dopamine (DA) receptor 2 (DR2) mRNA in the caudate-putamen and mu-opioid receptor (MOR) mRNA in the nucleus accumbens (NAc; core and shell) were quantified given their involvement in reward processing (Wise, 2006a) and evidence that operant IOSA of HFCS-55 alters their expression in rats (Levy et al., 2015).

**Material and methods**

**Subjects**

Adult male Sprague-Dawley rats (Charles River, St-Constant, QC, Canada) weighing 200-250 g were single-housed, maintained on reverse light/dark cycle (7:00 a.m. lights off, 7:00 p.m. lights on) and tested during the active (dark) phase. Unless otherwise indicated, rats had *ad libitum* access to chow/water in home cages. Experiments were approved by the Animal Care
Committee of the University of Guelph and carried out in accordance with the recommendations of the Canadian Council on Animal Care.

Drugs and food

Bupropion hydrochloride (BUP; Toronto Research Company, TO, Canada) and naltrexone-hydrochloride (NTX; Sigma Aldrich, USA) were dissolved in 0.9% physiological saline for drug administration. For continuous delivery via subcutaneous osmotic mini-pump (Theeuwes & Yum, 1976), BUP and NTX were administered at 40 and 4 mg/kg/day, respectively, and for acute subcutaneous injections administered at 30 and 3 mg/kg, respectively. These doses were selected because they fall within a range that have been reported to reduce voluntary food intake (Greenway et al., 2009a) as well as modulate operant responding for both food and drug reinforcers on FR1 and PR schedules of reinforcement (Bruijnzeel and Markou, 2003; Giuliano et al., 2012; Gosnell et al., 2010; Reichel et al., 2009). Finally, to closely model the ratio of BUP to NTX in Contrave® (i.e. 360 + 32 mg/day), the combination of BUP+NTX was administered at a ratio of 10/1, respectively.

Rats were fed standard laboratory chow (18% protein and 4% fat; Teklad Global Diets, Harlan Laboratories). Solutions of HFCS-55 (HFCS-55 formula for human consumption; Natures Flavors, California, USA) were stored at room temperature and made by diluting HFCS-55 (serving of 4g = 0g (0%) fat, 0g (0%) protein, and 3.08g (77%) carbohydrate) in reverse osmosis water to concentrations of 25% HFCS-55 (Experiments 1, 2 and 4) and 50% HFCS-55 (Experiment 3). These concentrations were selected because they support robust operant SA in rats (Levy et al., 2014, 2015).

Surgery

*Intraoral (IO) cannulation*
IO cannulas were surgically implanted as described in (Levy et al., 2014, 2015).

**Osmotic mini-pumps**

Subcutaneous osmotic mini-pumps (Alzet model 2ML2, 0.5 ul/hour for 12-days, Durect Corporation, Cupertino, CA, USA) were surgically implanted and removed as described in (Leri, Zhou, Goddard, Cummins, & Kreek, 2006).

**Apparatus**

**Operant intraoral self-administration (IOSA)**

Operant IOSA of HFCS-55 occurred in 26 operant chambers (model ENV-008CT, Med Associates, Lafayette, IN) equipped with retractable levers configured for IO infusions as described in (Levy et al., 2015).

**Taste reactivity (TR)**

TR tests occurred in clear Plexiglas chambers (22.5 X 26.0 X 20.0 cm) placed over a transparent glass surface. A digital video camera (Sony, DCR-HC48) was pointed at a mirror placed at a 45° angle under the glass surface to visualize the ventral surface of the rat and record orofacial reactions (Neath et al., 2010).

**Home cage self-administration (SA)**

Home cage SA of HFCS-55 was assessed from 100 ml no-drip water bottles (Thermo Scientific, Waltham, MA, USA) with metal spouts (Ancare, Bellemore, NY, USA) secured with rubber stoppers (Fisher Scientific, ON, Canada) placed alongside standard water bottles.

**Activity chambers**

Horizontal and vertical activity was automatically tracked using EthoVision (v3, Noldus, The Netherlands) in 12 semitransparent Plexiglas chambers (30.0 X 40.0 X 26.0 cm; University of Guelph, ON, Canada).
Quantification of mRNA

Solution Hybridization RNase Protection-TCA Precipitation

Prior to mRNA quantification, brains were harvested 6-days following the removal of osmotic mini-pumps to maintain consistency with laboratory protocols and to allow for drug clearance. To this end, animals were euthanized by CO2 and the brain regions of interest immediately dissected on ice for subsequent storage at -80°C. Subsequently, POMC mRNA in the hypothalamus, BDNF mRNA in the dentate gyrus (DG)/cornu ammonis (CA1), and CA3 regions of the hippocampus, DR2 mRNA in the caudate-putamen, and MOR mRNA in the NAc core and shell were quantified (attomoles/µg) using a solution hybridization RNase protection-TCA precipitation protocol described in (Leri et al., 2006).

Procedures

Experiment 1: Continuous BUP and/or NTX - operant intraoral self-administration (IOSA)

Rats were food-restricted for 18-hours prior to IOSA sessions and then fed chow ad libitum for 3-hr immediately thereafter (Levy et al., 2014). Sessions began with activation of a house light, entry of the active lever and illumination of the conditioned stimulus (CS) light above it for 30-sec. Presses on the active lever resulted in a 2.5-sec IO infusion of 80 µl of 25% HFCS-55 (caloric content = 0.08 kcal/infusion). A time-out period of 27.5-sec was imposed to allow sufficient time for ingestion. During this period, the active lever was retracted, and the CS light was activated. No limit was imposed on the number of IO infusions obtainable. Animals lever pressed for IO infusions of 25% HFCS-55 on fixed ratio 1 (FR1) and progressive ratio (PR) schedules of reinforcement during the initial training phase to acquire IOSA behaviour (experiment days 1-15), under conditions of continuous drug-administration (via subcutaneous
osmotic mini-pumps; experiment days 16-27), and following the removal of the osmotic mini-pumps (experiment days 28-33).

Initially, rats lever pressed on a FR1 schedule of reinforcement for 10-consecutive IOSA sessions, each lasting 180-min. Then, for the final five training sessions (total of 15 prior to drug-administration), rats were trained to lever press on a dual PR/FR1 schedule of reinforcement. Both SA schedules assess reinforcement (White & Milner, 1992) but FR1 may reflect appetitive consummatory behaviour as each lever response is reinforced (Spealman & Goldberg, 1978), while PR is considered to reflect incentive approach behaviour as the response requirement to obtain subsequent reinforcers increases exponentially (i.e. motivation; Arnold & Roberts, 1997). To this end, rats responded for IO infusions of 25% HFCS-55 on a PR schedule for the initial 30-min, followed by the FR1 schedule for the remaining 150-min, separated by a 5-min time-out period (levers retracted/lights turned off). On the PR schedule, the number of responses required to obtain each successive IO infusion increased exponentially according to the formula: $5e^{(injection\ number \times 0.2)}$ (Richardson & Roberts, 1996), and the breakpoint was indexed by the last infusion received prior to cessation of responding on the active lever for at least one hour.

On experiment day 16, rats were randomly assigned to groups (40 mg/kg/day BUP (n = 10), 4 mg/kg/day NTX (n = 10), or 40 mg/kg/day BUP + 4 mg/kg/day NTX (n = 13). Sham surgeries were performed on the control group (n = 11). Following 12-days of drug treatment during which time animals were tested once per day as described above on the dual PR/FR1 schedules, osmotic mini-pumps were removed (experiment day 27) and operant SA was re-assessed for an additional six days. Then, animals were euthanized and plasma and brain tissue extracted 24-hr following the last IOSA session.

*Experiment 2: Continuous BUP and/or NTX - taste reactivity (TR)*
Orofacial reactions in response to IO infusions of HFCS-55 were measured in TR tests on the first (Day 1) and last (Day 2) of continuous drug exposure. Animals received IO infusions of either 40 mg/kg/day BUP (n = 6), 4 mg/kg/day NTX (n = 6), or 40 mg/kg/day BUP + 4 mg/kg/day NTX (n = 6). Sham surgeries were performed on the control group (n = 6). TR tests were performed as described in (Levy et al., 2015) with the exception that habituation and tests lasted a duration of 1-min (not 3-min). To index palatability, the orofacial reactions scored were number of forward (i.e. extensions of the tongue out of the front of the mouth) and lateral (i.e. extensions of the tongue out of the sides of the mouth, sweeping along the lips) tongue protrusions. As the observed frequency of these measures were comparable they were combined to obtain a single index of palatability (Neath et al., 2010). To index any neutral or aversive responses, the number of passive drips (i.e. number of drops of the infused solution that fall from the oral cavity not ingested; Berridge & Grill, 1983) and gapes (i.e. large opening of the mouth with the corners of the mouth retracted; Sorge, Fudge, & Parker, 2002) were scored, respectively.

