The Effect of High-Fructose Corn Syrup on Ethanol Taking, Seeking, and Palatability in Rats

by

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ABSTRACT

THE EFFECT OF HIGH-FRUCTOSE CORN SYRUP ON ETHANOL TAKING, SEEKING AND PALATABILITY IN RATS

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Many alcoholic beverages contain sweeteners, such as high-fructose corn syrup (HFCS), which has been implicated in increasing alcohol consumption and encouraging hazardous drinking patterns. Therefore, four experiments were designed to investigate whether the addition of HFCS to an ethanol (EtOH) solution could alter its intake, hedonic taste, and sensitivity to food deprivation stress in Sprague Dawley rats. Experiments 1 and 2 revealed that HFCS caused a robust increase in EtOH intake during operant intraoral self-administration (IO SA), however an equally palatable, yet non-caloric sweetener (saccharin) could not replicate this effect. This suggests the caloric value of a sweetener mediates the effect of sweeteners on EtOH intake. Experiments 3 and 4 revealed food deprivation stress increased EtOH consumption during resumption following extinction, but only when HFCS was added to the solution. The palatability of 10% EtOH and 25% HFCS was also monitored across IO SA, and under conditions of food deprivation stress. It was found that the palatability of 10% EtOH and 25% HFCS increased following acquisition. More interestingly, food deprivation stress only increased HFCS palatability. Thus, it could be the heightened palatability of HFCS under stressful conditions that contributes to increased operant responding for solutions containing HFCS during resumption in rats. These results have implications for the use of highly palatable and caloric sweeteners in EtOH beverages because of the impact they may have on excessive EtOH intake.
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General rationale and summary of experiments

Heavy episodic drinking is prevalent in youth and has been shown to elevate the risk of developing an alcohol use disorder later in life (Chassin, Pitts, & Prost, 2002). Furthermore, youth who display such drinking patterns prior to the age of 18 are less likely to seek help, and have increased risks for chronic relapsing following treatment (Hingson, Heeren, & Winter, 2006). It has been suggested that the use of sweeteners in alcoholic beverages reduces the aversive taste of alcohol in naïve drinkers, and leads to increased consumption (Mosher & Johnsson, 2005; Lanier, Hayes, & Duffy, 2005; Romanus, 2000; Copeland, Stevenson, Gates, & Dillon, 2007). In fact, youth that primarily consume sweetened alcoholic beverages have four times the risk of experiencing alcohol-related injuries and report a higher frequency of alcohol binges (Albers, Siegel, Ramirez, Ross, DeJong, & Jernigan, 2015). Although this may seem intuitively obvious, the mechanisms by which sweeteners increase alcohol intake are not fully understood. More specifically, sweeteners not only change the taste of alcohol, but they also support reliable effects on behaviour and the brain. Thus, sweeteners also have the potential to synergistically alter alcohol's reinforcing value because they elicit approach and conditioned behaviour (Yasoshima & Shimura, 2015; Sclafani & Ackroff, 2017; White & Carr, 1985; Agmo & Marroquin, 1997), and maintain high levels of operant self-administration (Levy et al., 2015; Avena, Long, & Hoebel, 2005). Recently, our laboratory has explored the behavioural and neurobiological responses to high-fructose corn syrup (HFCS) in rats (Levy et al., 2014, Levy et al., 2015; Daniels, Marshall & Leri, 2016; Levy et al., 2018). In comparison to sucrose (which contains 50% glucose and 50% fructose), HFCS has a higher proportion of fructose (typically, 55%
fructose), and has been linked to several adverse health effects including obesity, heart disease, and poor metabolic health (Meyers, Mourra, & Beeler, 2017; Bray, 2013; Stanhope et al., 2015). The findings from our lab suggest that HFCS has strong reinforcing properties, and could possibly be a more potent behavioural reinforcer than sucrose. HFCS is highly prevalent in our diets, and can be found in many of the sweetened alcoholic beverages available for purchase (e.g. Palm Bay, Bacardi Silver, Smirnoff Ice, Mike’s Hard Lemonade). Thus, the current experiments were designed to investigate the effect of HFCS on ethanol (EtOH) intake, palatability, and persistence of operant responding in Sprague Dawley rats.

Experiment 1 used an operant intraoral self-administration (IO SA) technique to explore whether HFCS could alter EtOH intake. It was found that HFCS produced robust increases in IO SA behaviour and EtOH intake.

In Experiment 2, a similar experimental design was used to assess the effect of an equally palatable, yet non-caloric sweetener (saccharin; SACC) on EtOH intake. It was found that SACC increased EtOH intake, however this effect was minimal in comparison to what was observed by the addition of HFCS. These results suggest that the caloric value of a sweetener powerfully mediates the effect of sweeteners on EtOH intake in rats.

Experiment 3 tested the persistence of operant responding following behavioural extinction in rats trained to self-administer 10% EtOH in 25% HFCS. This experiment also included a stress manipulation, because stress is known to alter the reinforcing effects of drugs of abuse and facilitate drug-seeking in many species (Shaham, Erb, & Stewart, 2000). The stress selected was food deprivation because it has been shown to
have potent effects on animal drug-seeking behaviours (Shalev, Yap, & Shaham, 2001; Carroll, 1985) and because of the relevance to the behaviour being explored (i.e., ingestion of calories from both EtOH and HFCS). It was found that food deprivation stress increased persistent operant responding for EtOH, only when it was mixed in HFCS.

In Experiment 4, EtOH and HFCS taste reactivity was measured throughout the IO SA of EtOH + HFCS to assess how the palatability of these solutions are altered over subsequent exposure periods, and under conditions of food deprivation stress. This revealed two key findings. First, rats produced more palatable responses to EtOH and HFCS following IO SA acquisition. Second, food deprivation stress increased palatable responding to HFCS, but did not alter responding to EtOH. Thus, it could be the increased palatability of HFCS under stressful conditions that contributes to the increased operant responding for solutions containing HFCS during resumption following extinction in rats. In other words, the motivation to consume an EtOH beverage under normal or stressful conditions can be increased by the addition of HFCS.

Collectively, these results have implications for the use of highly palatable and caloric sweeteners in EtOH beverages because of the impact they have on EtOH intake in naïve drinkers. Below, I will sequentially review the key aspects of my research.

**Heavy Episodic Drinking in Youth Increases the Risk of AUD Development**

Heavy episodic drinking refers to the consumption of 60 or more grams of ethanol within a single occasion, occurring at least once per month (World Health Organization, 2014). This quantity of alcohol roughly equates to 5 standard drinks for men, and 4
standard drinks for women. Worldwide, heavy episodic drinking patterns are the most prevalent in young and often underaged drinkers (World Health Organization, 2014). A nationwide Canadian survey revealed 14.7% of youth aged 15-17 reported these consumption patterns, while this number increased to 33.4% between respondents aged 18-19 (Archie, Zangeneh-Kazemi, & Akhtar-Danesh, 2012). Furthermore, the US National Institute on Alcohol Abuse and Alcoholism (2015) estimates 1.45 million youth aged 12 to 17 ‘binge’ on alcoholic beverages at least once per month. These statistics are particularly concerning because young drinkers characterized as ‘bingers’ have an exceedingly elevated risk for subsequent alcohol use disorders (AUDs) compared to ‘non-binger’ youths (Chassin, Pitts, & Prost, 2002). Furthermore, early onset of alcohol drinking is correlated with increased risks of subsequent alcohol misuse and associated problem behaviours in adulthood (Gruber, DiClemente, Anderson, & Lodico, 1996; Higson, Heeren, & Winter, 2006; DeWit, Adlaf, Offord, & Ogborne, 2000; Hawkins, Graham, Maguin, Abbott, Hill, & Catalano, 1997). Thus, reducing excessive alcohol consumption in young, naïve drinkers could be an effective strategy to mitigate the high prevalence of disordered drinking worldwide.

**Alcohol Use Disorders**

Alcohol use disorders increase many health risks for the abuser and place a heavy burden on healthcare systems worldwide. In fact, the World Health Organization (2009) reported alcohol use as the third leading risk factor contributing to the global burden of disease. Heavy alcohol consumption is causally linked to liver and heart disease, birth defects, cancer, and unintentional injuries (Rehm et al., 2009). Additionally, AUDs are comorbid with a wide range of psychiatric disorders, including, anxiety disorders, major
depressive disorders, post-traumatic stress disorder, substance use disorders, and schizophrenia (Petrakis, Gonzalez, Rosenheck, & Krystal, 2002; Regier et al., 1990). Despite the negative consequences associated with excessive alcohol consumption, the prevalence rate for AUDs remains high at approximately 4% worldwide (The World Health Organization, 2014).

(1) Chronic Relapse

The high occurrence of AUDs is partially attributable to the inability of abusers to maintain abstinence following alcohol detoxification and in-patient treatment programs. Studies reveal high relapse rates following both short-term (Moos & Moos, 2006) and long-term remission periods (Jin, Rourke, Patterson, Taylor, & Grant, 1998), signifying that a relapse to excessive alcohol consumption can be triggered regardless of long-term periods of abstinence. For example, Jin et al., (1998) measured relapse rates in long-term abstainers, defined as individuals with 18-months of stable abstinence, and found 31% had relapsed prior to an 11-year follow-up period.

The high and frequent occurrence of relapses during recovery has led to the recognition of AUDs as chronic relapsing disorders. Given that the recovery from an AUD is a long and sometimes impossible road, preventative measures may be an effective method to reduce AUD prevalence. That is, preventing disordered drinking in naïve drinkers may reduce the incidents of AUDs.

(2) Triggers of Relapse

Identifying AUDs as chronic relapsing disorders has drawn attention to the identification of factors that provoke a relapse, and the neural correlates that underlie this behaviour. Exposure to alcohol-paired stimuli is one determinant of alcohol relapse
following abstinence (Perry, Zbukvic, Kim, & Lawrence, 2014). For example, detoxified alcoholics experience increased alcohol craving, and physiological responses, when exposed to alcohol-paired stimuli in a laboratory setting (Garland, Franken, & Howard, 2012; Cooney, et al., 1997). Furthermore, the magnitude of post-treatment reactivity to alcohol-paired stimuli accurately predicts future relapses (Garland et al., 2012). These findings suggest alcohol-paired stimuli possess incentive salience, acquired through repetitive pairings with alcohol’s reinforcing pharmacological properties. That is, through associative learning, alcohol-paired stimuli become conditioned cues, or conditioned reinforcers (CRs), capable of eliciting a craving response and motivating behaviour.

