2014

A Bittersweet Investigation of Availability and Nutritive Value as Determinants of Volitional Sucrose Consumption

Milan D. Valyear
Wilfrid Laurier University, milan.valyear@gmail.com

A Bittersweet Investigation of Availability and Nutritive Value as Determinants of Volitional Sucrose Consumption

by

Milan D. Valyear

Bachelor of Science, Wilfrid Laurier University, 2013

THESIS

Submitted to the Department of Psychology, Faculty of Science

in partial fulfilment of the requirements for

Master of Science in Psychology

Wilfrid Laurier University

Abstract

Rats given ~24 h access to a 4% sucrose solution every 3rd day (E3DA) consume about 100 g more solution than rats with continuous, everyday access (EDA). Under the same EDA-E3DA conditions rats will consume similar amounts of a more concentrated 8 or 16% sucrose solutions (Eikelboom, Hewitt, & Adams, Unpublished). It maybe that with these more concentrated solutions rats hit a satiety limit that prevents a difference between EDA and E3DA consumption from being evident. Experiment 1 was conducted to investigate the effect of adding quinine to 4% and 8% sucrose solutions with the intention of reducing consumption to allow the emergence of an EDA-E3DA consumption difference with 8% sucrose. Rats were given EDA or E3DA access to 4% or 8% sucrose with or without added .005% quinine. An EDA-E3DA consumption difference emerged with 8% sucrose + .005% quinine where the quinine addition lowered consumption. However, in this experiment the EDA-E3DA consumption profile of 4% sucrose was smaller than typically seen.

Rats show considerable variability in sucrose consumption and experiment 2 was conducted to explore the effects of using a pre-exposure period to determine group assignment. Rats were pre-exposed to 4% sucrose continuously for 6 days. Consumption during this pre-exposure was used to assign rats to 4% or 8% sucrose with or without added .005% quinine who were then assigned to EDA or E3DA conditions. In this experiment the pre-exposure helped create eight equal groups and the 4% sucrose group demonstrated an EDA-E3DA consumption difference. However, the .005% quinine concentration did not reduce 8% sucrose consumption as severely as it had in experiment 1 and failed to produce an EDA-E3DA difference with 8% sucrose.

Experiment 3 explored the effects of a pre-exposure on 8% sucrose + .005% quinine consumption as a pre-exposure was a critical difference in the first two experiments. The results of experiment 3 suggested that a 4% sucrose pre-exposure would not change future 8% sucrose + .005% quinine consumption levels.

Experiment 4 was conducted to investigate the effect of varying the quinine concentration on 8% sucrose + quinine consumption. Rats were given EDA or E3DA to 8% sucrose adulterated with .0025%, .005%, .01%, or .02% quinine. Quinine concentration-dependently reduced consumption levels, and allowed for an EDA-E3DA consumption difference to emerge.

Collectively these experiments suggest that rats' regulate their consumption of a supplementary sucrose solution based on the value of the solution and its availability in an integrated fashion. That is, the ability for intermittent access to elevate consumption depends on the value of the food being consumed. This is evidenced by the efficacy of quinine adulteration to degrade the value, and consequently intake, of an 8% sucrose solution which permitted an EDA-E3DA difference, normally only seen with 4% sucrose, to emerge.

Acknowledgements

I would like to thank my committee members Bruce McKay, Bill Hockley, and Elham Satvat for their helpful comments on this thesis. I would also like to thank my advisor Rudy Eikelboom for his compassionate and helpful mentorship.

It would not have been possible to complete this thesis without the help of my lab members Gehan Senthinathan, Angela Mastroianni, Benjamin Peckham, and Adam Celejewski.

I should also thank my father, Timothy D Valyear, for teaching me to read among many other parental duties. And my mother, Luisa Valyear, for her unwavering encouragement, also among many other parental duties. My sisters Arisa and Kiara Valyear have consistently monitored my well-being and, despite my sometimes apathetic responses, for this I am grateful. My grandparents Fortunato and Rose Fusco are an inspiration – thank you for everything you have taught me. Lastly, my partner Jaqueline D Brown, I am forever indebted to you for your unrelenting support throughout my education. Thank you.

It was funding from NSERC that made this research possible and I recognize them here to express my gratitude.