*Experiment 3: Continuous BUP and/or NTX - home cage self-administration and locomotion*

Non-food-restricted rats received *ad libitum* access to chow and 50% HFCS-55 in home cages for 21-days. On experiment day 22, osmotic mini-pumps were implanted releasing 40 mg/kg/day BUP (n = 10), 4 mg/kg/day NTX (n = 10), or 40 mg/kg/day BUP + 4 mg/kg/day NTX (n = 15). Sham surgeries were performed on the control group (n = 25). Daily intake of HFCS-55 was subsequently measured for 12-days. Osmotic mini-pumps were removed on experiment day 34 and weight-gain, HFCS-55, food, and water consumption were assessed for an additional six days. Then animals were euthanized, and plasma and brain tissue was extracted 24-hr following the last bottle measurement.
Locomotion was measured prior to (experiment day 18), during (experiment days 24 and 30) and post (experiment days 36 and 40) drug treatment in a subset of animals (n = 6) from each group described above. For these tests, rats were placed in activity chambers and locomotion was recorded for 2-hr.

Experiment 4: Acute BUP and/or NTX - operant intraoral self-administration

Animals (food-restricted, n = 21) were trained to self-administer IO infusions of 25% HFCS-55 (one 3-hour session/day) on a FR1 schedule of reinforcement for 14-consecutive days. Then, using a within subject design, rats were subcutaneously injected with: 30 mg/kg BUP, 3 mg/kg NTX, 30 mg BUP + 3 mg/kg NTX, and vehicle immediately prior to the start of four IOSA sessions responding on a PR schedule. The order of drug doses administered was counterbalanced according to a Latin Square design, and each test session was separated by one session of responding on the PR schedule after pre-treatment with vehicle (i.e. total of eight IOSA sessions were performed to assess the effects of these different doses on breakpoint achieved). Identical procedures were then employed to assess the effects of the same drug treatments on FR1 responding.

Statistical Analysis

Data were analyzed using mixed analysis of variance (ANOVA) and t-tests. Significant interactions and main effects were assessed by multiple comparisons using the Student–Newman–Keuls method (α = 0.05). Exact values of non-significant results were not reported and all analyses were performed using SigmaStat (v. 3.5 for Windows, SPSS Inc., NY, USA).

Results

Experiment 1: Continuous BUP and/or NTX on operant intraoral self-administration
Mean breakpoint on PR (Panels A and C) and infusions on FR1 (Panels B and D) schedules of reinforcement for 25% HFCS-55 are represented in Figure 1 on the first (D1) and last (D12) day of continuous drug treatment. On D1, the ANOVA on PR responding revealed no effect of treatment group on the breakpoint achieved for IO infusions of 25% HFCS-55. In comparison, the ANOVA on FR1 revealed a significant Group by Time interaction \( [F(42,560) = 1.6, p < 0.001]\) as well as significant main effects of Group \( [F(3,40) = 8.7, p < 0.001]\) and of Time \( [F(14,560) = 17.9, p < 0.001]\). Multiple comparisons indicated that operant responding for IO infusions of 25% HFCS-55 was significantly reduced by administration of BUP+NTX and NTX between the 30- and 70-min session marks. Similar results were observed on D12: on the PR, no effect of group on breakpoint was observed and on the FR1, there was a significant Group by Time interaction \( [F(42,560) = 1.5, p < 0.001]\) as well as main effect of Group \( [F(3,40) = 8.5, p < 0.001]\) and of Time \( [F(14,560) = 17.1, p < 0.001]\). By D12, only continuous treatment with BUP+NTX reduced IOSA of HFCS-55 between the 40- and 70-min session marks. Inactive lever responses did not differ between groups or across tests on either schedule of reinforcement tested (data not depicted).

Figure 2 illustrates expression of D2R mRNA in the caudate-putamen (Panel A) and MOR mRNA in the NAc core and shell (Panel B) of rats tested above. In the caudate-putamen, the ANOVA revealed a significant main effect of Group \( [F(3,24) = 15.16, p < 0.001]\) and multiple comparisons indicated that D2R mRNA expression was significantly higher in animals treated with BUP+NTX. In the NAc, the ANOVA revealed a significant Group by Region interaction \( [F(3,30) = 11.2, p < 0.001]\), as well as significant main effects of Group \( [F(3,30) = 10.9, p < 0.001]\) and of Region \( [F(1,30) = 268.8, p < 0.001]\). Across all drug treatments, expression of MOR mRNA was significantly higher in the core compared to shell, and in the
NAc core, MOR mRNA was significantly lower in rats treated with BUP+NTX and NTX. In the NAc shell, group differences in the expression of MOR mRNA were not observed.

Figure 2 also represents POMC mRNA expression in the hypothalamus (Panel C), and BDNF mRNA expression in the DG/CA1 and CA3 hippocampal regions (Panel D) in the same animals. In the hypothalamus, the ANOVA revealed a significant main effect of Group \( F(3,34) = 10.6, p < 0.001 \), and multiple comparisons indicated that POMC mRNA expression was higher in animals treated by continuous BUP+NTX and NTX. Additionally, the ANOVA on BDNF mRNA expression in the hippocampus revealed a significant Group by Region interaction \( F(3,34) = 13.4, p < 0.001 \), and significant main effects of Group \( F(3,34) = 15.3, p < 0.001 \) and of Region \( F(1,34) = 365.2, p < 0.001 \). Across all groups, BDNF mRNA expression was significantly higher in the DG/CA1 regions, and in this region, BDNF mRNA expression was significantly greater in those animals treated by continuous BUP+NTX and BUP. Expression of BDNF mRNA in the CA3 region of the hippocampus did not differ between groups.

Experiment 2: Continuous BUP and/or NTX on taste reactivity

Passive IO infusion of HFCS-55 did not elicit passive dripping or gaping reactions from animals, and the mean frequency of tongue protrusions did not significantly differ between groups or across repeated TR tests performed at the start and end of drug-treatment (Table 1).

Experiment 3: Continuous BUP and/or NTX on home cage self-administration and locomotion

Figure 3 represents mean voluntary drinking of 50% HFCS-55 (Panel A) and mean chow consumed (Panel B) on the first (D1) and last (D12) day of continuous treatment. The ANOVA on drinking 50% HFCS-55 revealed a significant Group by Session interaction \( F(3, 97) = 3.7, p < 0.001 \), as well as significant main effects of Group \( F(3,97) = 8.9, p < 0.001 \) and of Session \( F(1, 97) = 35.4, p < 0.001 \). Multiple comparisons indicated that BUP+NTX and NTX reduced
intake of 50% HFCS-55 on D1, and this effect remained significant by D12 only in the NTX group. With respect to consumption of chow, the ANOVA revealed a significant Group by Session interaction \( [F(3,97) = 4.1, p < 0.01] \), as well as significant main effects of Group \( [F(3, 97) = 8.3, p < 0.001] \) and of Session \( [F(1, 97) = 34.2, p < 0.001] \). On D1, consumption of chow was significantly lower in animals treated with BUP+NTX and NTX, and by D12, the observed reduction of chow intake remained significant only in the BUP+NTX treated group.

In a subset of these animals, locomotor activity was assessed on D1 and D12 of continuous treatment (Table 2) and the mean total distance moved did not significantly differ between groups or across repeated tests of locomotion.

Finally, Figure 2 also represents POMC mRNA expression in the hypothalamus (Panel E) and BDNF mRNA expression in the DG/CA1 and CA3 hippocampal regions (Panel F) in rats that voluntarily drank 50% HFCS-55 in home cages. In the hypothalamus, the ANOVA revealed a significant effect of Group \( [F(3,44) = 18.4, p < 0.001] \), and multiple comparisons indicated that POMC mRNA expression was significantly higher in animals treated with BUP+NTX and NTX. In the hippocampus, the ANOVA revealed a significant Group by Region interaction \( [F(3,44) = 10.9, p < 0.001] \) and significant main effects of Group \( [F(3,44) = 10.7, p < 0.001] \) and of Region \( [F(1,44) = 229.9, p < 0.001] \). Across all drug treatments, expression of BDNF mRNA was greater in the DG/CA1 region, and within the DG/CA1, expression of BDNF mRNA was significantly higher in animals treated with BUP+NTX and BUP. Expression of BDNF mRNA in the CA3 region of the hippocampus did not differ between groups.

**Experiment 4: Acute BUP and/or NTX on operant intraoral self-administration**

Figure 4 depicts the effect of acute drug treatments on the mean breakpoint (BP; Panel A) and mean infusions (Panel B) obtained responding for IO infusions of 25% HFCS-55 on PR and
FR1 schedules of reinforcement, respectively. The ANOVA on PR responding revealed a significant main effect of Group \[ F(3,69) = 32.8, p < 0.001 \] whereby compared to vehicle and NTX treated groups, breakpoints were significantly enhanced by acute pre-treatment with BUP+NTX and BUP (Figure 4 Panel A). On the FR1, the ANOVA likewise revealed a significant main effect of Group \[ F(3,69) = 22.7, p < 0.001 \] and it was found that operant responding for 25% HFCS-55 was significantly reduced by acute pre-treatment with BUP+NTX and NTX relative to treatment with vehicle or BUP (Figure 4 Panel B). Inactive lever responses did not differ between groups or across tests on either schedule of reinforcement (data not depicted).