Given the taste of alcohol is prominent during consumption, alcohol-paired taste cues may act as CRs to motivate alcohol consumption behaviour. Indeed, exposure to the taste of beer significantly increases alcohol craving in heavy drinkers when compared to other flavoured controls (i.e. Gatorade; Oberlin et al., 2013). Additionally, neuroimaging studies reveal that the taste of alcohol activates mesocorticolimbic structures in the brain (Filbey et al., 2008), and increases dopamine (DA) release in these areas (Oberlin et al., 2013). Given that mesolimbic DA signaling is implicated in reward, motivation (see Robins & Everitt, 1996 for a review) and goal-directed behaviour (Wise & Rompre, 1989; Horvitz, 2000), these findings suggest alcohol-paired tastes can drive appetitive behavioural responding for alcohol, and perhaps contribute to relapse following abstinence.

Exposure to stress is another major determinant of relapses following AUD treatment (Preston & Epstein, 2011). For example, the occurrence of severe stress following treatment is correlated with increased relapse risk (Brown, Vik, Patterson, Grant, &
Schuckit, 1995; Brown et al., 1990). Brown et al. (1990) found alcoholics who had relapsed during a three-month treatment follow-up had self-reported significantly more severe stress during the study period than those who remained abstinent. Additionally, stress induces alcohol cravings in abstinent alcoholics (Preston & Epstein, 2011). Fox, Bergquist, Hong, and Sinha, (2007) used a stress imagery technique to induce anxiety in former alcoholics within a laboratory setting. They found stress imagery significantly increased alcohol craving, as well as measures of blood pressure, in comparison to a neutral imagery condition. Similar to cue-induced craving, the magnitude of laboratory stress-induced alcohol craving can also predict abstinence following treatment (Sinha et al., 2011; Higley et al., 2011). For example, Higley et al. (2011) demonstrated greater laboratory stress-induced craving was associated with a significantly shorter duration of alcohol abstinence, fewer total days of abstinence, and a higher mean number of drinks per week following treatment.

In sum, both exposure to alcohol-paired stimuli acting as CRs, and stress, have been implicated in provoking alcohol relapse following AUD treatment. The data presented is largely derived from human laboratory studies of alcohol-induced craving, or from correlative measures of CR/stress exposure on subsequent relapse rates. Animal models of relapse can causally measure the influence of CRs and stress on relapse-like responding, thereby strengthening the assumptions made regarding CR and stress exposure on relapse vulnerability. Operant drug self-administration (SA) procedures model “drug-taking” and “drug-seeking” in animals, and are used to analyze the factors that contribute to drug addiction, including relapse-like responding (Panlilio & Goldberg, 2007).
Measuring Drug-Intake in Animals: Operant Self-Administration Models

The fundamental assumptions used by the operant SA model date back to the early works of Thorndike (1898) who proposed behaviours that produce favourable outcomes are more likely to be repeated than behaviours producing non-favourable outcomes. Using this theoretical framework, operant SA procedures allow an animal to perform a behavioural response for the administration of drug reinforcement; thus, if the drug is indeed a reinforcing substance, the operant response which led to its administration should continue to be repeated.

In rodents, operant drug SA models are conducted in operant conditioning chambers that are typically equipped with an active and inactive lever (O’Conner, Chapman, Butler, & Mead, 2011). Responding on the active lever (a lever-press) results in the delivery of drug reinforcement, thereby producing a measure of drug-reinforced responding, or “drug-taking”. A lever-press on the inactive lever results in no programmed consequences, and can therefore be used as a control measure of non-reinforced operant responding. Measures of drug intake (number of drug infusions) or operant responding (active versus inactive lever responding) are used as indications of SA behavior, and help researchers make inferences about the drug’s abuse potential.

(1) Extinction-Reinstatement Procedures

Extinction-reinstatement procedures stem from operant drug SA models, and measure “drug-seeking” in animals (Panlilio & Goldberg, 2007; Shaham, Shalev, Lu, deWitt, & Stewart, 2002). Following initial acquisition of SA, extinction of the drug-taking behaviour is accomplished by removing response-contingent drug reinforcement. As a result, responding under extinction conditions declines rapidly as the behaviour is no
longer being reinforced (Katner, Magalong, & Weiss, 1999). Thus, any behavioural responding in the absence of drug reinforcement is a measure of “drug-seeking” rather than drug-taking. Once drug-seeking is extinguished, a reinstatement test can be used to delineate the factors that provoke a reinstatement of the extinguished responding (i.e. drug-seeking) for that drug. Therefore, extinction-reinstatement procedures can measure relapse-like responding following a period of abstinence and are invaluable tools in drug addiction research (Panlilio & Goldberg, 2007; Martin & Weiss, 2013).

In corroboration with the human literature on relapse vulnerability, exposure to alcohol-paired stimuli reinstates extinguished alcohol-seeking in rats. For example, re-exposure to drug-taking environments potently reinstates previously extinguished drug-taking behaviour (see Crombag, Bossert, Kova, & Shaham, 2008 for a review). Chaudhri, Sahuque, Cone and Janak (2008) trained rats to self-administer alcohol in an environmental context distinguished by distinctive visual, olfactory and tactile stimuli. Subsequently, alcohol-taking behaviour was extinguished in a separate and perceptually altered context. During reinstatement, animals were given a small oral alcohol prime (i.e. re-exposure to oral alcohol) in the drug-taking context, or in the extinction context. Reinstated responding for alcohol was observed when rats were primed in the alcohol-taking context but not in the extinction context; thus, demonstrating alcohol-paired contextual stimuli modulate behavioural responding for alcohol after periods of abstinence.

The taste of alcohol, or flavours paired with alcohol, also hold conditioned reinforcing effects that affect alcohol-seeking behavior in rats. For example, oral alcohol primes (Bienkowski, Koros, Kostowski, & Bogucka-Bonikowska, 2000; Maccioni et al., 2007)
and alcohol-paired taste cues (Knight et al., 2016) reinstate responding for alcohol following behavioural extinction. Knight et al. (2016) paired a distinct flavour with alcohol during SA acquisition to create an excitatory conditioned stimulus (CS+). Therefore, the CS+ signaled the presence of alcohol reinforcement while rats acquired and stabilized alcohol-taking behaviour. Subsequently, a different flavour was paired with water during an extinction phase to create an inhibitory conditioned stimulus (CS-), which served to signal the absence of alcohol reinforcement. During a reinstatement session, rats were placed back into the conditioning chambers and allowed to self-administer either the CS+, CS- or a neutral taste stimulus (CS0) in water. Lever responding was significantly elevated in the CS+ group when compared to extinction baseline responding, demonstrating a flavour previously paired with alcohol reinstated extinguished alcohol-seeking behaviour in rats. Moreover, there was no reinstatement of lever responding found in the CS- or CS0 group. Taken together, these studies support the human literature that suggests the conditioned reinforcing properties of alcohol-paired cues, such as taste, provoke appetitive behavioural responding for alcohol following periods of abstinence.

Exposure to stress also reinstates alcohol-seeking behaviour in rats using the extinction-reinstatement model. Lê, Harding, Juzutsch, Funk and Shaham (2005) induced a stressed-state in rats by exposing animals to 10-minutes of intermittent footshock (0.8 mA). Footshock stress immediately preceding a reinstatement test potently increased alcohol-seeking behaviours following extinction. Moreover, stress may interact with the effect of conditioned cue exposure to potentiate alcohol-seeking behaviours. For example, Liu and Weiss (2002) demonstrated a brief exposure to
footshock stress exacerbated alcohol-seeking behaviours elicited by conditioned cue exposure in comparison to alcohol-seeking behaviours elicited by conditioned cue exposure alone.

(2) Extinction-Reacquisition Procedures

Extinction-reacquisition procedures also stem from SA models to measure the persistence of drug-taking following the extinction of this behaviour. During reacquisition testing, response reinforcement is restored, making it possible to explore the capacity for an animal to relearn extinguished operant responding. Typically, the reacquisition of extinguished operant drug-reinforced responding, otherwise called resumption, is more rapid in comparison to the original acquisition (Bouton, Winterbauer, & Todd, 2012; Willcocks & McNally, 2013). This suggests that the extinction of a reinforced behaviour does not equate with the unlearning of previously reinforced responding. Moreover, extinction-reacquisition procedures also support the notion that exposure to CRs and/or stress exacerbates relapse-like responding. For example, Yu, Chen and Sharp (2014) demonstrated exposure to restraint stress during a period of abstinence exacerbated the reacquisition of nicotine self-administration when animals were reintroduced to the operant chambers.

In sum, the same factors that have been implicated to provoke relapse in human populations (i.e. alcohol-paired cues, stress) also reinstate extinguished alcohol-seeking and alcohol-taking behaviours in rats using animal models of relapse. This signifies the translational efficacy of using operant alcohol SA procedures in animals to make inferences about human alcohol relapse behaviour. Furthermore, by producing alcohol-taking and alcohol-seeking behaviour in animal models, the neurobiology of this
responding can be investigated by pharmacological manipulation of different neurotransmitter systems. Understanding the neural mechanisms underlying alcohol-taking and alcohol-seeking behaviour will assist in the development of therapeutic treatment options for AUD patients who experience chronic relapses following treatment.

**Food Deprivation Stress**

Food deprivation increases behavioural and neurobiological biomarkers of stress in humans and animals. For example, the magnitude of dietary restriction is associated with increased urinary cortisol excretion (McLean, Barr, & Prior, 2001) and elevated salivary cortisol levels (Anderson, Shapiro, Lundgren, Spataro, & Frye, 2002) in healthy adults. Rodent studies corroborate these findings by showing food deprivation increases corticosterone levels (Nowland, Hugunin, & Rogers, 2011; Dietze, Lees, Fink, Brosda & Voigt, 2016). Thus food deprivation elicits a stress response that could produce reward-seeking behaviour in humans and animals.

In humans, being in a food deprived state is associated with increases in motivation to seek rewarding stimuli. For example, Leeman, O’Malley, White, and McKee (2010) found that daily smokers who participated in a 12-hour food deprivation period smoked more cigarettes during the study period and reported stronger cravings for nicotine than participants who were satiated. Moreover, studies support that food deprivation increases a healthy individual’s persistence of behavioural responding for rewarding stimuli (Hogenkamp, Shechter, St-Onge, Sclafani, & Kissileff, 2017; Bulik & Brinded, 1994). For example, food deprivation has been found to increase the amount of behavioural responding for sweetened beverages (Hogenkamp, Shechter, St-Onge,
Sclafani, & Kissileff, 2017; Bulik & Brinded, 1994) and cigarettes (Bulik & Brinded, 1994).