Table of Contents

List of Figures

Feeding is a biological imperative that must be tightly regulated as over- and under-consumption can be detrimental (Woods, 1991). The regulation of feeding is complicated in the natural world as the state of resources for many animals is in constant flux. A myriad of factors inform the regulation of feeding but decisions of how much to consume at a given time ultimately depend on two factors: the quality of the consumable and how often it is available. Nutritive value, or quality, is largely inferred from taste with evolutionarily conserved taste receptors for sweet, bitter, sour, salty and umami indicating different nutritive characteristics. The taste of a food may indicate the presence of necessary vitamins or mineral salts but importantly reflects the energy density of certain foods. Availability is also a complex issue as a food may become inaccessible for a number of reasons: multiple organisms may compete for the same resource, a food may be too geographically sparse to be encountered frequently, or seasonal changes limit success of certain flora and fauna. As such, the rat benefits from a relatively plastic feeding behaviour. Ultimately the rat must ingest more biochemical energy than is expended obtaining that energy and in achieving this balance nutrition and availability must be carefully considered.

Interestingly, manipulating the availability of certain food sources in non-deprived rats can lead to surprising changes in consumption. Rats given intermittent access to sweet solutions or palatable foods tend to elevate their consumption, sometimes permanently, compared to rats with continuous access. This access-dependent consumption difference appears to hold true only for moderately sweet sucrose solutions whereas with more concentrated, and calorically dense, sucrose solutions rats consume similar amounts regardless of access. The aim of my thesis is to understand why rats will consume elevated amounts of intermittently available sucrose solutions

at some concentrations and not others. To this end, I hope to explore how nutritive value, availability, and palatability collectively determine sucrose consumption.

In the lab taste and nutritive value can be easily manipulated with mixtures of non-nutritive flavourings and calorically or metabolically relevant additives. This methodology has elucidated many interesting properties of taste and food's value, for example, that saltiness can be both hedonic or aversive depending on the concentration (Young & Christensen, 1962) and that flavour preferences can be conditioned by manipulating the caloric density of ingestants (Bolles, Hayward, & Crandall, 1981). The availability of a food can also be manipulated simply and, likewise, has uncovered many interesting and surprising characteristics of feeding. For example, training rats to lever press for a food pellet on a 1-minute variable interval schedule induces excessive water consumption (Falk, 1961) and restricting water access also reduces food consumption (Bolles, 1961). Despite the relative ease and simplicity with which investigations of nutritive value and availability can be carried out it is unclear how these two factors interact to collectively determine intake.

Discerning the nutritive characteristics of a food from its taste is an important sensory ability which, if damaged, can lead to an unhealthy diet (Lees, 1999). Sugar, either as a solid or in solution, is a classic medium in feeding studies and has received more attention lately given the suggested link between sugar sweetened beverage consumption and obesity (Caprio, 2012). For sucrose every gram of refined cane sugar translates to 3.75 calories[1] (Redpath, n.d.) of immediately available energy. The nutritive value of a sugar solution is evident to the rat as its sweetness and caloric density are both a direct function of the percentage sugar content. From a

[1] Throughout this thesis, and on most food labels, a calorie is used to actually mean a kilocalorie. This misnomer is used because a true calorie, the energy required to raise 1 gram of water 1°C, is negligible in terms of feeding (Owen, 1980).

practical standpoint modifying the taste and nutritive value of a sugar solution by making more or less concentrated solutions is uncomplicated.

Changing the palatability of sugar solutions can weaken the correlation between taste (sweetness) and caloric density. Adding bitterness to a sweet solution degrades the taste of the solution, as bitterness is aversive, and lowers the perceived value of the solution. Altering palatability in this way offers the unique opportunity to degrade the value of the solution without modifying the caloric density of the solution. Thus sugar solutions with added bitterness separate the taste of the solution from its postingestive consequences. Manipulating the availability of sweet, and bittersweet, solutions can be accomplished by placing a sugar bottle onto a rat's cage for whatever duration of time the experiment requires. Together these simple manipulations of sucrose concentration, palatability, and access allow the effects of nutritive value, availability, and their interaction on feeding to be elucidated.