**Discussion**

A series of experiments were designed to investigate the pharmacological actions of Contrave® by assessing the effects of continuous and acute administration of BUP, NTX and BUP+NTX on consumption of HFCS-55 in rats and by assessing the expression of genes associated with its purported effects on appetite, mood, and reward. Continuous BUP+NTX (40+4 mg/kg/day; Figure 1 Panels A-D) significantly decreased 25% HFCS-55 IOSA on FR1 (but not PR) schedules and likewise reduced drinking of 50% HFCS-55 from bottles in home cages (Figure 3 Panel A). It was unlikely that these results were influenced by energy status as the anorexigenic effects of continuous BUP+NTX were similarly observed in both food-restricted rats tested in operant IOSA (Experiment 1) and in *ad libitum* fed rats drinking HFCS-55 from bottles in home cages (Experiment 3). Moreover, reduced intake of HFCS-55 could not be attributed to drug induced alterations of either the perceived palatability of 25% HFCS-55 (Table 1) or motor behaviour affecting operant responding (Table 2) as these measures were unchanged when assessed on the first and last day of continuous drug treatment. In response to
acute administration of BUP+NTX (30+3 mg/kg, SC; Figure 4) IOSA of 25% HFCS-55 was likewise reduced, yet when this same pre-treatment was administrated prior to testing on the PR schedule, breakpoint was significantly enhanced. This interesting finding, as well as the results of continuous and acute administration of BUP and NTX alone relative to its effects when administered in combination are discussed below.

In line with observed reductions in responding for food following NTX administered by osmotic mini-pump (Lang, Strahlendorf, Strahlendorf, Lutherer, & Barnes, 1982) or by acute injection (Blasio, Steardo, Sabino, & Cottone, 2014; Giuliano et al., 2012; Gosnell et al., 2010), continuous (4 mg/kg/day; Figure 1 Panel B) and acute (3 mg/kg; Figure 4 Panel B) NTX reduced operant responding for 25% HFCS-55 on FR1 schedules. The lack of effect of continuous or acute administration of NTX on PR responding was unexpected as Blasio et al., (2014) reported reduced breakpoints for chocolate-flavored sucrose pellets (0.1 and 0.3 mg/kg NTX). Perhaps, this discrepancy is due to the difference in sweet rewards used, as different sugars (i.e. sucrose or saccharin) stimulate different opioid receptor subtypes and taste pathways in the brain (Beczkowska, Bowen, & Bodnar, 1992; Beczkowska, Koch, Elizabeth Bostock, Leibowitz, & Bodnar, 1993; Frank et al., 2008; Hayward, Schaich-Borg, Pintar, & Low, 2006). It was also interesting that continuous NTX reduced operant IOSA of 25% HFCS-55 only on the first treatment day, while it retained its effect throughout the entire period of drug delivery in rats drinking 50% HFCS-55 from bottles in home cages. Why this may be the case remains elusive, it is possible that greater intake of HFCS-55 in animals that drank it in home cages induced sensitization to the anorexigenic effect of NTX (Kanarek, Mathes, Heisler, Lima, & Monfared).

Administered alone, BUP is reported to be minimally effective in reducing food intake or enhancing weight-loss in humans (Greenway et al., 2009b; Verpeut & Bello, 2014) and
consistent with these previous reports, continuous BUP (40 mg/kg/day) did not alter operant responding for 25% HFCS-55 on FR1 or PR schedules (Figure 1 Panels A-D), nor drinking 50% HFCS-55 from bottles in home cages (Figure 3 Panel A). Different from the effects of continuous treatments on operant SA, acute administration of BUP (30 mg/kg, SC) reduced operant responding for 25% HFCS-55 on FR1 schedules (Figure 4 Panel B) while conversely increasing breakpoint achieved on the PR schedule (Figure 4 Panel A). While these findings appear at odds, they may reflect differences in activation of the neurochemical substrates mediating satiety or reward processes that influence operant SA behaviour by the different routes of drug administration (continuous versus acute) as well as schedules of reinforcement employed.

On PR schedules, operant responding is particularly sensitive to acute elevations in brain monoamine levels (Evans & Vaccarino, 1987; Stafford, LeSage, & Glowa, 1998) that enhance sensitivity to the incentive value of rewarding stimuli (Robinson & Berridge, 1993; Wise, 2004). This may account for why BUP, a DA and norepinephrine reuptake inhibitor, enhanced breakpoints only when administered under acute conditions. This interpretation is consistent with findings that acute pretreatment with BUP (10 to 40 mg/kg, intraperitoneal) enhances PR responding for high carbohydrate pellets that was likewise associated with elevations in DAergic signaling within the NAc (Randall et al., 2015). Moreover, it is also plausible that the anorexigenic effects of BUP on food intake are best revealed when consumption is sufficiently high to engage satiety regulating mechanisms (i.e., on FR1 schedules believed to reflect incentive consummatory behaviour; Spealman & Goldberg, 1978) and not by measures of incentive approach behaviour (i.e. motivation; Arnold & Roberts, 1997; Wise, 2004) tested on the PR schedule where the availability of HFCS-55 infusions are limited. This is a possible
interpretation as BUP alone can reduce food intake in animals (Greenway et al., 2009; Sinnayah, Wallingford, Evans, & Cowley, 2007).

Gene expression analyses also revealed novel findings related to these drugs actions, although it should be noted that these results are limited to protein biosynthesis. With respect to appetite, continuous treatment with BUP+NTX as well as NTX alone led to significant elevations in hypothalamic POMC mRNA expression. It is possible then, that reduced operant responding for 25% HFCS-55 may be due, in part, to the actions of POMC. More specifically, upon its release, this peptide is cleaved into β-endorphin and the appetite suppressant, alpha melanocyte stimulating hormone (α-MSH; Millington, 2007; Zhan et al., 2013). The observation that NTX alone was able to likewise enhance the expression of POMC mRNA supports findings that the anorexigenic effect of α-MSH can be potentiated by NTX administration (Greenway, et al., 2010; Wang et al., 2014). That is, NTX blocks the action of β-endorphins at MORs expressed on POMC neurons where they function as autoreceptors to inhibit the firing rate and release of POMC (Pennock & Hentges, 2011). It is important to note, however, that continuous treatment by either BUP+NTX or NTX elicited comparable levels of POMC mRNA expression which is not consistent with their purported synergistic effects on enhancing POMC release in the hypothalamus (Greenway et al., 2009a). However, change in gene expression cannot be directly linked to behavioural responses in IOSA so it is plausible that enhanced release of POMC by the synergistic effects of BUP+NTX underlies its anorexigenic effects in clinical populations (Greenway et al., 2009b) and animals (Sinnayah et al., 2007; Wright & Rodgers, 2013).

Diminished expression of hippocampal BDNF serves as a marker for depression and the therapeutic effects of antidepressants are believed to be mediated in part, by enhancing the expression of BDNF in this region (Fuchikami et al., 2011; Nestler & Carlezon, 2006; Yu &
Consistent with the observation that Contrave® relieves depressive symptoms in obese individuals (Greig & Keating, 2015; Nathan, O’Neill, Napolitano, & Bullmore, 2011) continuous administration of BUP+NTX enhanced expression of hippocampal BDNF mRNA. However, this effect may be attributed to the actions of BUP alone, as expression of hippocampal BDNF mRNA was similar between BUP+NTX and BUP treated groups and NTX had no effect on the expression of BDNF mRNA relative to sham. The antidepressant properties of BUP (Welbutrin®; Patel et al., 2016) are often associated with its modulatory effects on dopaminergic and noradrenergic signaling (Ascher et al., 1995) and the results here may reflect an additional means through which BUP may alleviate symptoms of depression (Nestler & Carlezon, 2006).

Finally, it also appears that the combination of BUP+NTX alters gene expression of neural substrates involved in reward processing. The mRNA analyses revealed enhanced D2R mRNA expression in the caudate-putamen of animals continuously treated with BUP+NTX or BUP (attributed primarily to BUP+NTX; Figure 2A), as well as reduced MOR mRNA expression in the NAc core in animals continuously treated by BUP+NTX or NTX (primarily attributed to NTX; Figure 2B). These results are inconsistent with previous findings in our laboratory whereby operant IOSA of 25% HFCS-55 led to decreased D2R mRNA in the caudate-putamen and increased MOR mRNA in the NAc core (Levy et al., 2015). The latter findings (Levy et al., 2015) are similar to neural adaptations elicited by repeated administration of drugs of abuse that are implicated in the development of addictive behaviours (Wise, 2005; Leri et al, 2009). The current observation that continuous BUP+NTX leads to opposing effects on the expression of these genes despite likewise IOSA of 25% HFCS-55 (Levy et al., 2015) may speak to possible protective effects BUP+NTX may have on reducing vulnerabilities for addiction; in
line with the therapeutic use of these drugs alone for the treatment of SRADs (Sherman et al., 2016).