In animal models, food deprivation stress reliably increases drug-taking and drug-seeking behaviours. For example, 24- and 48-hour food deprivation has been shown to potently increase heroin-seeking and cocaine-seeking in rodents (Shalev, Yap, & Shaham, 2001; D’Cunha, Sedki, Macri, Casola, & Shalev, 2013; Tobin, Newman, Quinn, Shalev, 2009; Carroll, 1985; Highfield, Mead, Grimm, Rocha, & Shaham, 2002). Furthermore, animal researchers frequently use food deprivation as a reliable method to increase the intake of various drugs of abuse (Carr, 2007).

**The Self-Administration of Alcohol in Rats**

Rats will self-administer alcohol intravenously (IV; Smith & Davis, 1974; Gass & Olive, 2007), intragastrically (IG; Waller, McBride, Gatto, Lumeng, & Li, 1984; Fidler & Clews, 2006) or orally (Chaudhri et al., 2008). IV and IG self-administration are useful tools in studies of drug addiction, however their use in alcohol-related research is limited by the lack of face-validity provided when applying their findings to the prototypical oral consumption patterns found in humans. Additionally, IG and IV methods by-pass important orosensory aspects of alcohol consumption (such as smell and taste), which as previously mentioned, have been demonstrated to modulate alcohol craving in humans (Oberlin et al., 2013) and alcohol-seeking behaviours in rats (Katner, Magalong, & Weiss, 1999; Maccionia et al., 2007; Knight et al., 2016).

Although oral alcohol SA in rats can be established, there are procedural complications with the existing system which delivers alcohol reinforcement using a fluid receptacle (FL-R). More specifically, a dipper-cup raises a small quantity (0.1 ml) of an
alcohol solution from a fluid reservoir, into a trough within the operant conditioning chamber (Chaudhri, et al., 2008; Maccioni et al., 2007). The dipper-cup presents the alcohol solution for a predetermined time interval, and then recedes back into the Fl-R until the next reinforcement is delivered. Therefore, this approach requires an animal to perform the operant response, and then move to the Fl-R to consume the alcohol reinforcer. As such, the Fl-R delivery approach poses several caveats for measuring operant alcohol SA responding.

One limitation of the Fl-R delivery approach is the delay experienced between the operant response and delivery of reinforcement; a variable known to retard SA acquisition (Williams, 1976; Black, Belluzzi, & Stein, 1985). Williams (1976) measured the influence of varying response-reinforcement delays on the responding for grain in food-deprived pigeons. It was demonstrated that even at the shortest delay (3-secs) pigeons displayed a drastic reduction in responding when compared to a non-delayed baseline. Additionally, Black, Belluzzi and Stein (1985) found delays as minimal as 1-sec significantly impaired the acquisition of reinforcing brain self-stimulation in rats. Thus, the delay experienced between the performance of an operant response and the delivery of an oral alcohol reinforcer into a Fl-R may produce deficits in alcohol SA acquisition, and lower behavioural responding.

Another limitation of the Fl-R approach is the inability to accurately measure alcohol intake on a trial-by-trial basis, limiting the interpretation of consumption data. That is, following each alcohol presentation the dipper-cup recedes back into the fluid reservoir without measuring whether the liquid was consumed. The amount of alcohol intake can be calculated by measuring the remaining liquid in the fluid reservoir at the end of each
session; however, trial-by-trial consumption is left undetermined. Additionally, without trial-by-trial consumption data it is unknown whether each operant response is being reinforced, thereby devaluing the capacity for SA behaviour to be a reliable measure of alcohol-reinforced responding. Furthermore, unreinforced responding can lead to an extinction of the operant response (Katner, Magalong, & Weiss, 1999), hence disturbing the acquisition and maintenance of SA behaviour.

An additional shortcoming of the FI-R technique is the variability in timing of liquid prime deliveries. Oral alcohol primes are often delivered during reinstatement/reacquisition procedures, as they have been demonstrated to reinstate extinguished alcohol-seeking in rats (Bienkowski et al., 2000; Maccioni et al., 2007). Using the FI-R technique, an effective oral prime delivery is contingent upon the animal’s approach response to the FI-R. However, a rat must be responsive to the FI-R to receive the administered prime. If rats differ in their receptiveness to the FI-R, the timing of obtaining the oral prime could vary between-subjects. It has been demonstrated that as many as 15 dipper-cup presentations are required to reinstate extinguished responding for alcohol in rats (Bienkowski et al., 2000), an effect perhaps attributable to inconsistencies in prime delivery between-subjects.

A Novel Technique Approach to the Oral Self-Administration of Alcohol in Rats.

Recently, a novel intraoral (IO) delivery technique was developed to administer liquid reinforcement directly into the oral cavity of a rat during operant SA sessions (Levy et al., 2014). By surgically implanting an oral cannula into the cheek, the IO delivery technique overcomes many of the caveats posed by the FI-R approach. First, IO administrations will strengthen the response-reinforcement contingency by rapidly
delivering alcohol reinforcement upon the behavioural response. Second, in IO SA rats directly consume each liquid reinforcer, providing consumption data for each trial. Third, IO SA permits passive IO liquid infusions, allowing for a priming technique that does not require movement toward the FI-R, thereby decreasing between-subject variability.

In addition to strengthening the measurement of operant alcohol SA responding, IO SA allows alcohol’s taste properties to be more efficiently investigated during reinstatement and reacquisition procedures. That is, rather than the presentation of a liquid prime in a FI-R (visual + orosensory cues), IO primes are delivered directly into the oral cavity to permit the analyses of taste and orosensory stimulation selectively.

The Reinforcing Properties of Sweeteners

Sweet substances have substantial and reliable effects on behavior. For example, people describe the taste of sweeteners as pleasant (Thai et al., 2011; Frank et al., 2008) and show preference for beverages that have been sweetened (Tatzer, Schubert, Timischl, & Simbruner, 1985; Ventura & Mennella, 2011). In addition, individuals display the motivation to consume sweetened beverages by exerting effort to gain access to its administration (Schebendach et al., 2017; Guttman, 1954). The liking and preference for sweeteners may be linked to activity in cortical regions that process reward functions (Frank et al., 2008). Indeed, a variety of sweeteners increase the release of dopamine into the nucleus accumbens (NAc; Rada, Avena & Hoebel, 2005; Wheeler et al., 2011; Hajnal, Smith, & Norgren, 2004), a brain region implicated in attributing incentive salience to rewarding stimuli (Wyvell & Berridge, 2000; Steinberg et al., 2014).

Although individuals show difficulty in discriminating between caloric and non-caloric sweetness (Frank et al., 2008), caloric sweeteners are known to be more potent
reinforcers in terms of their behavioural and neurobiological effects (Domingos et al., 2011; White & Carr, 1985). Some have even argued that caloric sweeteners may have “addictive-like” properties, similar to drugs of abuse (Levy et al., 2015; Lenoir, Serre, Cantin, & Ahmed, 2007; Madsen & Ahmed, 2014).

(1) High fructose corn syrup

High-fructose corn syrup (HFCS) is highly prevalent in our diets (Barlow, McKee, Basu, & Stuckler, 2017; Bray, Nielsen, & Popkin, 2004), and has been linked to several adverse health effects including obesity, heart disease, and poor metabolic health (Meyers, Mourra, & Beeler, 2017; Bray, 2013; Stanhope et al., 2015). In comparison to sucrose (which contains 50% glucose and 50% fructose), HFCS has a higher proportion of fructose (typically, 55% fructose), and this is sufficient to engender different behaviours during operant intraoral self-administration, as well as different patterns of gene expression in the brain (Levy et al., 2015). Despite the recognition that the reinforcing properties of HFCS differs from that of sucrose, (Ackroff & Sclafani, 2011; Levy et al., 2015) research investigating the effect of HFCS on behaviour and brain is quite limited.

Recently, this laboratory has explored behavioral and neurobiological responses to high-fructose corn syrup in laboratory rats (HFCS; Levy et al., 2014; Levy et al., 2015; Levy et al., 2018; Daniels, Marshall, & Leri, 2016). In Levy et al. (2015), rats were trained to intraorally self-administer isocaloric solutions of 25% HFCS and 20% sucrose under multiple schedules of reinforcement. It was found that rats self-administered much more sucrose than HFCS during IO SA. Following IO SA, mRNA for dopamine receptor 2 and mu-opiod receptor genes were quantified in brain regions that are
implicated in addictive behaviours. Results indicated that although rats self-administered much more sucrose than HFCS, only HFCS led to similar neural adaptations that are present following repeated exposure to drugs of abuse. Thus, Levy et al. (2015) concluded that HFCS may be a more potent reinforcer than sucrose in terms of the behavioural and neural adaptations it produces following exposure.

**Sweeteners (HFCS) in alcoholic beverages**

It has been suggested that the use of sweeteners in alcoholic beverages reduces the aversive taste of alcohol, and leads to increased consumption in naïve drinkers (Mosher & Johnsson, 2005; Lanier et al., 2005; Romanus, 2000; Copeland, Stevenson, Gates, & Dillon, 2007). Indeed, youth that primarily consume sweetened alcoholic beverages report a higher frequency of binge drinking episodes (Albers, Siegel, Ramirez, Ross, DeJong, & Jernigan, 2015). Furthermore, it is well established that animals consume greater quantities of alcohol when it is mixed in a sweet solution. In fact, rats fail to acquire the self-administration of EtOH without the use of sucrose-pairings, sucrose-fading or other lengthy initiation procedures (i.e. intermittent access schedules, food/water deprivation; Samson, 1986; Samson, Pfeffer, & Tolliver, 1988).

Although this may seem intuitively obvious, the mechanisms by which sweeteners increase alcohol intake are not fully understood. That is, sweeteners not only change the taste of alcohol, but they also support reliable effects on behaviour and the brain. Thus, sweeteners also have the potential to synergistically alter alcohol’s reinforcing value. For example, in addition to increasing alcohol intake in animals, sweet substances are also capable of eliciting approach behaviour (Yasoshima & Shimura, 2015), conditioning neutral stimuli (Sclafani & Ackroff, 2017; White & Carr, 1985; Agmo
& Marroquin, 1997), and maintaining high levels of operant self-administration (Levy et al., 2015; Avena, Long, & Hoebel, 2005).
THE EFFECTS OF HIGH-FRUCTOSE CORN SYRUP ON ETHANOL TAKING, SEEKING AND PALATABILITY IN MALE RATS.