Historically, feeding studies have largely focused on the rats' consumption of one food source. For example, in Skinner's (1930) first operant conditioning experiments feeding was restricted to 1 or 2 h a day during which rats reached into a covered compartment to extract food pellets individually. This procedure showed that the food consumption steadily declined throughout the 2 h period of food availability, presumably as rats became sated. In later experiments the covered lid became a lever which triggered the dispersal of food pellets (Skinner, 1932) and, although initially focused on eating reflexes, ultimately uncovered the basic principles of reinforcement (Skinner, 1938). Skinner's early work set the precedents of manipulating access to the rats' single food source to elicit operant responses for individual pellets. Collier, Hirsch, & Hamlin (1972) veered slightly from this method by allowing rats to lever-press for access to unlimited food instead of for single pellets. Rats were not food

restricted *per se* but were required to complete a number of lever-presses to then eat freely but, upon cessation of the meal, would have to lever-press to regain access. Collier et al. (1972) argued that this procedure was more analogous to the natural condition as rats in nature would work for a meal, not a mouthful, and showed that as operant costs grew rats would eat fewer, larger, meals. Skinner's early work and the plethora of studies encouraged by it concentrated on manipulating access to a rat's single source of food. As such, considerably more work has been done with food deprivation than conditions where rats are to choose between multiple food sources.

The distinction between eating under conditions of deprivation and food choice without deprivation is an important one. When the rat has the opportunity to choose between different calorie sources the quality of the foods available inform diet choice. In some cases, where many high-value food choices are available, the rat will over-consume and become obese (Sclafani & Springer, 1976). In the current thesis rats are given supplementary access to a sugar solution; chow and water are available *ad lib*. Much of the recent work on feeding control has been done under conditions of choice. Although the current investigation is focused on the interaction between availability and nutritive value of a sucrose solution, much of the comparable work on food consumption has focused less on this basic question and more on the patterns of eating in these situation as a model of binge eating disorder (BED) (Corwin, Avena, & Boggiano, 2011). In an effort to imitate human palatable-food binging these models have induced fat, sugar, and even Oreo™ cookie binging in rats (Corwin & Babbs, 2012). Typically presenting a food intermittently induces binging during a discrete period when compared to that of continuous access controls. However, total caloric intake averaged over a longer time period is often similar across these access conditions. The foods and access schedules used in these models are diverse

but the well-founded feeding behaviours elicited by these models have been confirmed by an abundance of studies. A selection of models that are relevant to the current investigation will be introduced below.

Corwin and associates have a well-established model of excessive fat consumption where their "continuous" group has access to solid vegetable shortening for 2 h daily, and their intermittent group has access for 2 h on Mondays, Wednesdays, and Fridays (MWF) (reviewed in Corwin & Wojnicki, 2006). In this model the MWF group consumes substantially more shortening than the continuous group in a 2 h period; a difference that emerges over ~4-6 weeks (Corwin et al., 1998). Wojnicki, Stine, & Corwin (2007) have used this model to explore sucrose consumption where they made 3.2, 10, or 32% sucrose solutions available to rats on the same daily vs MWF schedule for 1.5 h. Under these conditions rats given MWF access to 3.2% and 10% sucrose consumed significantly more than rats with daily access. Interestingly, rats consumed similar amounts of the 32% sucrose solution across both access conditions.

Hoebel and associates work with a model where a sugar solution (either glucose or sucrose) is always available to their continuous group and their intermittent group has access to that solution, and food, on an alternating 12 h schedule (12 h food deprivation:12 h food + sugar) beginning 4 h into the night cycle (reviewed in Avena, Rada, & Hoebel, 2006). This procedure has been carried out with 25% glucose (Colantuoni et al., 2001) and 10% sucrose (Avena & Hoebel, 2003) showing similar results. Across access conditions rats in both groups consume similar amounts over the 24 h period but the intermittent groups come to consume significantly more, during the first hour of access, than the continuous group. Rats maintained on the intermittent access schedule show elevated first hour consumption and signs of "dependence" after just over 20 days of this feeding schedule (Avena et al., 2006).

Eikelboom and associates have developed a similar protocol for studying the consumption of sweet solutions (Celejewski, 2011; Eikelboom et al., Unpublished; Senthinathan, 2013; Valyear, 2013). The continuous group is given everyday 24 h access (EDA) to a 4 % sucrose solution (ad lib access) whereas the intermittent group is given access for 24 h every third day (E3DA). Typically EDA and E3DA rats quickly settle into consuming ~100-150 g and ~200-250 g in a 24 h period, respectively. This pattern of access is usually carried out for 12 cycles (34 EDA exposures; 12 E3DA exposures) at which point E3DA rats stably consume ~100 g more than EDA rats (Eikelboom, Hewitt, & Adams, Unpublished). The endurance of the EDA-E3DA consumption difference can then be assessed by giving both groups equivalent alternate-day access (E2DA). Alternate-day access, being one day more frequent than E3DA and one day less frequent than EDA, may be a similar change in availability for both groups. The E3DA-EDA consumption difference has been shown to remain stable in the alternate-day phase with 4% sucrose for as long as the rats were tested (Eikelboom, et al., Unpublished). Interestingly, the robust EDA-E3DA consumption difference seen with 4% sucrose in this protocol is not evident with higher sucrose concentrations. Rats given 16% sucrose tend to consume less than rats given 8% sucrose and with EDA and E3DA access neither solution demonstrated the emergence of an appreciable access-induced consumption difference.