The data reported in this manuscript support the use of Contrave® as adjunctive therapy for weight-loss given its ability to reduce caloric intake (Anderson et al., 2002; Greenway et al., 2010). When testing behaviour reinforced by HFCS-55, continuous and acute BUP+NTX and NTX reduced consummatory behaviour and correspondingly enhanced mRNA expression of anorexigenic peptides. Contrary to evidence that these effects on appetite relate to the synergistic effects of combining BUP+NTX (Reece, 2011; Greig and Keating, 2015) the results of IOSA of HFCS-55 or drinking HFCS-55 from bottles could likely be attributed primarily to an effect of NTX alone. These data also suggest Contrave® may relieve depressive symptoms in obese individuals via its actions on enhancing mRNA expression of hippocampal BDNF. Again, this observation may be attributed primarily to an effect of BUP alone and not the synergistic effects of these drugs. It is possible that the doses selected were too high (i.e. 30 and 40 mg/kg BUP and 3 and 4 mg/kg NTX) and or the incentive stimuli (Hayward et al., 2006) selected for IOSA experiments may have limited the ability to reveal such synergistic drug effects and should be investigated in subsequent studies. Importantly, it will also be of interest to test the effects of continuous BUP+NTX in animal models of compulsive food- or drug-seeking and taking behaviour (Avena, Rada, et al., 2008; Edwards & Koob, 2013; Shaham, Shalev, Lu, De Wit, & Stewart, 2003) given the novel results that Contrave® may alter reward processing of highly incentive stimuli.

Acknowledgments
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Conflict of Interest

The authors have no conflict of interest to disclose.
Table 1: Mean (sem) frequency of tongue protrusions in response to passive IO infusions of 25% HFCS-55 observed in taste reactivity tests within the first (D1) and last day (D12) of continuous treatment with: 40 mg/kg/day BUP (n = 6), 4 mg/kg/day NTX (n = 6), 40 mg/kg/day BUP + 4 mg/kg/day NTX (n = 6), or 0.0 mg/kg/day sham (n = 6).

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<th>D1</th>
<th>D12</th>
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<tr>
<td>Sham</td>
<td>75.3 ± 13.6</td>
<td>76.7 ± 21.4</td>
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<tr>
<td>BUP</td>
<td>78.8 ± 15.2</td>
<td>85.7 ± 24.8</td>
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<tr>
<td>NTX</td>
<td>93.0 ± 15.4</td>
<td>100.7 ± 19.0</td>
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<tr>
<td>BUP + NTX</td>
<td>84.7 ± 27.9</td>
<td>91.3 ± 30.1</td>
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Table 2: Mean (sem) total distance (cm) moved during tests of locomotion performed on day 1 (D1) and again on day 12 (D12) of continuous treatment with: 40 mg/kg/day BUP (n = 6), 4 mg/kg/day NTX (n = 6), 40 mg/kg/day BUP + 4 mg/kg/day NTX (n = 6), or 0.0 mg/kg/day sham (n = 6).

<table>
<thead>
<tr>
<th>Group</th>
<th>D1</th>
<th>D12</th>
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<tr>
<td>Sham</td>
<td>35011.8 ± 2024.3</td>
<td>36659.1 ± 3964.3</td>
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<tr>
<td>BUP</td>
<td>32727.5 ± 3882.3</td>
<td>28422.3 ± 3817.7</td>
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<tr>
<td>NTX</td>
<td>24860.0 ± 3402.2</td>
<td>26156.2 ± 3090.5</td>
</tr>
<tr>
<td>BUP + NTX</td>
<td>36308.7 ± 4030.5</td>
<td>35434.4 ± 2276.5</td>
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Figure 1: Operant intraoral self-administration of 25% HFCS-55 on the first (D1; Panels A and B) and last (D12; Panels C and D) day of continuous treatment with: 40 mg/kg/day BUP, 4 mg/kg/day NTX, 40 mg/kg/day BUP + 4 mg/kg/day NTX or sham. Panels A and C: Mean (sem) breakpoint achieved when responding on PR schedules for 25% HFCS-55. Panels B and D: Mean (sem) infusions of 25% HFCS-55 obtained on the FR1 schedule. Within D1, * indicates a significant difference between sham and BUP+NTX groups and ** a significant difference between sham and NTX groups. Within D12, * indicates a significant difference between sham and BUP+NTX.
Figure 2: Mean (sem) mRNA (attomole/μg) measured in the caudate-putamen (Panel A), nucleus accumbens core and shell (Panel B), hypothalamus (Panel C) and DG/CA1 and CA3 regions of the hippocampus (Panel D) of rats self-administering 25% HFCS-55 in operant chambers as well as hypothalamus (Panel E) and DG/CA1 and CA3 regions of the hippocampus (Panel F) of rats self-administering 50% HFCS-55 in home cages following continuous treatment with: 40 mg/kg/day BUP, 4 mg/kg/day NTX, 40 mg/kg/day BUP + 4 mg/kg/day NTX or sham. The * indicates a significant difference from sham.
Figure 3: Home cage drinking of 50% HFCS-55 from bottles (Panel A) and consumption of chow (Panel B) within the first (D1) and last day (D12) of continuous treatment with: 40 mg/kg/day BUP, 4 mg/kg/day NTX, 40 mg/kg/day BUP + 4 mg/kg/day NTX or sham. Panel A: Mean (sem) HFCS-55 intake (ml) within D1 and D12 of continuous treatment. The * indicates a significant difference between sham and BUP+NTX groups and ** a significant difference between sham and NTX groups. Panel B: Mean (sem) chow intake (g) within D1 and D12 of continuous treatment. The * indicates a significant difference between sham and BUP+NTX groups and ** a significant difference between sham and NTX groups.
Figure 4: Operant intraoral self-administration of 25% HFCS-55 following acute (subcutaneous) injection of: 30 mg/kg BUP, 3 mg/kg NTX, 30 mg/kg BUP + 3 mg/kg NTX and vehicle. Panel A: Mean (sem) breakpoint achieved on the PR schedule following acute pretreatments. The * indicates a significant difference from vehicle and NTX and ** a significant difference between BUP and BUP + NTX. Panel B: Mean (sem) infusions of 25% HFCS-55 obtained on the FR1 schedule following acute pretreatments. The * indicates a significant difference from vehicle and ** a significant difference from BUP.
Chapter 5:
General Discussion and Conclusions
**General Discussion**

**Evidence for the abuse liability of HFCS-55**

This dissertation provides novel evidence for the abuse liability associated with consumption of HFCS-55 as measured by methods and criteria traditionally used to assess the addictive potential of drugs of abuse. The abuse potential of a substance is measured in part, by its ability to reinforce the acquisition and maintenance of operant behaviour in studies of SA (Balster, 1991). Therefore, to assess the hypothesis that HFCS-55 may be addictive, animals self-administered different concentrations of HFCS-55 on both FR1 and PR schedules using IOSA (Levy et al., 2014). With respect to the critical assessment of reinforcement, both food-restricted and *ad libitum* fed animals readily acquire and maintain operant SA of HFCS-55 over a range of concentrations indicating that it is reinforcing. On FR1 schedules, substances with purported abuse liability typically elicit an inverted-U pattern of intake (Carter & Griffiths, 2009; Lynch & Carroll, 2001; Panlilio et al., 2003; Sclafani & Ackroff, 2003). IOSA of HFCS-55 produces such a pattern, whereby 8% and 25% made up the ascending limb while 50% HFCS-55 was self-administered along the descending limb of the concentration-response curve. Animals also received a PR test prior to (Test 1) and following (Test 2) 15-consecutive IOSA sessions on the FR1 schedule. Group differences emerged on Test 2 whereby lever pressing for IO infusions of 50% and 25% HFCS-55 elicited higher breakpoints than 8%, which remain unchanged.

Addictive-like behaviours emerged in IOSA when animals were given the opportunity to self-administer 25% HFCS-55. That is, over three-weeks of testing, escalation of HFCS-55 intake and specifically, a progressive elevation of HFCS-55 SA during the initial 90-minutes of sessions was observed. Escalation of drug intake, especially following extended access to self-administer drugs of abuse is a hallmark of substance related abuse disorders (SRADs) and
criterion for abuse liability (Cummins & Leri, 2008; Edwards & Koob, 2013). Similarly, the increased magnitude of the HFCS-55 “loading” phase at the start of sessions suggests that like drugs of abuse and sucrose, exposure to HFCS-55 can precipitate the development of binge-behaviour implicated in the transition from voluntary to compulsive intake (Koob & Le Moal, 2001; Tornatzky & Miczek, 2000) and associated with a food-addicted phenotype in rats (Avena et al., 2008a). Additionally, the significant increase in breakpoint achieved between PR tests 1 and 2 provides evidence that repeated exposure to HFCS-55 may increase motivation to self-administer higher concentrations of the sugar. Enhanced motivation is considered one of three primary criteria for addiction-like behaviour in rats likewise observed in response to repeated access to drugs of abuse and sucrose (Deroche-Gamonet et al., 2004; la Fleur et al., 2007; Paterson & Markou, 2003; Robinson et al., 2015; Schlosburg et al., 2013).