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Abstract

Many alcoholic beverages contain sweeteners, such as high-fructose corn syrup (HFCS), which has been implicated in increasing alcohol consumption and encouraging hazardous drinking patterns. Therefore, four experiments were designed to investigate whether the addition of HFCS to an ethanol (EtOH) solution could alter its intake, hedonic taste, and sensitivity to food deprivation stress in Sprague Dawley rats. Experiments 1 and 2 revealed that HFCS caused a robust increase in EtOH intake during operant intraoral self-administration, however an equally palatable, yet non-caloric sweetener (saccharin) could not replicate this effect. This suggests the caloric value of a sweetener mediates the effect of sweeteners on EtOH intake. Experiments 3 and 4 revealed food deprivation stress increased EtOH consumption during resumption following extinction, but only when HFCS was added to the solution. The palatability of 10% EtOH and 25% HFCS was also monitored across IO SA, and under conditions of food deprivation stress. It was found that the palatability of 10% EtOH and 25% HFCS increased following acquisition. More interestingly, food deprivation stress only increased HFCS palatability. Thus, it could be the heightened palatability of HFCS under stressful conditions that contributes to increased operant responding for solutions containing HFCS during resumption in rats. These results have implications for the use of highly palatable and caloric sweeteners in EtOH beverages because of the impact they may have on excessive EtOH intake.

Key words

Operant self-administration, intraoral, alcohol intake, high-fructose corn syrup
Introduction

Heavy episodic drinking refers to the consumption of 60 or more grams of alcohol within a single occasion, occurring at least once per month (World Health Organization, 2014). This quantity of alcohol roughly equates to 5 standard drinks for men, and 4 standard drinks for women and is estimated to occur amongst 16% of drinkers worldwide (World Health Organization, 2014). This statistic is concerning given that heavy alcohol consumption is linked to numerous adverse health effects including, liver and heart disease, birth defects, cancer, and unintentional injuries (Rehm et al., 2009). In fact, the World Health Organization (2009) reported alcohol use as the third leading risk factor contributing to the global burden of disease. In addition to the adverse health effects associated with binge episodes (Massey & Arteel, 2012; Bala, Marcos, Gattu, Catalano, & Szabo, 2014; Mathurin & Deltenre, 2009), the chronic consumption of large quantities of alcohol increases an individual’s risk for subsequent disordered drinking patterns and associated problem behaviours (Chassin, Pitts, & Prost, 2002; Hingson, Heeren, & Winter, 2006).

Although there are several factors that facilitate the overconsumption of alcohol, a key element appears to be the addition of sweeteners to alcoholic beverages (Mosher & Johnsson, 2005; Copeland, Stevenson, Gates, & Dillon, 2007; Metzner & Kraus, 2008; Albers, Siegel, Ramirez, Ross, DeJong, & Jernigan, 2015). For example, individuals that primarily consume sweetened alcoholic beverages have four times the risk of experiencing alcohol-related injuries and report a higher frequency of heavy episodic drinking (Albers et al., 2015). Furthermore, animal models show that sweeteners increase the amount of alcohol self-administered in both home-cage and
operant self-administration procedures (Samson, Pfeffer, & Tolliver, 1988). In fact, most species fail to acquire the self-administration of EtOH without the use of sucrose-fading or other lengthy initiation procedures (i.e. intermittent access schedules, food/water deprivation; Samson, Pfeffer, & Tolliver, 1988).

Sweet substances are known to have substantial and reliable effects on behavior. For example, people describe the taste of sweeteners as pleasant (Thai et al., 2011; Frank et al., 2008) and show preference for beverages that have been sweetened (Tatzer, Schubert, Timischl, & Simbruner, 1985; Ventura & Mennella, 2011). In addition, individuals display the motivation to consume sweetened beverages by exerting effort to gain access to its administration (Schebendach et al., 2017; Guttman, 1954). The liking and preference for sweeteners may be linked to activity in cortical regions that process reward functions (Frank et al., 2008). Indeed, a variety of sweeteners increase the release of dopamine into the nucleus accumbens (NAc; Rada, Avena & Hoebel, 2005; Wheeler et al., 2011; Hajnal, Smith, & Norgren, 2004), a brain region implicated in attributing incentive salience to rewarding stimuli (Wyvell & Berridge, 2000; Steinberg et al., 2014). Although individuals show difficulty in discriminating between caloric and non-caloric sweetness (Frank et al., 2008), caloric sweeteners are known to be more potent reinforcers in terms of their behavioural and neurobiological effects (Domingos et al., 2011; White & Carr, 1985). Some have even argued that caloric sweeteners may have “addictive-like” properties (Levy et al., 2015; Lenoir, Serre, Cantin, Ahmed, 2007; Madsen & Ahmed, 2014).

Recently, this laboratory has explored the behavioral and neurobiological responses to high-fructose corn syrup in laboratory rats (HFCS; Levy et al., 2014; Levy
et al., 2015; Levy et al., 2018; Daniels, Marshall, & Leri, 2016). This sweetener was selected for investigation because its consumption is highly prevalent in our diets (Barlow, McKee, Basu, & Stuckler, 2017; Bray, 2013), and linked to several adverse health effects including obesity, heart disease, and poor metabolic health (Meyers, Mourra, & Beeler, 2017; Bray, 2013; Stanhope et al., 2015). In comparison to sucrose (which contains 50% glucose and 50% fructose), HFCS has a higher proportion of fructose (typically, 55% fructose), and this is sufficient to engender different behaviours during operant intraoral self-administration in rats, as well as different patterns of gene expression in the brain (Levy et al., 2015).

The overall aim of this study was to explore the impact of HFCS on operant intraoral self-administration of ethanol (EtOH) in outbred male rats. This is a relevant question because HFCS is used as sweetener in many alcoholic beverages available for purchase (i.e. Smirnoff Ice, Mike’s Hard Lemonade, Bacardi Silver). It is generally known that sweeteners change the taste of EtOH (Blednov et al., 2008) and facilitate consumption in various species (Tolliver, Sadeghi, & Samson, 1988; Mandillo, Titchen, & Miczek, 1998; Lanier, Hayes, & Duffy, 2004), but how HFCS interacts with EtOH’s reinforcing effect is currently unknown.

Therefore, Experiment 1 was designed to establish whether HFCS can modify operant intraoral self-administration (IO SA) of alcohol (EtOH; 5%, 10% and 20% alc/vol) in outbred male rats. Experiment 2 was designed to contrast the effects of HFCS and saccharin (SACC; a non-caloric sweetener) on EtOH IO SA. Experiment 3 tested the persistence of operant responding following extinction conditions in animals trained to IO SA EtOH & HFCS. This experiment also included a stress manipulation,
which is known to alter the reinforcing effects of drugs of abuse and facilitate drug-seeking in many species (Shaham, Erb, & Stewart, 2000). We selected food deprivation stress (Shalev, Marinelli, Baumann, Piazza, & Shaham, 2003; Sinha, 2008) because of the relevance to the behaviour explored (i.e., ingestion of calories from both EtOH and HFCS). Finally, Experiment 4 expanded the findings of Experiment 3 by also investigating how changes in EtOH and HFCS palatability, assessed by taste reactivity (Grill & Norgren, 1978), may play a role in persistent drug-taking, drug-seeking and the effect of food deprivation stress.

**Methods**

**Subjects**

Male Sprague Dawley rats were acquired at 175-200 grams (Charles River Laboratories, Saint-Constant, Quebec) for all experiments. Animals were single-housed and located in a colony room maintained on a 12-hour light/dark cycle (7:00/19:00h). Standard rat chow and water were provided ad-libitum, unless indicated otherwise below. Rats were acclimated to the colony room and handled daily for one week prior to any experimental conditions. All procedures were reviewed and approved by The University of Guelph Animal Care Committee and adhere to sanctions put forth by the Canadian Council on Animal Care.

**Apparatus**

**Self-administration chambers.** Plexiglas operant conditioning chambers (model ENV-008CT, Med Associates, Georgia, VT) were used for all SA procedures. Each conditioning chamber was located within a larger sound-attenuating partition, with a built-in fan to mask noise and provide constant ventilation. An overhead houselight (28-
V) was situated on the back wall of each chamber. On the front wall, a retractable lever (8-cm above floor level) was situated just below a cue-light (28-V; 11-cm above floor level). The retractable lever served as an active lever during SA sessions, and was linked to a liquid infusion pump (Razel Scientific Instruments, Stamford, Connecticut). On the opposite side of the front wall, there was a non-retractable lever (8-cm above floor level) that served as an inactive lever during SA sessions. Stainless steel bars provided the foundation of the conditioning chambers (30.5-cm x 24.1-cm x 21.0-cm). A MED-PC interface controlled all conditioning chambers through MED-PC compatible software.

Taste reactivity chambers. Taste reactivity chambers were constructed of clear Plexiglas (22.5 x 26.0 x 20.0 cm) that sat atop a table with a clear glass top. A mirror was placed beneath the chamber, tilted at a 45° angle to facilitate adequate viewing of the rat’s ventral surface. Polyethylene tubing connected the IO cannula to an infusion pump (KDS100; KD Scientific Inc., USA) which was set to deliver liquid for two minutes at a rate of 0.5 ml/min. All taste reactivity sessions were recorded with a video camera for experimenter scoring.

Surgery

IO cannulas were surgically implanted following a week of acclimation to the colony room. The materials and procedures used for IO cannulation surgery have been previously described in detail (Levy et al., 2014). Briefly, cannulas were constructed by flanging polyethylene-90 tubing (approximately 80-mm in length) using a soldering iron. Rats were then anesthetized using isoflurane, and cannulas were surgically implanted. Using a 15-G stainless steel needle, cannulas were woven subcutaneously through the
skin at the back of the neck to protrude intraorally from the left cheek. A head-cap was constructed using dental cement to uphold the bolt used to secure IO tubing during SA sessions. Rats were given a week to recover following all surgical procedures.

**General Procedures**

**Operant self-administration:** Rats were food-restricted for all IO SA sessions by allowing unlimited access to standard rat chow for only three hours daily. Feeding occurred immediately following IO SA sessions. All IO SA sessions began 4-hrs into the rat's dark (active) cycle and ran for 180-min duration. Cannulas were flushed at the beginning of every session with reverse osmosis water to ensure there were no obstructions in the delivery line.