The Hoebel model of sugar binging fails to show 24 h access-induced consumption differences with 10% sucrose and 25% glucose solutions (Avena et al., 2006). Similarly the Corwin group saw elevated sucrose consumption in the MWF group at 3.2% and 10% sucrose, not 32% (Wojnicki et al., 2007). These findings, taken with the EDA-E3DA consumption difference seen at 4%, but not 8% or 16% sucrose, suggest that access-induced consumption

differences are most prominent at moderate sugar concentrations (Eikelboom et al., Unpublished).

At present it is unclear how rats regulate their intake of sweet solutions around nutritive density and availability. If rats are given *ad lib* access to a sucrose solution, chow, and water the amount of calories consumed from sugar in a 24 h period will increase with the concentration of the sucrose solution to a plateau (Collier & Bolles, 1968). This caloric plateau has been argued to represent a preference by the rat to consume only a certain portion of its calories from sugar (Collier & Bolles, 1968). It may be that some caloric or fluid-volume limit on sucrose intake prevents an EDA-E3DA consumption difference from emerging at higher sucrose concentrations. Access-induced consumption differences may be most apparent at moderate sugar concentrations. The greater caloric consumption seen with more concentrated sugar solutions may limit access effects from presenting because of the activation of satiety mechanisms. Thus if consumption of concentrated sugar solutions could be reduced (lowering volume and calorie intake) rats may demonstrate access effects even with highly concentrated sucrose solutions.

Adding quinine, a bitter tastant, to sucrose solutions has been shown to reduce the palatability, and intake, of sucrose solutions (Kappauf, Burright, & Demarco, 1963; Young, Burright, & Tromater, 1968). With pure sugar solutions, sweetness and caloric density are the same. As such increased sweetness signals more calories. Adding quinine to a sugar solution separates the taste from the caloric consequences of ingestion but maintains the caloric density. The current investigation tests the hypothesis that reducing the palatability of more concentrated sucrose solutions with added bitterness will reduce the intake of sucrose calories and allow for access-induced consumption differences to emerge under EDA-E3DA conditions.

The Consumption of Sweet Solutions

Throughout the 1930's Curt P. Richter performed a number of experiments looking at the dietary changes of rats in response to physiological perturbations. Adrenalectomized rats excreted excessive amounts of salt and would die shortly after the surgery if not administered sodium in some form. With this knowledge Richter made a sodium chloride solution available to adrenalectomized rats and found that the rats would volitionally consume the salt and maintain their health as long as this supplementary salt solution was available (Richter, 1936). Richter posited that rats would make beneficial dietary selections of available nutrients as long as they were presented in detectable concentrations. In an effort to transfer his work on purified tastants to natural foods Richter sought to determine the detection thresholds of many substances. The basic methodology for these studies was to give rats two water bottles then, provided they consumed equally out of both, augment one water bottle with a gradually increasing amount of some tastant, each day, until the rat consumed more or less of the flavoured solution. A change in consumption indicated that the rat could detect the taste and had an affective reaction to it. This methodology is not without flaws and more recent psychophysical measures which count licks, not only volume consumed, have proven to be more sensitive in determining taste thresholds (Thaw & Smith, 1992).

Despite the methodological issues with Richter's technique he uncovered considerable information about the rat's volitional sugar consumption. In a study to identify taste thresholds for maltose, glucose, galactose, and lactose rats showed an inverted-U profile of 24 h consumption as the sugar concentration increased; volume consumed first increased and then decreased (lactose was an exception and was consumed only minimally; Richter & Campbell, 1940). The peak of the volumetric inverted-U ranged from 9% to 11% depending on the sugar.