Operant IOSA was developed to address procedural issues with existing methods of food SA that may impede accurate or necessary manipulations to assess the addictive potential of food (Levy et al., 2014). The evidence collected using IOSA supports the notion that this method can be used to assess the abuse liability of any soluble test solution in a manner similar to those employed to study and label drugs as addictive (Levy et al., 2014, 2015, 2018; Maccioni & Colombo, 2017; Paterson, 2012). Firstly, operant SA behaviour in IOSA appears to be reinforced by the nutritional value of the test solutions. This is consistent with evidence that the reinforcing efficacy ascribed to sugar is primarily determined by its post-ingestional consequences, that is, its caloric content and not just sweet taste (Beeler et al., 2012; Frank et al., 2008; Tellez et al., 2016; van Rijn et al., 2015). While both 25% HFCS-55 and the sweet but non-caloric solution of 0.1% w/v saccharin elicited similar hedonic responses in TR tests, substitution of 25% HFCS-55 by 0.1% w/v saccharin significantly reduced responding and failed to reinforce operant SA at the
same level as 25% HFCS-55. Like any reinforcer, dopaminergic responses elicited by a food stimulus largely mediates its reinforcing efficacy and even though saccharin can reinforce operant behavior it only has weak effects on DA release in the NAc in rats (Blackburn et al., 1986; Gosnell et al., 2010; Mierzejewski et al., 2009; Miller & Frattali, 1989; Weiss et al., 1993). In fact, intra-gastric infusion of glucose (but not saccharin) elicits fMRI BOLD activation of the VTA, NAc, and amygdala in rats (Tsurugizawa & Uneyama, 2014). Not surprisingly then, dopaminergic responses to sugar by these midbrain regions reportedly direct appetitive behaviour based on its nutritional value; and this may explain in part, low levels of IOSA elicited by this sweet but non-caloric sweetener (Tellez et al., 2016).

The concentration-response relationships observed in IOSA also reflects those produced by IV operant SA of drugs of abuse as well as sucrose self-administered from sipper tubes and receptacles (Sclafani & Ackroff, 2003; Panlilio et al., 2003). On the PR schedule, there was a concentration dependent increase in breakpoints achieved consistent with the observation that reinforcing efficacy typically increases as a linear function of the magnitude of the reinforcer (Baron et al., 1992; Hodos, 1961). Whereas on the FR1 schedule, the cumulative effects of exposure to substance may alter operant responding for reasons unrelated to its reinforcing effects (Lynch & Carroll, 2001). With respect to food, this often relates to post-ingestive satiety, whereby as food accumulates there are reciprocal homeostatic responses that reduce consumption such as the synthesis and release of anorexigenic peptides (Davis & Campbell, 1973; Sclafani & Ackroff, 2003). While producing the highest breakpoints on a PR schedule, 50% HFCS-55 produced low responding on the FR1 schedule suggesting that it may have more rapidly initiated satiety and as a consequence was self-administered less (Sclafani & Ackroff, 2003). Finally, ad libitum feeding only reduced rates of lever pressing for IO infusions of HFCS-
55 on FR1 schedules while the pattern of acquisition and maintenance remained comparable to those observed following food-restriction. This suggests that IOSA is useful tool to study the development of behaviours associated with addictive-like eating including the development of bingeing, escalation of intake and sugar-seeking in the absence of food-restriction. This is a significant advantage to IOSA as food-restriction itself can enhance these responses to incentive stimuli (Raynor & Epstein, 2003; Stafford et al., 1998).

**Not all sugars are equal with respect to abuse liability**

The abuse liability of an unknown substance can also be evaluated by comparing its reinforcing efficacy relative to that of stimuli with similar pharmacokinetic and pharmacodynamic properties and importantly, known abuse potential (Ator & Griffiths, 2003). Therefore, the behavioural and neurobiological consequences of exposure to HFCS-55 was also compared to that of sucrose. While composed of the same monosaccharides, sucrose possesses a ratio of 50% fructose: 50% glucose while HFCS-55 possess a slightly higher ratio of 55% fructose: 42% glucose plus 3% polycose sugars (Bray et al., 2009). Unlike HFCS-55 however, the abuse liability associated with sucrose has been well described with respect to its ability to reinforce operant behaviour and elicit behavioural and neurobiological indices of addiction (Alsiö et al., 2009; Bobadilla et al., 2017; Di Ciano & Everitt, 2004; Diergaarde et al., 2009; Grimm et al., 2012; Nieh et al., 2015a, 2015b; Riga et al., 2015).

Differences in fructose glucose ratios appear to have a significant impact on operant SA. Even though the sugars were perceived as equally palatable in TR tests, animals responded significantly less on the FR1 schedule for 25% HFCS-55 compared to isocaloric solutions of 20% sucrose. This difference was primarily due to a gradual but significant decline in lever pressing for 25% HFCS-55 over the latter half of IOSA sessions. The respective level of
responding that these sugars maintain on FR1 schedules suggests that the optimal set-point for reinforcement may be achieved by consuming less HFCS-55 than sucrose (Lynch & Carroll, 2001; Norman & Tsibulsky, 2001). In other words, animals may have achieved optimal intake with respect to its reinforcing effects and consequently titrated lever pressing for the remainder of the session to maintain such levels.

This implies that HFCS-55 may be a more potent reinforcer and hence possess greater abuse liability than sucrose. Such an interpretation would be in line with the observation that satiety can be conceived not only with respect to nutritional status but also with respect to reward (Norman & Tsibulsky, 2001). The notion of reward set-point refers to the observation that animals regulate operant SA of drugs of abuse to achieve some level of stable concentration of drug in blood/brain (Yokel & Pickens, 1974). The tendency to attain a reward set-point is observed in response to alterations in the available unit dose per infusion, duration of access to the drug, reinforcement schedule employed, and pharmacological pre-treatments (Gerber & Wise, 1989; Richardson & Roberts, 1996; Woods & Schuster, 2009). It is plausible that an optimal reward set-point also applies to the reinforcing properties of food stimuli (perhaps beyond its caloric value) that likewise regulates operant responding (Lynch & Carroll, 2001; Norman & Tsibulsky, 2001).

Despite consuming less HFCS-55 than sucrose, alterations in the expression of reward-related genes associated with enhanced vulnerability for addictions were also more pronounced following IOSA of HFCS-55. Like drugs of abuse, operant SA of HFCS-55 led to diminished expression of D2R mRNA in the dorsal striatum and elevated MOR mRNA in the core of the NAc (Besson et al., 2013; Georges et al., 1999; Leri et al., 2009). These neuroadaptations are associated with deficits in reward sensitivity, learning, as well as enhanced impulsive and
compulsive behaviours (Bassareo et al., 2007; Besson et al., 2013; Hall et al., 2001; Johnson & Kenny, 2010; Kelley, 1999). Recent evidence likewise indicates that ad libitum access to drink 10% HFCS-55 in rats diminishes evoked DA release in the dorsal striatum (Meyers, et al., 2018). When considered along with the novel gene expression results here, it suggests that HFCS-55 exposure may produce a state of dopaminergic hypofunction that is implicated in reduced reward sensitivity and enhanced risk for developing addictive-like behaviour (Meyers, et al., 2018; Wise, 2005).

Notably, while a similar pattern of operant SA was observed when animals responded on a FR1 schedule in the 8-arm radial arm maze for sugar pellets composed of fructose and glucose in ratios that approximate sucrose and HFCS-55, group differences were not significant. Despite this lack of difference, consumption of the high fructose ratio pellets was associated with lower Fos activation in the perifornical area of the LH and ARC nucleus. Perturbations in energy status and or food intake lead to homeostatic responses by insulin, leptin, and ghrelin that facilitate the synthesis and release of appetite regulating substrates including NPY and melanocortins from cells in these same hypothalamic regions (Brown et al., 2000; Pandit et al., 2013). In addition to these neural responses, structural and functional connectivity between the hypothalamus and mesocorticolimbic brain regions involved in reward facilitate cross-talk between the homeostatic and non-homeostatic processes that dynamically control appetite (Figlewicz et al., 2006; Naleid, et al., 2005; Palmiter, 2008). Accordingly, these data support the idea that a higher ratio of fructose in sugar may promote overconsumption, a primary feature of addictive disorders, because of its diminished ability to generate appropriate post-prandial responses that regulate satiety and hunger (Avena & Gold, 2011).
Parallel patterns of intake were observed under conditions of *ad libitum* access to drink the same isocaloric solutions of HFCS-55 or sucrose from bottles in home cages. Once again, even though less HFCS-55 was consumed, exposure to HFCS-55 elevated levels of the pro-inflammatory omega-6 polyunsaturated fatty acid in the liver. Additional studies comparing isocaloric solutions of HFCS-55 and sucrose note that even though similar quantities of sugar are consumed across 8-weeks, HFCS-55 exposure enhances *de novo* lipogenesis and triglyceride accumulation in the liver (Mock et al., 2017). This suggests that higher ratio of fructose may be more detrimental to hepatic function consistent with current findings. In fact, Simopoulos (2013) argues that the adverse consequences associated with high fructose diets parallel those linked to the recent dietary shift in the ratio of essential polyunsaturated fatty acids to one that is high in omega-6 and low in anti-inflammatory omega-3 (Simopoulos, 2002; 2013). The evidence described here supports this hypothesis as well as the suggestion that the addition of fructose to western diets via HFCS-55 may contribute not only to the development but also severity of conditions including insulin resistance (Stanhope & Havel, 2008b), non-alcoholic fatty liver disease (Ouyang et al., 2008; Samuel, 2011; Ter Horst & Serlie, 2017), and metabolic disorders (Collison et al., 2009; Goran et al., 2013).