Rats were placed into the conditioning chambers and allowed to habituate for 5-min prior to each session. Illumination of the house light signaled the beginning of a session, with the active lever extending out after a 10-sec delay. Subsequently, responding on the active lever resulted in a 0.1-ml IO infusion of liquid reinforcement on a fixed-ratio 1 (FR1) or fixed-ratio 3 (FR3) schedule of reinforcement, and the presentation of a cue-light (27.5-secs). During this time, responding on the active lever was recorded but did not lead to additional reinforcement. Responding on the inactive lever had no programmed consequences under any experimental conditions as it served as a baseline of non-reinforced operant responding.

For the first three sessions of each IO SA experiment the lever-press response was shaped by experimenter-induced priming. During priming sessions, rats were allowed 15-min to perform an active lever-press response. If no response occurred during this time, the experimenter held the rat up to the active lever and made the
animal press down to receive liquid reinforcement. This was repeated every 15-min, up to six times per session.

**Extinction, cue-induced reinstatement and resumption:** Once IO SA was stable on a FR3 schedule of reinforcement (see below), rats received one 180-min extinction session per day, for five days. Previous IO SA studies in our laboratory indicated that after 5 days of extinction, responding on the active and inactive levers is comparable. To maintain the oral sensation of liquid delivery during extinction, rats responded on the active lever for a 0.1-ml IO delivery of reverse osmosis water. There was no presentation of a cue-light during IO water delivery.

Cue-reinstatement testing was conducted during a 180-minute session following the last day of extinction. During reinstatement, rats could respond on the active lever for a 0.1 ml IO delivery of reverse osmosis water and the illumination of the cue light.

Resumption tests were conducted in an identical manner, but rats could respond on the active lever for a 0.1 ml IO delivery of EtOH, HFCS or EtOH + HFCS and the presentation of a cue-light.

Extinction, reinstatement and resumption sessions were tested on a FR3 schedule of reinforcement. Active and inactive lever responding, and number of infusions earned were recorded.

*Experimental procedures*

**Experiment 1: IO SA of EtOH and combined EtOH + HFCS.**

Rats (n=8) self-administered 5% EtOH on a FR1 schedule of reinforcement for 16 consecutive 180-min sessions. The response requirement was increased to a FR3 schedule of reinforcement for the next four sessions (17-20), and then reduced back to
an FR1 schedule for four sessions (21-24). This alternation in schedule was performed to ascertain baseline level of EtOH self-administration. On session 25, 5% EtOH was mixed in a 25% HFCS solution and animals self-administered the mixed solution on a FR1 schedule. The FR3 schedule was then reintroduced for 8 additional sessions (26-33). Following this period, the concentration of EtOH in 25% HFCS was varied in blocks of four sessions to complete a dose-response curve. That is, rats self-administered a 10% EtOH-HFCS solution for four sessions (34 – 37), then a 20% EtOH-HFCS solution for four sessions (38 – 41), and then a 5% EtOH-HFCS solution for another four sessions (42 – 45). To conclude the experiment, HFCS was removed and rats reverted to self-administering 5% EtOH for four sessions (45-49).

**Experiment 2: IO SA of EtOH and combined EtOH + SACC**

Rats (n=14) self-administered a 5% EtOH solution mixed in 0.1% saccharin for 10 180-min sessions. Next, the schedule of reinforcement was increased to a FR3 schedule for seven more sessions (11-17). Subsequently, a dose-response curve was performed as in Experiment 1: the concentration of EtOH was successively increased (5%, 10%, 20%, 5%) in the 0.1% saccharin solution (sessions 17-32). Following the dose-response determination, rats received a single session (33) in which they could respond for 25% HFCS. This session tested whether this group of rats could emit more responses for a more effective reinforcer (Levy et al. 2016).

**Experiment 3: Effect of food deprivation stress on IO SA acquisition, extinction and resumption of EtOH + HFCS**

Rats (n=28) self-administered 10% EtOH in 25% HFCS on an FR1 schedule for 10 180-min sessions (1-10), and then the schedule of reinforcement was increased to
an FR3 schedule for 7 sessions (11-17). The concentration of EtOH was selected based on the results of Experiments 1-2 which determined 10% EtOH + 25% HFCS led to the highest g/kg alcohol intake (0.91 g/kg). Following 5 180-min extinction sessions, rats were randomly assigned to food restriction (n=14) or food deprivation (n=14) conditions.

Food restricted rats continued their typical food restriction regimen of 3-hours of free access to standard rat chow, and the test of cue-induced reinstatement was followed by two tests of resumption 10% EtOH and 25% HFCS intake, which occurred on two counterbalanced sessions.

Food deprived rats did not receive any food after their last extinction session, but they were fed after the test of cue-induced reinstatement. To control for food deprivation stress carry over effects, they received one more extinction session followed by EtOH-only or HFCS-only resumption test, and they were fed immediately after the test. This was repeated once more to counterbalance for the substance self-administered.

**Experiment 4: Effect of food deprivation on resumption of EtOH + HFCS IO SA responding-modulation by palatability of components.**

A within-subjects design was used to investigate the effect of food deprivation stress on 10% EtOH + 25% HFCS intake during IO SA resumption testing. Additionally, a taste reactivity (TR) test was given at four different time points throughout the SA period to assess possible changes in palatability of 10% EtOH and 25% HFCS under pre-acquisition, post-acquisition, food restriction and food deprivation conditions. The timeline of procedural events that occurred during Experiment 4 is represented in Figure 4A.

The day preceding the first TR test, rats were habituated to TR chambers by
intraorally infusing reverse osmosis water at a flow rate of 0.5 ml/min for two minutes. During TR tests rats received 10% EtOH and 25% HFCS separately over a two-min exposure period. The appropriate liquid was dispensed at the same flow rate of 0.5 ml/min, resulting in 1 ml of the appropriate solution dispensed over a two-min period. EtOH and HFCS solutions were presented to rats an hour following an IO SA session and these solutions were tested in a counterbalanced order across rats. All test sessions were captured on video and tapes were scored for the mean number of hedonic reactions of tongue protrusions (protrusions of the tongue from the mouth) and mouth movements (summed). Additionally, tapes were scored for the mean number of aversive reactions of gaping (wide triangular shaped open mouth exposing lower incisors) and the avoidance reaction of passive dripping (passive dripping of liquid from the mouth to the floor). The aversive reactions of chin rubbing (rubbing chin along the floor of the cage) and paw treading (movement of forepaws back and forth) were also assessed, but these behaviors were not seen so they are not discussed further.

Rats (n=18) self-administered 10% EtOH + 25% HFCS on a FR1 schedule of reinforcement for 10 consecutive 180-min sessions. The response requirement was increased to a FR3 schedule of reinforcement for the next seven sessions (11-17). On session 25, rats received five extinction sessions (18-22), followed by tests of cue-induced reinstatement and resumption of 10% EtOH + 25% HFCS IO SA in both a food deprived and food restricted state. Extinction sessions were interposed between each test day as described above.

Drugs

Ethanol solutions (5%, 10%, 20%) were prepared by diluting ethyl alcohol (95%
vol; Commercial Alcohols, Tiverton, Ontario) in reverse osmosis water. High-fructose corn syrup (25%; HFCS-55 formula; Natures Flavors, CA, USA) and saccharin (0.1%; sodium salt hydrate 99+ %, ACROS Organics, New Jersey) solutions were also prepared by dilution in reverse osmosis water. Solutions were freshly prepared every three days to prevent evaporation.

Statistical analyses

One factor analyses of variance (ANOVA) and t-tests with independent or repeated subjects were conducted as appropriate. Significant main effects or interactions were further analysed by multiple comparisons using the Student-Newman Keuls method (α=0.05). All analyses were performed using SigmaStat (v. 12.5).

Results

Experiment 1: IO SA of EtOH and combined EtOH + HFCS.

Rats demonstrated higher IO SA of 5% EtOH + 25% HFCS than 5% EtOH alone. Figure 1A represents the mean (SEM) infusions rats earned across 25 days of IO SA acquisition for 5% EtOH (sessions 1-24) and 5% EtOH + 25% HFCS (session 25). An ANOVA revealed a main effect of session [F(7, 24) = 20.934, p < 0.001]. Multiple comparisons indicated that the number of infusions rats earned on session 25 was significantly higher than sessions 1 – 24, p < 0.001. No other significant differences were found.

In a dose-response assessment, when 20% EtOH was added to HFCS, rats showed suppressed IO SA. Figure 1B represents the mean (SEM) infusions rats earned across sessions 26 – 45, during which the concentration of EtOH in 25% HFCS was varied to create a dose-response (intake) curve. Blocks of four sessions were used to
allow IO SA behaviour to stabilize and the last session of which each concentration was presented were compared (sessions 33, 37, 41 and 45). An ANOVA revealed a main effect of session \( [F (3, 15) = 19.872, p < 0.001] \). Multiple comparisons indicated rats earned significantly less infusions on session 41 (20% EtOH) than on sessions 33 (5% EtOH; \( p < 0.001 \)), 37 (10% EtOH; \( p < 0.001 \)) and 45 (5% EtOH; \( p < 0.001 \)). No other significant differences were found.

Rats displayed enhanced IO SA of 5% EtOH following training with 5% EtOH + 25% HFCS. Figure 1C represents the mean (SEM) infusions rats earned on the last day of acquisition for 5% EtOH prior to HFCS pairing (FR3; session 20) compared to the four sessions after HFCS was removed (FR3; sessions 46-49). An ANOVA revealed a significant effect of session \( [F (4, 16) = 4.675, p = 0.11] \). Multiple comparisons indicated rats consumed less 5% EtOH on session 20 than sessions 46 (\( p < 0.01 \)), 47 (\( p < 0.05 \)), and 49 (\( p < 0.05 \)). No other significant differences were found.