When the volume consumed was plotted as grams of solute, or calories, consumed the inverted-U translated to a more sigmoidal curve where grams consumed increased and then plateaued or decreased slightly. Richter, neglecting solute consumption, concluded that the peak of the volumetric inverted-U represented the point at which the rats exhibited the greatest preference for the solution.

Collier & Bolles (1968b) examined, more closely, the contribution of solute consumption to total energy intake and whether the inverted-U apex reflected a preference peak for sucrose solutions. In an effort to replicate the volumetric inverted-U with a between subjects design Collier and Bolles maintained rats on *ad lib* chow, and a 4, 8, 16, 32, or 64% sucrose solution for 40 days while consumption of all ingestants and body weight was closely monitored. They found that peak volume consumption occurred at 8%, replicating Richter's findings, but the solute, or calories, consumed increased to 16% and then plateaued through 64%. Pure water consumption was negligible. Total calorie consumption also remained stable across the sugar concentrations but at the higher sucrose concentrations rats allocated a larger portion of their daily caloric intake to the sugar solution and reduced chow intake accordingly. That is, at sucrose concentrations of 16% or greater rats attributed approximately 60% of their total caloric intake to sugar-calories.

To address the question of whether the apex of the volumetric inverted-U reflected optimal preference Collier & Bolles (1968b) gave rats 24 h two-bottle choice tests of every permutation of 4, 8, 16, & 32% sucrose. Importantly these concentrations span the inverted-U seen with volume consumption peaking at 8% sucrose. When given the choice between two sucrose solutions in this experiment rats consumed more of the sweeter solution, which translates to higher solute consumption from the sweeter solution as well. The importance of the results

with this choice test are two-fold: 1) it demonstrates that the apex of the volumetric inverted-U does not represent peak preference and 2) that rats minimized *consummatory* energy expenditure by favouring the more concentrated solution. Rats with access to 16, 32, or 64% sucrose solutions stably consumed 60% of their total caloric intake from sugar and during the two-bottle choice test rats preferentially consumed from the more concentrated solutions to meet this dietary allocation. The rat appeared to consume a maximum fixed proportion of total caloric intake from sucrose. Collier and Bolles suggest that this proportion could not be achieved at 4% or 8% sucrose because of a limit on the rate of water excretion; these solutions were too dilute for the rat to excrete the water required to consume enough solute to meet this proportion and maintain their normal calorie intake.

The concentration-dependent inverted-U profile of volume consumption and complimentary sigmoidal curve of calorie consumption have been widely replicated with sucrose solutions (Sclafani & Nissenbaum, 1987; Smith & Sclafani, 2002; Spector & Smith, 1984; Young, 1948). These general patterns have even been shown to remain stable throughout the lifespan of the rat (Smith & Wilson, 1989). Collier & Bolles (1968b) and Young & Greene (1953) concluded that the one-bottle exposures measured the *acceptability* of solutions whereas the two-bottle exposures directly measured *preference*.

Further reinforcing this point are the results seen when rats are sham-fed sucrose solutions. Sham-feeding is a procedure where a fistula is implanted into the rats' oesophagus or stomach that, when opened, allows any food or fluid consumed to exit the rat, leaking onto the floor, before it can be digested. This procedure allows rats to consume liberally while obtaining little or no calories from the solution, so taste is disconnected from energy intake. Nissenbaum & Sclafani (1987) maintained rats at 85% of their body weight so they were always food

deprived and exposed them for 30 minutes to 1, 4, 16, and 32% sucrose solution under normal- and sham-feeding conditions. When normally fed, even these food deprived rats showed an inverted-U profile of volume consumption typical of that seen with 24 h sugar exposures (Sclafani & Nissenbaum, 1987). In contrast, rats increased their "consumption" as the concentration of the solution increased from 4, to 16, and 32% sucrose when sham-fed during the same 30 min exposure; 1% sucrose was sampled minimally during both exposures. The difference in volume consumption between sham- and normal-feeding grew larger as the concentration of sucrose increased. This was in part due to the decrease in normal consumption on the higher concentrations of the inverted-U but also a result of sham-consumption increasing monotonically with sucrose concentration. Weingarten & Watson (1982) showed that, in single-bottle 30 min exposures, rats would sham-consume significantly more 40% than 30 %, 30% than 16%, and 16% than 6% sucrose solution. Rats sham-consume substantially more sucrose than would otherwise be possible and maintain a preference for more concentrated sucrose solutions (Nissenbaum & Sclafani, 1987; Sclafani & Nissenbaum, 1985; Weingarten & Watson, 1982).