Across experiments, group differences in weight-gain were never observed. Interestingly though, consuming 20% sucrose altered body composition leading to greater adiposity primarily due to a significant increase in the size of epidydimal fat pads. Fat accrual and not necessarily weight-gain is cited to better indicate obesity suggesting that sucrose consumption had an obesogenic effect on these animals and supports the hypothesis that sugar rich diets may promote obesity (Dinicolantonio & Berger, 2016). However, this was inconsistent with reports that in rats, drinking less 8% HFCS-55 than 10% sucrose solutions lead to greater weight-gain and
adiposity (Bocarsly et al., 2010). Rather, these data appear to be in line with the recent proposal that HFCS-55 may be more pro-inflammatory than pro-obesogenic in rats (Ma et al., 2017). Ma and colleagues (2017) similarly report that drinking isocaloric solutions of these sugars leads to weight-gain in animals consuming sucrose while HFCS-55 enhances epidydimal tissue macrophages associated with insulin resistance, the release of pro-inflammatory cytokines, and critically, suppresses anti-inflammatory responses. Such pro-inflammatory states are likewise associated with metabolic disturbances and may reflect an indirect means through which HFCS-55 may promote obesity over time.

**Pharmacotherapy for weight-loss reduces the abuse liability associated with HFCS-55**

Central to the diagnoses of FA, some eating disorders, and a subset of overweight and obesity is the development of excessive and or compulsive behaviour driven by food (Davis, 2013; Parylak et al., 2011; Schulte & Gearhardt, 2017). Not surprisingly, the physical and psychological consequences of a diagnoses of FA coupled with overweight and obesity are cited as more severe and treatment resistant (Brewerton, 2017; de Vries & Meule, 2016; Ivezaj et al., 2016). Contrave®, a novel pharmacotherapy prescribed to enhance and sustain weight-loss, purportedly reduces cravings for processed food as well as the tendency to binge and compulsively eat (Anderson et al., 2002; Greenway et al., 2010; Guerdjikova et al., 2017). It combines sustained release formulas of BUP and NTX that synergistically enhance the release of anorexigenic peptides in the hypothalamus that regulate homeostatic and hedonic responses to food.

To test the hypothesis that Contrave® may reduce appetitive consummatory behaviour and hence the abuse liability associated with HFCS-55, both food-restricted and *ad libitum* fed animals self-administered 25% HFCS-55 in IOSA on FR1 and PR schedules of reinforcement or
drank 50% HFCS-55 from bottles in home cages while continuously exposed (via subcutaneous osmotic mini-pumps) to 40 mg/kg/day BUP, 4 mg/kg/day NTX, or 40+4 mg/kg/day BUP+NTX for 12-consecutive days. Food-restricted animals also self-administered 25% HFCS-55 in IOSA immediately following acute administration (subcutaneous injection) of 30 mg/kg BUP, 3 mg/kg NTX, and 30+3 mg/kg BUP+NTX to assess acute drug effects relative to continuous exposure.

Under conditions of both continuous and acute administration, the combination of BUP+NTX reduced appetitive consummatory behaviour in both food-restricted and ad libitum fed animals. To our knowledge, these data reflect the first evidence for the anorexigenic effect of continuous administration of the combination of these drugs in rats (Levy et al., 2018). The interpretation of these findings is somewhat limited by the fact that only three studies in rodents have compared the relative efficacy of the combination of BUP+NTX to BUP or NTX on reducing consummatory behaviour (Clapper et al., 2013; Greenway et al., 2009a; Wright & Rodgers, 2013). Nevertheless, the doses selected for combined administration (30-40 mg/kg BUP and 3-4 mg/kg NTX, respectively) fall within the range of doses identified to reliably elicit at least 50% reduction in food intake when acutely administered alone (35 mg/kg BUP and 3.3 mg/kg NTX; Greenway et al., 2009a). These novel data extend the range of known doses for which this combination of drugs may reliably reduce consummatory behaviour. Moreover, the combination effectively reduced SA of a range of reinforcing concentrations of HFCS-55 indicating it may be an effective therapeutic tool for reducing the abuse liability associated with this sugar (Levy et al., 2014, 2015).

Novel findings from the gene expression analysis revealed one possible mechanism through which the combination of BUP+NTX may reduce the addictive potential of HFCS-55. It was previously reported that IOSA of 25% HFCS-55 produced alterations in MOR and D2R
mRNA typically observed following repeated exposure to drugs of abuse (Levy et al., 2015). It appears that continuous administration of BUP+NTX may attenuate and or prevent those HFCS-55 induced alterations in gene expression associated with such addictive-phenotypes in rats (Bassareo et al., 2007; Hall et al., 2001; Kelley, 1999). More specifically, administration of the combination of BUP+NTX or NTX reduced MOR mRNA expression in the NAc core in animals while BUP+NTX and BUP enhanced D2R mRNA expression in the caudate-putamen, and the magnitude of the effect was most pronounced in animals treated with the combination of drugs.

This interpretation is of course limited by comparisons between studies and importantly, by the lack of saccharin or water exposed groups to compare the effects of HFCS-55 alone on mRNA expression here. That said, the results are consistent with evidence that BUP and NTX are effective monotherapies for treating SRADs. These drugs are prescribed to reduce the reinforcing properties of drugs of abuse in a manner that may diminish subjective pleasurable effects, reduce intake, and vulnerability for relapse (Paterson, 2009; Volpicelli et al., 1995). The novel finding that combined, BUP+NTX were most effective in enhancing D2R mRNA expression suggests additional applications for the use of Contrave® in the treatment of not only pathological eating behaviours associated with FA but also compulsive drug-seeking and taking associated with SRADs. In fact, it was recently found that the combination of BUP+NTX was more effective in reducing alcohol consumption in rats relative to BUP or NTX alone (Nicholson et al., 2017).

**Interpretation of findings and limitations**

 Novel evidence collected in this dissertation suggests that not only does HFCS-55 possess abuse liability, but that compared to sucrose, consumption of HFCS-55 (even in lesser amounts) may enhance vulnerability for addictions and risk of metabolic disease (Bocarsly et al.,
2010; Mock et al., 2017; Sadowska & Bruszkowska, 2017). This is alarming given that HFCS-55 has largely replaced sucrose as the primary added sweetener in processed food and drink that are cited to promote not only overconsumption but also addictive-like behaviours in both humans and animals (Beaver et al., 2006; Ifland et al., 2009; Schulte et al., 2017; Yokum et al., 2014).

There are however, several limitations and alternative explanations to consider with respect to the interpretation of the results of the IOSA experiments. Firstly, the evidence described here was collected following considerably less exposure to HFCS-55 (i.e. 3-hours/day for 3-weeks) than the 6 to 12-hours/day exposure to drugs of abuse employed in long access studies (Ahmed & Koob, 1998). In fact, in response to short access to drugs of abuse, many of the behavioural indices of dependence are less pronounced or fail to emerge. That said, escalation of intake, binge-like behaviour, and enhanced motivation have been reported following comparable short-term and or intermittent access to both drugs of abuse and food stimuli (Corwin et al., 2011; Goeders et al., 2009; Johnson & Kenny, 2010; Klenowski et al., 2016; Liu et al., 2005). In fact, a sugar addicted-phenotype as well as neuroadaptations associated with SRADs develop within three-weeks of intermittent access to 10% sucrose solutions in rats (Avena et al., 2008). It was likely that the behavioural effects that emerged following repeated IOSA of HFCS-55 were also dependent on experience self-administering HFCS-55. That is, on PR tests, animals only displayed enhanced responding and motivation for 25% and 50% HFCS-55 following two-weeks of IOSA. Moreover, exposure to HFCS-55 over three-weeks of testing was necessary for the development of escalation of intake and bingeing behaviour.

With respect to IOSA of HFCS-55 and sucrose, it is feasible that palatability of 25% HFCS-55 was reduced in discrete IOSA sessions by the development of sensory specific satiety
(SSS) within the first hour of testing (Rolls, 1986). Consequently, reduced palatability may explain the gradual decline in lever pressing within the sessions. While this was not measured, it would be possible to assess change in orofacial reactions to the sugars by measuring TR immediately prior to and following 3-hours of IOSA, as well as terminating the IOSA session at the 1-hour mark when significant group differences emerge to test TR. The development of SSS to 25% HFCS-55 may be less likely though, given that cycles of food-restriction coupled with binge-behaviour (that are similarly employed for IOSA and observed in response to 25% HFCS-55) abolish SSS for palatable foods (Ahn & Phillips, 2012). Moreover, palatability is cited to play a primary role in determining SSS along with volume ingested (not caloric content; Bell et al., 2003; Johnson & Vickers, 1992) and its onset is typically rapid (Hetherington et al., 1989).

Over 60-minutes of testing, the volume and caloric content of the sugar solutions consumed was equivalent, suggesting factors alternative to palatability are likely responsible for group differences in IOSA.