**Experiment 2. IO SA of EtOH and combined EtOH + SACC.**

Saccharin was much less effective than HFCS in enhancing IO SA of EtOH. Figure 2A represents the mean (SEM) infusions rats earned across 16 days of IO SA acquisition for 5% EtOH in 0.1% SACC. An ANOVA revealed a main effect of session \( [F (13, 208) = 2.038, p = 0.012] \). Multiple comparisons indicated that infusions earned by rats did not differ between session 1 and session 17. In other words, although infusions earned varied across sessions, there was not a significant increase in 5% EtOH in 0.1% SACC intake between the first and last day of IO SA. Figure 2B represents the mean (SEM) number of infusions rats earned on the last session of Experiment 1 (session 49; FR3; 5% EtOH) and the last session of Experiment 2 (session 30; FR3; 5% EtOH in
0.1% SACC). An independent samples t-test revealed rats earned more infusions when EtOH was mixed in 0.1% SACC, \( t(20) = -2.68, p = 0.01 \). Figure 2C represents the mean (SEM) number of infusions rats earned across sessions 14 to 29 during which the concentration of EtOH in 0.1% SACC was varied to create a dose-response (intake) curve. Blocks of four sessions were used to allow IO SA behaviour to stabilize and the last session of which each concentration was presented were compared (sessions 17, 21, 25, and 29). An ANOVA revealed main effect of session \( [F (3, 39) = 3.741, p < 0.019] \). Multiple comparisons indicated rats earned significantly fewer infusions on session 25 (20% EtOH) than on session 17 (5% EtOH; \( p < 0.001 \)). No other significant differences were found. Figure 2D represents the mean (SEM) number of infusions rats earned during the IO SA dose-response (intake) curve for EtOH in SACC (Experiment 2) and EtOH in HFCS (Experiment 1). An ANOVA revealed a solution by concentration interaction \( [F (3, 72) = 8.324, p < 0.001] \), a main effect of solution \( [F (1, 72) = 127.387, p < 0.001] \) and a main effect of concentration \( [F (3, 72) = 16.392, p < 0.001] \). Multiple comparisons on marginal means indicated that higher concentrations of EtOH significantly reduced intake and that animals consumed significantly more solution when HFCS was added to EtOH than when SACC was added.

Experiment 3. Effect of food deprivation stress on cue-induced and EtOH or HFCS-induced resumption of responding.

Rats displayed enhanced responding for EtOH + HFCS across acquisition sessions. Following extinction, food deprivation stress enhanced cue-induced and HFCS (but not EtOH)-induced resumption of responding. Figure 3A represents the mean (SEM) infusions rats earned across 17 days of IO SA of 10% EtOH in 25% HFCS.
An ANOVA across sessions revealed a main effect of session \[ F(1, 27) = 4.690, p < 0.05 \]. Multiple comparisons indicated rats significantly increased their intake from FR1-1 to FR1-10 \( (p < 0.001) \) and from FR3-1 to FR3-7. Furthermore, the number of infusions earned did not significantly differ across the last four sessions of each schedule of reinforcement. Figure 3B represents the mean (SEM) active lever responses rats made on the last day of IO SA acquisition (Baseline; B) and the following 5 extinction sessions. An ANOVA revealed a main effect of session \[ F(5, 162) = 100.178, p < 0.001 \]. Multiple comparisons indicated response rates were lower across all extinction sessions when compared to the baseline responding during IO SA acquisition. Furthermore, extinction sessions 2 – 5 produced significantly lower lever responding when compared to the first extinction session. No other significant differences were found. Figure 3C represents the mean (SEM) active lever responses made on the test of cue reinstatement in food restricted and food deprived rats. A t-test revealed food restricted rats had lower levels of responding compared to food deprived rats when tested for cue-induced reinstatement, \[ t(26)= -2.166, p < 0.05 \]. Figure 3D represents the mean (SEM) infusions rats earned on the tests of EtOH and HFCS resumption. Independent samples t-tests revealed food deprivation stress increased HFCS intake during resumption, \[ t(26)= 4.068, p=0.001 \] but had no effect on EtOH intake \[ t(26)= 0.148, p=0.884 \].

**Experiment 4: Effect of food deprivation on resumption of EtOH + HFCS IO SA responding-modulation by palatability of components.**

Food deprivation stress enhanced EtOH+HFCS resumption of responding following extinction. Both EtOH and HFCS individually elicited enhanced hedonic
responses in the TR test following acquisition training, and food deprivation stress during resumption testing enhanced hedonic responses of HFCS, but not EtOH. Figure 4B represents the mean (SEM) active lever responses made during EtOH + HFCS resumption following extinction across food restricted and food deprived conditions. A paired samples t-test revealed that food deprived rats consumed more EtOH + HFCS during resumption than rats under food restriction conditions [t(17)= -2.213, p < 0.05]. Figure 4C represents the mean (SEM) hedonic taste responses elicited by EtOH or HFCS during baseline testing compared to post-acquisition taste reactivity testing. A paired samples t-test revealed hedonic taste reactions were increased during post-acquisition compared to baseline TR testing for EtOH [t(19)= -5.098, p < 0.001] and HFCS [t(19)= -2.311, p < 0.05]. There was no effect on aversive taste reactions to EtOH and HFCS (data not shown). Figure 4D represents the mean (SEM) hedonic taste responses elicited by EtOH or HFCS during taste reactivity testing for food restricted and food deprived rats. Paired samples t-tests revealed that food deprivation stress increased HFCS palatability [t(19) = -3.733, p < 0.001] but not EtOH palatability [t(19)= 1.027, p =0.318].

**Discussion**

Young, inexperienced and often under-aged drinkers report a high frequency of heavy episodic drinking patterns (Archie, Kazemi, & Akhtar-Danesh, 2012) and adding sweeteners to alcohol may increase the risk of problematic drinking in this population. Therefore, we designed four experiments in naïve Sprague Dawley rats to investigate whether the addition of a caloric sweetener (HFCS) to an EtOH solution could alter its intake, hedonic taste, and sensitivity (intake under) to food deprivation stress. In
Experiment 1 (to study the effect of HFCS on EtOH intake) rats were trained to intraorally self-administer 5% EtOH under multiple schedules of reinforcement. The addition of 25% HFCS caused a robust increase in EtOH intake during IO SA. Additionally, when HFCS was removed and rats reverted to self-administering the 5% EtOH solution, their intake was elevated from levels seen before the HFCS pairings. Using similar methods, Experiment 2 revealed an equally palatable, yet non-caloric sweetener (SACC) did not increase EtOH intake to the degree that was observed with HFCS. In Experiments 3 and 4 (to study the effect of HFCS on EtOH’s sensitivity to food deprivation stress) rats were trained to self-administer 10% EtOH in 25% HFCS. This concentration of EtOH was chosen based on results from Experiment 1. Following acquisition, rats underwent behavioural extinction to test the persistence of operant responding during cue-induced reinstatement and resumption (of 10% EtOH, 25% HFCS, and 10% EtOH + 25% HFCS) under food deprivation stress. Food deprivation stress increased 25% HFCS and 10% EtOH + 25% HFCS intake, but did not alter the intake of 10% EtOH during resumption. Finally, the changes in palatability of 10% EtOH and 25% HFCS were assessed using taste reactivity at multiple time points throughout the IO SA of 10% EtOH + 25% HFCS. Rats elicited more hedonic orofacial responses to 10% EtOH and 25% HFCS following IO SA of 10% EtOH + 25% HFCS. Furthermore, food deprivation stress increased hedonic orofacial responses to the 25% HFCS solution, but did not alter 10% EtOH palatability. Thus, it could be the increased palatability of HFCS under food deprivation conditions that contributes to the increased motivation to consume HFCS during operant IO SA resumption in rats. These results
have implications for the use of highly palatable and caloric sweeteners in EtOH beverages because of the impact they may have on excessive EtOH intake.

When rats could self-administer 5% EtOH during IO SA, intake was minimal. However, when the same concentration of EtOH was sweetened with 25% HFCS, a robust increase in EtOH intake was observed (see Figure 1A). Following this, a dose-response (intake) curve was produced by varying the concentration of EtOH within HFCS. Rats maintained the number of infusions earned when the concentration of EtOH was increased from 5% to 10%, however a further increase to 20% EtOH reduced the amount of solution consumed (see Figure 1B.). In other words, the 10% EtOH + 25% HFCS solution produced the highest levels of EtOH intake during IO SA. Finally, when rats reverted to self-administering 5% EtOH, their intake was elevated from levels seen before the HFCS pairings (see Figure 1C.). It is important to note that although SACC increased the intake of EtOH during IO SA, this effect was miniscule in comparison to what was observed when HFCS was used to sweeten the EtOH solution (see Figure 2).

Caloric sweeteners such as sucrose and glucose increase EtOH intake during SA in rodents (Samson, Pfeffer, & Tolliver, 1988); however, to our knowledge, we are the first to demonstrate this effect using HFCS. HFCS was selected as its use is rapidly increasing throughout North America, yet its effects on behaviour and the brain are still not well understood. Additionally, HFCS is found in many of the alcoholic beverages available for purchase (i.e. Bacardi Silver, Palm Bay, Mike’s Hard Lemonade). In addition to increasing EtOH intake, caloric sweeteners are also capable of eliciting approach behaviour (Yasoshima & Shimura, 2015) conditioning neutral stimuli (Sclafani
& Ackroff, 2017; White & Carr, 1985; Agmo & Marroquin, 1997), and maintaining high levels of operant self-administration (Levy et al., 2015; Avena, Long, & Hoebel, 2005). That is, caloric sweeteners are known to have strong behavioural reinforcing properties. Therefore, it is not surprising that rats increased their EtOH intake when HFCS was mixed into the EtOH solution. We suggest this increase was mostly caused by caloric reinforcement, because an equally palatable yet non-caloric sweetener did not cause such a drastic increase in IO SA behaviour. Although non-caloric sweeteners hold similar hedonic taste properties as do caloric sweeteners (Frank et al., 2008), it is known that their behavioural reinforcing properties are minimal in comparison (Domingos et al., 2011; White & Carr, 1985).