Access Conditions and Consumption

Although the current thesis looks at sucrose consumption, investigations looking at the effects of access conditions on feeding span many types of solutions. Some of the earliest work on how availability affected intake was done with alcohol and only later expanded to sweet solutions and other flavours. In an effort to provide some history on access manipulations the work with alcohol will be introduced and the transition to sugar, and other solutions, discussed.

LeMagnen (1960) provided the first evidence that the removal and reintroduction of ethanol to rats with *ad lib* food and water caused a transient but marked increase in ethanol

consumption. At the time Amit, Stern, & Wise (1970) reported that the presentation of alcohol solutions on alternating days lead to elevated ethanol consumption and its preference over water. However, the Amit et al. (1970) study was complicated by coincident electrical stimulations of the lateral hypothalamus. In an effort to elucidate the contribution of intermittent availability to elevated consumption Wayner et al. (1972) presented rats with a variety of solutions, in separate experiments, at first on a continuous basis and then on alternating daily or 2-day schedules. Under these conditions rats increased their alcohol consumption when moved from a continuous to intermittent access schedule. Wise (1973) revisited his original study showing that intermittent availability was largely responsible for the increased alcohol consumption seen in previous studies. The ability of intermittent access to increase fluid consumption was extended to saccharin, quinine, citric acid, and saline solutions (Wayner & Fraley, 1972; Wayner et al., 1972).

When rats are presented with an artificially sweetened solution, devoid of any calories, they will increase consumption with intermittent access. Wayner and colleuges (1972) made a .05% saccharin solution continuously available to rats for 20 days and consumption was minimal. When saccharin was then made available every other day on 13 occasions (over 26 days) rats increased their consumption drastically. Throughout the alternate day period water was only consumed in any appreciable amount on days when saccharin was not present. Following this period of intermittent saccharin both saccharin and water were available continuously for 25 days and saccharin consumption remained substantially higher than that of water. A history of intermittent exposure to saccharin resulted in a long-lasting preferential consumption of saccharin over water. A similar finding was demonstrated by Celejewski (2011) where rats given E3DA to 1%, 0.5%, .25%, and 0.125% saccharin solutions maintained higher

consumption levels than rats given EDA to the same solutions. For both schedules the volume of solution consumed decreased as saccharin concentration increased which may be caused by saccharin's bitter aftertaste becoming more salient at higher concentrations. The EDA-E3DA consumption difference seen with .25% saccharin was maintained when all rats were switched to E2DA.

All of Wayner's work (Wayner & Fraley, 1972; Wayner et al., 1972) was based on a within-rat designs and so performed in the absence of continuous access controls. That is, consumption during periods of intermittent access and abstinence was compared with a preceding and subsequent periods of continuous access. Much of this work has since been replicated comparing continuous and intermittent access concurrently and perhaps most notably demonstrating the potent effect of intermittency on consumption of both rewarding and aversive substances (Celejewski, 2011; Pinel & Huang, 1976; Wise, 1973).

It is important to note the diversity of the solutions tested by Wayner and colleagues. Aside from representing a variety of flavours with different affective properties, these solutions have variable postingestive consequences. Sugar, for example, has been described as having a 'dual reinforcing effect' as it is rewarding because it is sweet, but also because it is rich in calories (Smith & Duffy, 1957b). Quinine, however, possesses only negative affective qualities as bitterness is widely regarded as aversive and quinine is non-nutritive. Ethanol solutions, at least initially, are aversive but have considerable calories. Agreement between the orosensory and postingestive consequences of a solution does not seem to interfere with access-induced consumption elevations. Sucrose and quinine solutions, which have different orosensory and postingestive consequences are both susceptible to access-induced increases in consumption (Eikelboom et al., Unpublished; Wayner et al., 1972). Furthermore, saccharin, which has a

sweet taste but no associated calories and ethanol, which has an aversive taste but offers readily accessible calories, are also consumed in greater amounts when presented intermittently (Pinel & Huang, 1976; Wayner et al., 1972). Taken together, solutions representing many permutations of orosensory, affective, and postingestive qualities display similar intake elevations as a result of intermittent availability.