Group differences that emerged at the 1-hour mark may also reflect the point at which the cumulative effects of consuming isocaloric HFCS-55 or sucrose influenced for example, locomotor behaviour and consequently operant SA (Lynch & Carroll, 2001). While not assessed, evidence suggests that ad libitum access to drink 8% HFCS-55 elevates general locomotor behaviour in mice relative to isocaloric solutions of 10% sucrose (Ma et al., 2017). Change in locomote behaviour induced by repeated exposure to 25% HFCS within IOSA sessions may have detracted from lever pressing, in a manner not unlike stimulant exposure leading to stereotypy that interferes with operant SA (Lynch & Carroll, 2001). It is also conceivable that within the first hour of IOSA, the cumulative effects of consuming isocaloric solutions of HFCS-55 or sucrose elicited different post-ingestional responses. Relative to sucrose, reduced
responding for 25% HFCS-55 may suggest a faster onset of satiety that limits intake. In fact, there are well described differences in the digestion and metabolism of fructose and glucose in both their bound (sucrose) and unbound (HFCS-55) forms (for review please see; Le et al., 2012; Parker et al., 2010; Rippe & Angelopoulos, 2013). These factors may influence differences that not only contribute to satiety responses but also may elicit states of discomfort that reduced lever pressing for HFCS-55 (Lynch & Carroll, 2001; Orlandi et al., 2015). A comprehensive evaluation of the behavioural satiety sequence elicited by isocaloric solutions of these sugars in rats would best address whether differences in consumption can be associated with locomotor behaviour and or adverse ingestive responses (Berridge & Grill, 1983; Ishii et al., 2003).

The interpretation that HFCS-55 is a more potent reinforcer than sucrose is also at odds with the results of PR tests. Lever pressing for either sugar on the PR schedule did not differ between groups or across PR tests 1 or 2. While breakpoint achieved increases alongside concentration and caloric content for both sucrose and HFCS-55 this is the first study, to the authors knowledge, comparing the relative reinforcing efficacy of these sugars using this schedule of reinforcement (Levy et al., 2014; Sclafani & Ackroff, 2003). Lack of group differences may relate then, to the use of isocaloric solutions of HFCS-55 and sucrose. Moreover, differences in breakpoint for sugar tend to diminish at higher concentrations (Sclafani & Ackroff, 2003), possibly due to ceiling effects that are likewise reported to affect breakpoints achieved in response to escalating doses of drugs of abuse (Richardson & Roberts, 1996). Therefore, we may have selected concentrations of HFCS-55 and sucrose that were too high to detect differences. Moreover, failure to observe differences in breakpoint achieved may indicate that the PR schedule and or criteria to measure breakpoint may have lacked the sensitivity required to detect differences in the relative reinforcing effects of these sugars given the limited
number of reinforcers obtained (Richardson & Roberts, 1996). In fact, additional aspects of operant responding such as differences in post-reinforcement pause or run-time responding are believed to more accurately address the question of satiety and reinforcement than the criteria employed for breakpoint here (Lynch & Carroll, 2001). It would be optimal to also employ for example, manipulations to study the effects of sugar substitution on operant responding for these isocaloric solutions to better characterize the relative reinforcing efficacy and abuse liability of sucrose and HFCS-55 (Ator & Griffiths, 2003; Lynch et al., 2010). This was however, not feasible as brain tissue was to be subsequently processed for differences in gene expression.

It is also important to note that differences in experimental parameters may also account for the differential effects of these sugars. In fact, isocaloric solutions of HFCS-55 and sucrose typically fail to produce differences in sugar intake or weight-gain in animals across a range of feeding schedules (i.e. ad libitum versus intermittent fed), energy status, and length of exposure to the sugars (Melanson et al., 2007; Mock et al., 2017; Sadowska & Bruszkowska, 2017; Soto et al., 2017). Moreover, preference for sugars with different ratios of fructose to glucose and vulnerability to develop DIO can vary with respect to the strain of rodent, type of sugar, and concentration employed (Glendinning et al., 2010). The concentrations employed herein also exceed those typically used to reflect isocaloric concentrations of sucrose and fructose common to sugar sweetened beverages (8% HFCS-55 and 10% sucrose; Bocarsly et al., 2010) or selected to reflect the recommended daily caloric intake from sugar (isocaloric solutions of 10-13% sugar; Mock et al., 2017). This may have limited the interpretation and generalizability of these findings; however, selecting a higher concentration for operant SA may be warranted. That is, sugar sweetened beverages are reported to contain higher concentrations of fructose than what is typically reported and individuals consume on average more added sugar than the daily
recommended intake (Walker et al., 2014). Finally, from a procedural point of view, while 8% HFCS-55 supports voluntary drinking of HFCS-55 from bottles, it was comparatively less reinforcing than 25% HFCS-55 when employed in operant IOSA.

When considering the anorexigenic effects of combining BUP+NTX on HFCS-55 intake, it was surprising that these effects appeared unrelated to the purported synergistic effects of the drugs. This was inconsistent with previous reports indicating that relative to NTX or BUP, the combination of BUP+NTX has synergistic effects on reducing food intake and fat loss in animals (Clapper et al., 2013; Greenway et al., 2009a; Wright & Rodgers, 2013) as well as weight-loss in humans (Greenway, et al., 2009b, 2010). Gene expression analysis likewise supported the hypothesis that the combination of these drugs enhances the activity of melanocortins in the hypothalamus to reduce appetitive consummatory behaviour (Greig & Keating, 2015). That is, continuous administration of BUP+NTX enhanced hypothalamic POMC mRNA expression relative to sham. However, again, the degree of upregulation was still no different than what was observed following continuous administration of NTX. Taken together, these findings imply that reduced consummatory behaviour may be primarily attributable to NTX. However, this interpretation is unlikely given that in vitro electrophysiology studies indicate that the frequency of action potentials elicited from POMC neurons in hypothalamic slices are higher in response to bath application of BUP+NTX relative to BUP and NTX (Greenway et al., 2009a). Moreover, acute administration of the combination of 20+1 mg/kg BUP+NTX is purported to protect against alterations in the expression of melanocortin genes within the hypothalamus that occur in response to weight-loss that favor maintaining a positive energy balance and re-gaining weight (Clapper et al., 2013; la Fleur et al., 2009).
While evidence for the synergistic anorexigenic effects of BUP+NTX have been reported in lean subjects, it is noted that its synergistic effects on the melanocortins and associated reductions in food-intake and weight-loss are significantly greater in those animals with DIO (Clapper et al., 2013; Greenway et al., 2009; Sinnayah et al., 2007) and likewise participants classified as over-weight or obese (Billes & Greenway, 2011; Greenway et al., 2009b; 2010). It is feasible that the lack of synergistic effect observed in the current experiments was related to the presumed lack of DIO induced by HFCS-55 consumption in these groups; however, a saccharin or water exposed group was not employed to verify this possibility. For future studies, establishing a full dose-response curve for BUP and NTX to determine the subthreshold doses necessary to reduce HFCS-55 intake and then measuring whether, when combined these doses are more effective would better address if these anorexigenic effects are synergistic.

Route of drug administration also appears to play an important role in determining the effects of pre-treatment on HFCS-55 intake. The blood plasma concentrations of the drugs were likely different in response to administration of the daily drug dose in a single subcutaneous injection (acute) relative to subcutaneous hourly release from the mini-pump (continuous). Even though both procedures promote a more sustained drug effect, the relative magnitude or onset of the drug’s effects on physiological, neurobiological and behavioural responses may have differed (Davidson et al., 2005; Turner et al., 2011a; 2011b). For example, on FR1 schedules, only acute BUP reduced HFCS-55 intake suggesting that its anorexigenic effects may not be observed under continuous administration, in line with evidence that it is only minimally effective in reducing food-intake or enhancing weight-loss in humans taking sustained release formulas (Jain, et al., 2002; Anderson et al., 2002). That said, 10 mg/kg/hour continuous BUP administration for 7-days is reported to enhance locomotion and thermogenesis in the absence of
any effects on reducing food intake in rats, possibly accounting then for its ability to promote weight-loss over time in rats (Billes & Cowley, 2008).

On a similar note, responding on PR schedules is reported to be particularly sensitive to acute elevations in brain monoamine levels (Evans & Vaccarino, 1987; Stafford et al., 1998). This may explain in part, why only acute administration of 30 mg/kg BUP, a DA and norepinephrine reuptake inhibitor, significantly enhanced motivation for HFCS-55 on PR schedules while its continuous administration either alone or in combination with NTX had no effect on IOSA of HFCS-55. It is possible that continuous administration did not elevate brain monoamine levels necessary to enhance incentive value and responding on the PR tests. This interpretation is consistent with evidence that methamphetamine which likewise enhances brain monoamine levels by blocking or reversing the direction of the DA and norepinephrine transporters, leads to greater elevations in DA in response to acute relative to continuous administration (Davidson et al., 2005).

Future studies exploring the pharmacological applications of Contrave® should investigate the synergistic effects of BUP+NTX on food- or drug-motivated behaviour using models of compulsive-seeking and taking (Edwards & Koob, 2013; Shaham et al., 2003) as well as on tasks that assess aspects of cognitive function or reward processing compromised by depression (Herrera-Guzmán et al., 2008; Slattery & Cryan, 2012). The evidence collected here highlights the importance however, in selecting an appropriate dosing procedure. The advantages of using continuous or acute administration should be weighed against findings that it may differentially affect aspects of reward measured for example, by FR1 and PR schedules of reinforcement (Davidson et al., 2005). Moreover, it is also imperative to consider that like sugars, the intrinsic properties of the drug in question may determine the efficacy of a
pharmacotherapy (Beczkowska et al., 1993; Kanarek et al., 1997). That is, the limited studies exploring the synergistic effects of BUP+NTX on SRADs to date, indicate that it is more effective than its monotherapy for reducing alcohol intake in rats but not for reducing the reinforcing properties associated with intranasal methamphetamine use in humans (Nicholson et al., 2017; Stoops et al., 2015).