Interestingly, our study also suggests that HFCS may have indirect effects on EtOH intake. That is, once HFCS was removed from EtOH following multiple pairings, EtOH intake was elevated in comparison to initial IO SA levels. While we understand that many factors could have contributed to this effect (increased palatability of EtOH, tolerance) it could be that HFCS conditioned or altered EtOH’s reinforcing value. After all, caloric sweeteners are known to modify the reinforcing value of neutral stimuli (Sclafani & Ackroff, 2017; Sclafani, Glass, Margolskee, & Glendinning, 2010; Fedorchak & Bolles, 1987; White & Carr, 1985; Agmo & Marroquin, 1997; Delamater, Sclafani, & Bodnar, 2000). To test this, future research could employ methods that are more suitable for measuring the reinforcing value of stimuli, such as, progressive ratio operant testing or choice procedures (Reilly, 1999; Sclafani & Ackroff, 2003; Butler, Irons, Bassett, & Correia, 2018).
Once the effect of HFCS on EtOH consumption was determined, our next objective was to assess how HFCS could alter EtOH’s sensitivity to food deprivation stress. Food deprivation stress has been shown to elevate corticosterone levels in rats (McGhee, Jefferson, & Kimball, 2009) and facilitate the reinstatement of operant self-administration for drugs of abuse (Shalev, Marinelli, Baumann, Piazza, & Shaham 2003; Highfield et al, 2002; Mantsch, Baker, Funk, Le & Shaham, 2016). Food deprivation stress was also chosen based on its relevance to the behaviour being explored (i.e., ingestion of caloric substances). First, rats acquired IO SA of 10% EtOH + 25% HFCS (see Figure 3A) then this behaviour was extinguished (see Figure 3B). The absence of the cue-light during extinction conditions is important to establish that this stimulus only represents sweetened EtOH reinforcement. The use of water during extinction conditions is also crucial to maintain the orosensory stimulation that is experienced during IO SA acquisition. During cue-induced reinstatement food deprivation stress increased responding for water reinforcement (see Figure 3C). During resumption testing, food deprivation stress did not increase 10% EtOH intake, however it increased the intake of 25% HFCS. Finally, Experiment 4 revealed that food deprivation stress increased the intake of 10% EtOH + 25% HFCS (see Figure 4A) and the palatability of 25% HFCS, but not 10% EtOH.

In this study, the lack of an effect of food deprivation stress on EtOH intake during resumption was surprising. This is because stress has long been implicated in exacerbating relapse-like responding for EtOH in humans. For example, the experience of a stressor in abstinent alcoholics is associated with increased EtOH cravings (Clay et al., 2018; Fox, Bergquist, Hong, & Sinha, 2007). Additionally, the occurrence of severe
stress following treatment is correlated with increased relapse in recovering alcoholics (Brown, Vik, Patterson, Grant, & Schuckit, 1995; Brown et al., 1990). Finally, exposure to acute stress increases alcohol consumption in heavy drinkers (Thomas, Bacon, Randall, Brady, & Ronald, 2011; McGrath, Jones, & Field, 2016). In rodents however, the effect of stress on EtOH consumption is inconsistent (Pohorecky, 1990) with some researchers reporting decreases (Sprague & Maickel, 1994; Vengeliene, Siegmund, Singer, Sinclair, Li, & Spanagel, 2003) while others report increases (Bahi, 2013; Gomez, Lewis, & Luine, 2012; Vengeliene, et al., 2003). Interestingly, food deprivation stress increased EtOH intake only when HFCS was mixed in the solution. These results imply that HFCS increases EtOH’s sensitivity to the effect of food deprivation stress. Thus, HFCS alters EtOH intake under normal and stressful conditions and this should be considered when employing efforts to reduce excessive EtOH intake.

The last objective of this study was to assess how the palatability of 10% EtOH and 25% HFCS is altered throughout the IO SA of 10% EtOH in 25% HFCS. More specifically, taste reactivity was used to test the palatability of each solution pre-acquisition (naïve responding), post-acquisition (experienced responding) and under conditions of food deprivation stress (see Figure 4A). The number of palatable orofacial responses to EtOH and HFCS increased following IO SA of 10% EtOH in 25% HFCS (see Figure 4 C.). Interestingly, the number of aversive orofacial responses (i.e. gaping) did not change for either solution following IO SA experience (data not shown). While rats displayed no aversive responses to the taste of 25% HFCS, gaping occurred during pre- and post-acquisition for the 10% EtOH solution (data not shown). During
resumption, food deprivation stress increased palatable orofacial responses to 25% HFCS, but did not alter EtOH palatability (see Figure 4D).

This data suggests that the IO SA of 10% EtOH in 25% HFCS alters the hedonic palatability of EtOH, without altering its aversive taste properties. In contrast, Kiefer, Bice, and Badia-Elder (1994) have shown no change in hedonic taste, but a decrease in aversive taste responses following continuous home-cage access to a variety of EtOH concentrations. The lack of difference between aversive taste responses during pre- and post-acquisition may be explained by the use of 25% HFCS during EtOH exposure. That is, our rats never experienced EtOH by itself during the exposure period as it was always mixed in 25% HFCS. The effect of food deprivation on EtOH and HFCS palatability may be the most interesting finding from the taste reactivity data because it corroborates with the self-administration results from Experiments 3 and 4. Food deprivation stress had no effect on EtOH intake under resumption testing, nor did it affect palatability. On the other hand, food deprivation stress significantly increased HFCS intake under resumption and increased its palatability. We suggest that the increased palatability of HFCS under stressful conditions may contribute to the increased motivation to consume HFCS during operant responding.

These data imply the use of HFCS in alcoholic beverages encourages EtOH consumption, increases its sensitivity to food deprivation stress, and increases its hedonic taste. Efforts aimed at reducing the harmful consumption of EtOH in naïve drinkers should include removal of HFCS from the list of ingredients.
References


Clinical and Experimental Research, 31(3), 395-403.


Figure Legends

Figure 1 A. Timeline of procedural events that occurred during Experiment 1. Rats could self-administer 5% EtOH under multiple schedules of reinforcement, then 25% HFCS was added to the solution. Following this, a dose-response curve was created by varying the concentration of EtOH within HFCS, then rats reverted to self-administering 5% EtOH. B. Mean (SEM) infusions earned across 25 days of IO SA of 5% EtOH (sessions 1-24) and 5% EtOH + 25% HFCS (session 25). The labels below the x-axis refer to the schedule of reinforcement. The *** indicates a significance increase from all IO SA sessions of 5% EtOH, p <0.001. C. Mean (SEM) infusions earned across sessions 26 to 45 during which the concentration of EtOH in the 25% HFCS solution was manipulated to create a dose-response (intake) curve: 5%, 10%, 20%, 5%. Animals were tested on a FR3 schedule of reinforcement. The *** indicates a significance decrease from sessions 33 (5%), 37 (10%) and 45 (5%), p <0.001. D. Mean (SEM) infusions rats earned on the last IO SA acquisition day of 5% EtOH prior to the addition of HFCS (session 20), compared to the four sessions following HFCS removal (sessions 46–49). The * indicates a significant increase from Pre-HFCS (session 20), p <0.05. The ** indicates a significant increase from Pre-HFCS, p< 0.01.

Figure 2 A. Timeline of procedural events that occurred during Experiment 2. Rats could self-administer 5% EtOH in 0.1% SACC. Following this, a dose-response curve was created by varying the concentration of EtOH within SACC. On the final session, rats could self-administer 25% HFCS. B. Mean (SEM) infusions earned across 17 sessions of IO SA of 5% EtOH in 0.1% SACC. The labels below the x-axis refer to the schedule of reinforcement. C. Mean (SEM) infusions earned during acquisition for 5%
EtOH (data taken from Experiment 1; session 20) compared to 5% EtOH + 0.1% SACC (data taken from Experiment 2; session 17). D. Mean (SEM) infusions earned across sessions 14 - 29, during which the concentration of EtOH in 0.1% SACC solution was manipulated to create a dose-response (intake) curve: 5%, 10%, 20%, 5%. The *** indicates a significance difference from session 17 (5% EtOH). E. Mean (SEM) infusions rats earned on the last day each concentration (5%, 10%, 20%, 5%) of EtOH was presented in the SACC solution and in the HFCS solution (Experiment 1) during the dose-response (intake) curve. The *** indicates a significance difference from Exp 1 to Exp 2 solutions.

**Figure 3 A.** Timeline of procedural events that occurred during Experiment 3. Rats could self-administer 10% EtOH in HFCS during acquisition, then this behaviour was extinguished by replacing sweetened EtOH reinforcement with reverse osmosis water deliveries. Following extinction, a cue-reinstatement test was used to test drug-seeking for the drug-paired cue light. Following this, two resumption tests were used to test the resumption of 10% EtOH and 25% HFCS (across two counterbalanced sessions) under conditions of food restriction and food deprivation. B. Mean (SEM) infusions earned across 17 days of IO SA of 10% EtOH in 25% HFCS. The labels below the x-axis refer to the schedule of reinforcement. The * indicates a significant difference from session 1. C. Mean (SEM) active lever responses made on the last day of IO SA acquisition (Baseline; B) and the following five extinction sessions. The * indicates a significant decrease from Baseline, p < 0.001. The # indicates a significant decrease from Ext1, p <0.001. D. Mean (SEM) active lever responses made on the tests of cue reinstatement between food restricted and food deprived rats. The * indicates a significant increase in
food restricted relative to food deprived rats. E. Mean (SEM) infusions earned during the tests of EtOH and HFCS resumption across food restricted and food deprived rats. The ** indicates a significant difference from extinction responding within stressed rats, p < 0.01. The # indicates a significant difference from cue-reinstatement responding within non-stressed animals.

**Figure 4 A.** Timeline of procedural events that occurred during Experiment 4. Each taste reactivity (TR) session tested EtOH and HFCS palatability separately over a two-min liquid exposure. Please see Figure 3 A for a more detailed explanation of the IO SA protocol used. B. Mean (SEM) infusions earned during the test of 10% EtOH + 25% HFCS resumption across food restricted and food deprived rats. The * indicates a significant difference from food restriction conditions, p < 0.05. C. Mean (SEM) hedonic taste responses elicited to EtOH and HFCS during baseline (pre-acquisition) and post-acquisition taste reactivity testing. The * indicates a significant increase from baseline responding, p < 0.05. The *** indicates a significant increase from baseline responding, p < 0.001. D. Mean (SEM) hedonic taste responses elicited to EtOH and HFCS across food restricted and food deprived rats. The *** indicates a significant increase from food restriction conditions, p < 0.001.
Figure 1.

A. 

5% EtOH → 5% EtOH + HFCS → Dose Response: EtOH (5%, 10%, 20%) in HFCS → 5% EtOH

B. 

![Graph showing mean (SEM) infusions for 5% EtOH and 5% EtOH + 25% HFCS over sessions FR1, FR3, and FR1.](image)

C. 

![Graph showing mean (SEM) infusions FR3 for 5%, 10%, and 20% conditions over sessions.](image)

D. 

![Bar graph showing mean (SEM) infusions FR3 for different conditions.](image)
Figure 2.

A. Dose Response: EtOH (5%, 10%, 20%) in SACC

B. Mean (SEM) Infusions

C. Mean (SEM) Infusions on FR3

D. Mean (SEM) Infusions on FR3

E. EtOH Concentration (Session)
Figure 3.

A. 

<table>
<thead>
<tr>
<th>Acquisition</th>
<th>Extinction</th>
<th>Reinstatement</th>
<th>Resumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% EtOH + HFCS</td>
<td>Water</td>
<td>Water</td>
<td>10% EtOH / 25% HFCS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Food R. / Food Dep.</td>
</tr>
</tbody>
</table>

B. 