Models of Elevated Palatable Food Consumption

Periodic substance availability has a robust effect on consumption and thus is the focus of many excessive and binge-like food consumption models. The exact parameters vary across a number of models but all models use intermittent access to elevate palatable food consumption during a portion or the entirety of food availability. These models usually contrast this intermittent access group with continuous access controls. Coarsely, these models can be divided into two categories: those where the rats are consuming under energy-deprived (food restricted) and energy-replete (*ad lib* fed) conditions. For simplicity, these labels will be used to dichotomize the models as discussed below.

Intermittent Access to Palatable Foods Under Energy-deprived Conditions

The late Bart Hoebel boasted a career of studying the regulation of feeding (Hoebel & Teitelbaum, 1962). Hoebel and associates defined a now well-established protocol to produce binge-like consumption in rats. The intermittent group in this protocol have access to a 10% sucrose or 25% glucose solution and food for 12 h beginning 4 h into the night cycle and are food-deprived the other 12 h; water is available *ad lib*. Continuous access controls have access to the sugar solution, food, and water *ad lib*. Daily consumption is measured for all rats upon cessation of the 12 h access period for the intermittent access rats for ~21 to 28 days. Rats with

intermittent and continuous access to the sugar solution consume similar amounts over a 24 h period, taking in ~65 ml for 25% glucose (Colantuoni et al., 2001) and ~85 ml for 10% sucrose (Rada, Avena, & Hoebel, 2005). However, intermittent rats consume, in a binge-like manner, ~12 ml of 25% glucose and ~20 ml of 10% sucrose solution during the first hour of access whereas continuous access controls consume only ~6 ml and ~15 ml of the same solutions (Colantuoni et al., 2001; Rada et al., 2005). This intermittent profile of consumption is paralleled by an increased motivation to consume, as assessed by bar-pressing, after a 14 day abstinence period (Avena, Long, & Hoebel, 2005).

The Hoebel model of binge-like sugar consumption has been studied in the context of addiction. Rats given intermittent access under this protocol show signs of binging, withdrawal, craving, and cross-sensitization which has been suggested as evidence that sugar addiction exists.

Boggiano & Chandler (2006) have defined a model of binge eating that incorporates food restriction combined with differential stress conditions. Stressful or negative emotional events can trigger episodes of binge eating in humans (Parylak, Koob, & Zorrilla, 2011). The food restriction + stress model involves three phases: 1) food restriction, 2) refeeding, and 3) a stress episode and feeding test. Throughout all phases water is available *ad lib* and 24 h consumption measurements are made 3-4 h into the light cycle. Typically four groups of rats are used in this protocol: a control group (C) that is never food restricted; a food restricted only group (R); a stressed only group (S); and a food restricted + stressed group (R+S). During the restriction phase, which lasts 5 days, R and R+S rats are maintained on 66% of their normal chow diet while C and S rats are fed *ad lib*. The refeeding phase follows consisting of *ad lib* access to Oreo™ cookies and chow for all rats for two days and then ad lib chow for four more days which can be extended until total chow consumption is similar across all groups. The last phase is the

stress and feeding test. After the last *ad lib* feeding day S and R+S rats are exposed to four brief 0.6 mA shocks separated by 15-second intervals in a shock chamber; C and R rats are exposed to the same environment in the absence of the shocks. Afterwards all rats are given 24-h *ad lib* access to Oreo cookies and chow and consumption is measured at 2, 4, and 24 h. This last day's protocol is repeated for a number of cycles but typically on the third or fourth cycle, on the stress and feeding tests, R+S rats will consume significantly more cookies in the first 2 h of access and also over the total 24 h period than any of the other groups (Hagan et al., 2002). Importantly, chow consumption is similar across all groups and the C, S, and R rats consume a similar number of cookies.

The restriction + stress model highlights the synergistic action of food restriction and stress which produce elevated consumption of highly palatable foods. This interaction may be involved in other models of binge eating but can be more clearly isolated and studied in this model. It has been pointed out that the stress + restricted feeding model may be most relevant to reports of stress-induced binging in humans (Corwin et al., 2011; Corwin & Babbs, 2012). In addition, this model represents a well-documented demonstration of access-induced increased palatable food consumption. A variation of this model has also been carried out with a 10% sucrose solution and restraint stress showing elevated sucrose consumption when these factors are combined (Martin & Timofeeva, 2010).

Intermittent Access to Palatable Foods Under Energy-replete Conditions

The Corwin binging model has been carried out with a variety of consumables including shortening (Corwin et al., 1998), sucrose (Wojnicki et al., 2007), sucrose-fat mixtures (Wong, Wojnicki, & Corwin, 2009), and cream-oil-sucrose mixtures (Lardeux, Kim, & Nicola, 2013).