Implications and conclusions

The evidence collected for this thesis contribute to the growing body of literature that strongly supports the hypothesis that sugar may be addictive and consequently, likely to promote its overconsumption when consumed by vulnerable individuals (Avena et al., 2008; Barbano & Cador, 2005; Beaver et al., 2006; Ifland et al., 2009; 2015; Moubarac et al., 2017; Oginsky et al., 2016). Although these findings also indicate that a higher ratio of fructose in sugar may be more hazardous, it would be premature to label HFCS-55 as addictive or conclude that its abuse liability is greater than sucrose. Firstly, only a subset of the criteria for abuse liability were assessed. It will be necessary to also measure whether following operant IOSA of HFCS-55, animals experience withdrawal (Avena et al., 2008b), develop insensitivity to punishment (Vanderschuren & Everitt, 2004), display vulnerability to relapse (Deroche-Gamonet et al., 2004), and importantly, develop neuroadaptations associated with these behavioural indices of dependence. Furthermore, only male rats were employed in the experiments performed and there are important sex differences in the homeostatic and hedonic responses to sugar that may influence vulnerability to develop not only addiction but also over-weight or obesity in response to sugar consumption (Burke et al., 2016; Swithers et al., 2013). It would also be beneficial to study these features of HFCS-55 abuse liability with respect to sucrose and in response to short and long access schedules of IOSA (Ahmed & Koob, 1998). Analysis of whether individual
differences emerge is also necessary, as it is well known that only a subset of those exposed to substances with abuse liability develop addiction (Deroche-Gamonet et al., 2004). Importantly, a range of concentrations of HFCS-55 should be studies to address issues of potency and exposure as they relate to the emergence of addictive-like behavioural and neuroadaptive responses.

There is much clarity to be gained by identifying what specific food(s) or in the case of sugar, food additives(s) have abuse potential. Such evidence would support the use of a classic substance use framework (Albayrak et al., 2010) to guide methods for researching the behavioural and neurobiological basis of FA, inform its diagnoses and treatment, and advance knowledge of conditions associated with maladaptive eating such as eating disorders and obesity (Horvath, 2005). This may also improve the management of pathological eating associated with these conditions by extending and adopting treatment strategies effective for SRADs (Gearhardt et al., 2014). There is however resistance within both the lay public and research community to label food as addictive and or simply recognize FA as a construct or disorder (Westwater, et al., 2016; Ziauddeen & Fletcher, 2013). This largely stems from arguments that either approach may impart a sense of lack of control or responsibility over the selection and consumption of food and or promote overeating of foods implicated as addictive; however, evidence suggests this is not the case (Gearhardt et al., 2011; Hardman et al., 2015; Hebebrand et al., 2014). In fact, strategies to improve public awareness surrounding nutrition, and policy initiatives aimed at preventing the development of over-weight and obesity are viewed more favorably and effective by individuals who likewise believe that foods may be addictive and that FA exists (Latner et al., 2014; Moran et al., 2016; Schulte et al., 2016).

Perhaps most importantly, identifying and labelling food with addictive potential may also change the policies that regulate the ease with which such stimuli are produced, sold, and
made ubiquitously available (Latner et al., 2014; Moreira et al., 2015). Such regulations emerged in response to identifying the consequences of exposure to the legal but addictive drugs alcohol and nicotine. This highlights the importance of identifying if sugars such as HFCS-55 are addictive, and possibly the upper limit of its consumption associated with abuse liability (Christensen, 2016; Rippe et al., 2017). This may allow for the development and use of warning labels on food, not unlike those found on cigarette packages, to improve the public's ability to make informed food choices (Miller & Cassady, 2015). Such policy initiatives, as well as those aimed at raising taxation or imposing restrictions on the availability and advertising of processed foods (Dilk & Savaiano, 2017; Seiders & Petty, 2004) have been proposed as harm reduction measures to help those in treatment for FA or eating disorders avoid foods with known abuse liability and in general, curb rising rates of overweight and obesity (Latner et al., 2014; Schulte et al., 2016). Such initiatives may be critical for achieving these goals as recent evidence suggests early life exposure to sugar and or cafeteria style diets during labile developmental periods leads to learning, behavioural, and neural adaptations that enhance vulnerability for not only addictions but also overweight or obesity later in life (Franklin et al., 2017; Kendig et al., 2013; Naneix et al., 2016; 2018; Noble et al., 2017; Robertson & Rasmussen, 2017; Speight et al., 2017).
Chapter 6

References


Beeler, J. a., Mccutcheon, J. E., Cao, Z. F. H., Murakami, M., Alexander, E., Roitman, M. F., &


13.


Brewerton, T. D. (2017). Food addiction as a proxy for eating disorder and obesity severity,


Clapper, J. R., Athanacio, J., Wittmer, C., Griffin, P. S., D’Souza, L., Parkes, D. G., & Roth, J.


characteristics, and impact on bariatric surgery outcomes, and proposing a standardized
definition. Surgery for Obesity and Related Diseases, 10(5), 973–982.

(2013). Differences in brain responses between lean and obese women to a sweetened drink.
Neuagastroenterology and Motility, 25(7), 579-e460.

Cook, J. B., Hendrickson, L. M., Garwood, G. M., Toungate, K. M., Nania, C. V, & Morikawa,
H. (2017). Junk food diet-induced obesity increases D2 receptor autoinhibition in the
ventral tegmental area and reduces ethanol drinking. PloS One, 12(8), e0183685.

doses of naltrexone reduce palatability and consumption of ethanol in outbred rats. Alcohol,

three rat models of binge eating. Physiology & Behavior, 104(1), 87–97.


diabetic, and impaired glucose tolerance subjects. Diabetes Care, 3(5), 575–82.

Crespi, L. P. (1942). Quantitative Variation of Incentive and Performance in the White Rat. The

Cummins, E., & Leri, F. (2008). Unreinforced responding during limited access to heroin self-


responses and preferences for sweet high-fat foods: Evidence for opioid involvement.

*Physiology and Behavior, 51*(2), 371–379.


Evans, K. R., & Vaccarino, F. J. (1987). Effects of d- and l-amphetamine on food intake:


Behavior, 98(5), 618–624.


Giuliano, C., Robbins, T. W., Nathan, P. J., Bullmore, E. T., & Everitt, B. J. (2012). Inhibition of
opioid transmission at the μ-opioid receptor prevents both food seeking and binge-like eating. *Neuropsychopharmacology*, 37(12), 2643–52.


Gold, M. S., & Avena, N. M. (2013). Animal models lead the way to further understanding food addiction as well as providing evidence that drugs used successfully in addictions can be successful in treating overeating. *Biological Psychiatry, 74*(7), e11.


Administration. *National Institute on Drug Abuse, Monograph.*


Heden, T. D., Liu, Y., Kearney, M. L., & Kanaley, J. A. (2014). Weight classification does not influence the short-term endocrine or metabolic effects of high-fructose corn syrup-


Neuron, 51(6), 801–810.


Johnson, P. M. (2010). Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats.


effects of chronic administration of naltrexone on appetite and water exchange in rats.

*Pharmacology, Biochemistry, and Behavior, 16*(6), 909–13.


https://doi.org/10.1016/j.appet.2007.07.002


Meule, A., & Kübler, A. (2012b). The translation of substance dependence criteria to food-
related behaviors: Different views and interpretations. *Frontiers in Psychiatry.*


Murray, S., Tulloch, A., Criscitelli, K., & Avena, N. M. (2016a). Recent studies of the effects of sugars on brain systems involved in energy balance and reward: Relevance to low calorie


Nathan, P. J., & Bullmore, E. T. (2009). From taste hedonics to motivational drive: central µ-


Association, 309(1), 63–70.


Rolls, B. J., Rowe, E. A., & Turner, R. C. (1980). Persistent obesity in rats following a period of


Simopoulos, A. P. (2013). Dietary omega-3 fatty acid deficiency and high fructose intake in the development of metabolic syndrome, brain metabolic abnormalities, and non-alcoholic fatty liver disease. *Nutrients, 5*(8), 2901–23.


83.


https://doi.org/10.1007/s002130050702


sucrose or high fructose corn syrup (HFCS-55) on adiposity and hepatic lipid composition in rat offspring. *Journal of Physiology, 595*(13), 4379–4398.


Wright, F. L., & Rodgers, R. J. (2013). Acute behavioural effects of bupropion and naltrexone,
alone and in combination, in non-deprived male rats presented with palatable mash.


Yu, Z., Lowndes, J., & Rippe, J. (2013). High-fructose corn syrup and sucrose have equivalent effects on energy-regulating hormones at normal human consumption levels. *Nutrition*