Mean (SEM) Infusions

FR1

Session 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

FR3

Mean (SEM) Responses FR3

B Ext1 Ext2 Ext3 Ext4 Ext5

C.

D. 

E.

Mean (SEM) Infusions FR3

EtOH

Food Restriction Food Deprivation

HFCS

Food Restriction Food Deprivation

**
Figure 4.

A. Diagram showing the experimental protocol with phases of acquisition, extinction, and resumption. Treatments include 10% EtOH + HFCS and water or food restriction/food deprivation.

B. Bar graph showing mean (SEM) infusions FR3 for Food Restriction and Food Deprivation conditions. The graph indicates a significant difference (*).

C. Bar graphs for EtOH and HFCS showing mean (SEM) Hedonic Taste Responses for baseline and post-acquisition conditions. Asterisks indicate significant differences (*).

D. Bar graphs for EtOH and HFCS showing mean (SEM) Hedonic Taste Responses for Food Restriction and Food Deprivation conditions. Asterisks indicate significant differences (*).
General Discussion

Summary of results

Excessive alcohol consumption by young, naïve drinkers is prevalent and has been associated with an increased risk for developing alcohol use disorders later in life (Chassin, Pitts, & Prost, 2002; Albers et al., 2015). Furthermore, the addition of sweeteners to alcoholic beverages have been implicated in increasing alcohol intake of naïve drinkers (Albers et al., 2015). Thus, the current study sought to investigate whether the addition of HFCS to an EtOH solution could alter EtOH’s intake, hedonic taste, and sensitivity to food deprivation stress in naïve rats. HFCS caused a drastic increase in EtOH intake across multiple concentrations; an effect that was not observed by the addition of a non-caloric sweetener (SACC). HFCS also altered rat’s sensitivity to the effect of food deprivation stress on EtOH consumption. More specifically, food deprivation stress increased EtOH intake only when HFCS was mixed in the solution. In terms of EtOH and HFCS palatability, our study revealed two important findings. First, multiple exposures to 10% EtOH in 25% HFCS increased the palatability of 10% EtOH and 25% HFCS. Second, food deprivation stress increased the palatability of HFCS, but did not alter the palatability of EtOH. Taken together with the results from self-administration, we suggest that the increased palatability of HFCS under food deprivation stress may contribute to the increased motivation to seek HFCS under stressful operant conditions.

Methodological limitations

One methodological limitation to consider when interpreting the results of the presented study is the selective use of male rats throughout all experiments. Male rats...
were chosen because our laboratory has already established the consumption patterns of HFCS in the home-cage and during operant IO SA. This limits the generalizability of our findings since differences in alcohol intake and alcohol preferences have been reported across male and female rodents. Generally, females tend to consume larger quantities, and demonstrate a higher preference for alcohol in comparison to males (Lancaster & Spiegel, 1992; Lancaster, Brown, Coker, Elliot, & Wren, 1996). In corroboration with these behavioural findings, female rats show increased responsiveness to mesolimbic dopamine stimulation by EtOH exposure (Blanchard, Steindorf, Wang, & Glick, 1993). Human research has also indicated that women reach a higher peak blood alcohol concentration (BAC) than men after consuming equivalent amounts of EtOH (Sutker, Tabakoff, Goist, & Randall, 1983). These findings have been suggested to reflect the differences in EtOH pharmacokinetics between the two sexes (Taylor, Dolhert, Friedman, Mumenthaler, Yesavage, 1996; Mumenthaler, Taylor, O’Hara, Yesavage, 1999).

In addition to differences in alcohol intake and preference, the effect of stress on EtOH intake might also differ between female and male rodents. For example, restraint stress has been shown to increase homecage EtOH consumption in male mice, while decreasing consumption in females (Chester, Gustavo, Demaria, & Finegan, 2006). Taken together, these results highlight the importance of using both males and females while investigating rodent models of EtOH intake.

Although our laboratory has frequently studied the reinforcing properties of HFCS, the current experiments represent our first attempt to assess ethanol reinforcement. As such, our laboratory was not adequately prepared to measure the
blood alcohol concentration (BAC) of rats throughout IO SA. During operant SA of EtOH in rats, BACs are frequently assessed in addition to the total amount of EtOH consumed (Doherty & Gonzales, 2015; Carnicella, Yowell, & Ron, 2011). Using BACs as a biomarker of EtOH consumption is necessary to provide a measure of EtOH exposure that accounts for other factors that have been shown to affect EtOH consumption in animals (i.e. patterns of drinking, metabolism, sex differences). Thus, our experiments could have drawn stronger conclusions about the effects of HFCS on EtOH consumption if BACs were presented along with consumption data. However, when the consumption patterns of animals responding for 10% EtOH in 25% HFCS were calculated in ten-minute time bins (Experiment 1), rats consumed 0.91 g/kg within the first 30 minutes of each session. Briones and Woods (2013) found that home-cage self-administration of approximately 0.75 g/kg EtOH in rats produced BACs greater than 0.08 g%, which constitutes as a binge consumption pattern according to the National institute of Alcohol Abuse and Alcoholism (2015). Therefore, although the current study lacked measures of BACs throughout the IO SA of EtOH + HFCS, we are confident that animals consumed quantities of EtOH that produced recognized levels of binge intoxication.

**Future directions**

This study could be expanded upon by future research. As previously mentioned, one limitation of the presented experiments is the inability to draw conclusions about how HFCS changes the relative reinforcing value of EtOH. A particularly useful way to measure the reinforcing value of a stimulus while using an operant self-administration model is by applying a progressive-ratio schedule of reinforcement. When a
progressive-ratio schedule is applied, the amount of effort required to earn reinforcement successively increases across each trial, until responding for the stimulus ceases and a ‘breakpoint’ has been achieved (Jones & Comer, 2013; Panlilio & Goldberg, 2007). Given that breakpoints quantify an organism’s persistence/desire to achieve reinforcement, with higher breakpoints associated with higher persistence, they can be used to indicate the relative reinforcing value of different stimuli. Furthermore, the application of a progressive ratio schedule also stabilizes possible confounds between solutions used during operant self-administration such as, levels of satiety or intoxication.

Choice procedures are another method that can be employed to delineate changes in a stimulus’ reinforcing value; in fact, some researchers argue choice procedures are the only method by which changes in the reinforcing value of a stimulus can be determined (Panlilio & Goldberg, 2007). During choice testing, participants are required to choose between a fixed magnitude of the particular reinforcer of interest, or a scaling amount of an alternative reinforcer. The point at which the participant stops choosing the reinforcer of interest over the alternative reinforcer is then used to compare the relative reinforcing value of the reinforcer of interest (in comparison to the alternative).

Although the use of progressive ratio or choice testing could allow us to make stronger inferences about changes in the reinforcing efficacy of EtOH, both procedures would be complicated to employ as rats do not self-administer reliable amount of EtOH without the use of sucrose-fading or lengthy initiation procedures (as reviewed by Samson, 1986; Samson, Pfeffer, & Tolliver, 1988). Ultimately the use of sucrose-fading
would confound the variable of interest; that is, changes in EtOH reinforcing efficacy as a result of HFCS pairings. Furthermore, alternate initiation procedures could take upwards of 6-weeks, and do not lead to strong responding for EtOH alone. Therefore, although our lab has considered alternate ways to investigate the changes in EtOH’s reinforcing value, there are multiple limitations in using these methods to measure a reinforcer that is not reliably self-administered on its own (e.x. EtOH).

Our laboratory is also interested in examining the role of arginine vasopressin in EtOH intake, palatability and persistence of responding under conditions of food deprivation stress. Arginine vasopressin (AVP) is synthesized in the supraoptic nucleus of the hypothalamus and acts as a key regulator of the hypothalamic-pituitary-adrenal (HPA) axis, the body’s central stress response system (Aguilera & Rabadan-Diehl, 2000; Tanoue et al., 2004). More specifically, following exposure to stress, AVP binds to V1b receptors (V1bR) in the anterior pituitary to promote the secretion of adrenocorticototropic hormone (ACTH) into the bloodstream. ACTH subsequently travels down to the adrenal cortex to promote the synthesis and release of glucocorticoids (see Beurel & Nemeroff, 2014 for a review). Correspondingly, V1bR antagonists decrease multiple anxiogenic behaviours in animal models (Griebel et al., 2002; Stemmelin, Lukovic, Salome, & Griebel., 2005).

Given that AVP modulates anxiogenic responding, it may contribute to stress-induced relapse. Indeed, AVP secretion is heightened during periods of drug withdrawal in humans and animals. For example, Eisenhofer, Lambie, Whiteside, and Johnson (1985) measured AVP levels in alcoholic patients during a drug detoxification program. Patients displaying withdrawal symptoms had significantly elevated urine and plasma
AVP levels in comparison to patients without withdrawal symptomology, or healthy controls. Furthermore, rats show heightened AVP mRNA gene expression during periods of acute heroin and cocaine withdrawal (Zhou, Leri, Cummins, Hoeschele, & Kreek, 2008; Zhou et al., 2005).

There are few studies that have investigated the effect of V1bR antagonists on relapse-like responding in animals. Zhou, Leri, Cummins, Hoeschele, and Kreek (2008) found that the administration of a V1bR antagonist (1 mg/kg and 30 mg/kg; SSR149415) decreased stress induced relapse-like responding for heroin in rats. Interestingly, the function of AVP in modulating relapse-like responding for alcohol is unknown. It would be interesting to investigate whether the V1bR antagonist SSR149415 would decrease stress-induced increases in resumption responding for EtOH + HFCS and HFCS.

Conclusions

In conclusion, results from Experiment 1 and Experiment 2 suggest that the addition of a sweet and calorically dense sweetener (HFCS) drastically elevated EtOH intake in rats, in comparison to an equally palatable, yet non-caloric sweetener. Furthermore, when HFCS was removed from the EtOH solution, rats demonstrated increased EtOH intake levels then what was observed prior to the HFCS pairings. Finally, Experiment 3 and Experiment 4 demonstrated that food deprivation stress only increased EtOH intake when HFCS was mixed into the solution. Furthermore, results from taste reactivity measures suggest that food deprivation stress causes increase in HFCS palatability, which may explain the observed self-administration data. Taken together, our results suggest that HFCS increases EtOH intake under normal and
stressful conditions, and should be reconsidered as an acceptable addition to alcoholic beverages.
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