The model was originally defined with shortening where rats would be maintained on *ad lib* food and water with vegetable shortening made available for 2 h either every day or only on three days of the week (i.e., on MWF). The 2 h access period in these studies generally ends just before the rat's dark cycle begins. Rats with MWF access in this protocol begin to consume significantly more shortening (~45 kcal) during the 2 h access period than the daily access controls (~25 kcal); a difference that stabilises at ~4-6 weeks. Along with elevated consumption, the MWF rats are more motivated to consume the shortening as assessed by progressive ratio test (Wojnicki, Babbs, & Corwin, 2010).

Lardeux et al. (2013) extended Corwin's access conditions to a liquid mixture of corn oil and sugar. The aim of this study was to offer this highly palatable solution through a sipper tube so that 1) latency to begin licking, 2) initial lick rate, and 3) changes in lick rate could be measured. These three measures were taken to indicate motivation, palatability, and the development of satiety, respectively. Under these conditions rats with intermittent access to the COS mixture showed increased motivation and palatability for the solution when compared to daily access control rats.

Particularly germane to the current thesis are the results seen by Corwin with sucrose solutions. Wojnicki et al. (2007) exposed rats to the typical Corwin protocol with 3.2, 10, or 32% sucrose; concentrations which were chosen to span the inverted-U *ad lib* consumption pattern discussed earlier (Spector & Smith, 1984). Rats with MWF access to 3.2% or 10% sucrose solutions came to consume significantly more of the solution in a 1.5 h period than daily access controls. Interestingly, rats with MWF and daily access to 32% sucrose consumed similar amounts of solution. In this study Wojnicki et al., (2007) successfully emulated their shortening results with less concentrated sucrose solution and found conflicting results with highly

concentrated sucrose solutions. This discrepancy begs the question of why the robust and reliable increases in consumption seen with intermittent access are non-existent with concentrated sucrose solutions. Given that rats are more motivated to consume 32% sucrose than weaker solutions Wojnicki et al. (2007) proposed that access manipulations are less salient at more concentrated sucrose solutions.

Eikelboom and associates have defined a protocol for studying access-induced elevations of sucrose consumption. In this model rats given ~24 h access to a 4% sucrose solution every 3rd day (E3DA) consume ~100 g more solution than continuous, everyday access (EDA), controls; a difference which emerges after 2-3 cycles and then stabilizes (Eikelboom et al., Unpublished). Typical 4% sucrose consumption levels for EDA and E3DA rats are ~100-150 g and ~200-250 g, respectively. Perhaps the most striking feature of this model is that after 12 cycles of this schedule, when all rats are given E2DA, rats previously under E3DA conditions will maintain higher consumption levels than rats previously under EDA conditions. The endurance of the EDA-E3DA consumption difference is taken as evidence that a long-lasting change, which may reflect an increased value for the solution, has occurred in the rat. Interestingly, this access-induced consumption difference is either small and emerges slowly, or not at all, with 8%, or 16% sucrose solution under EDA-E3DA conditions. That is, in a 24 h period, rats with EDA or E3DA to more concentrated sucrose solutions will generally consume similar amounts of solution across access conditions.

In the EDA-E3DA protocol the lack of a consumption difference with more concentrated sucrose solutions does not necessarily reflect the lack of an underlying (possibly learned) difference between the EDA and E3DA groups. In one experiment rats were given 12 cycles of EDA or E3DA to 16% sucrose, then two cycles of E2DA to 16% sucrose, and finally 8 cycles of

E2DA to 4% sucrose. When consuming 16% sucrose a small consumption difference emerged between the EDA and E3DA groups and was maintained for the two 16% E2DA cycles. This consumption difference became much larger when the rats were given E2DA to 4% sucrose. Importantly, this large EDA-E3DA difference was evident under conditions of similar access. That is, the introduction of a less concentrated 4% sucrose solution allowed for an already developed EDA-E3DA difference to emerge.

The emergence of an EDA-E3DA difference upon the introduction of E2DA to a 4% sucrose solution may reflect a learning performance distinction. Rats allowed to explore a maze unrewarded show no performance improvements in reaching the "goal box" as there is no incentive for completing the maze. Conversely, rats given food rewards for completing the maze will become increasingly efficient at finding the goal box. However, when rats with a history of unrewarded exploration are given a food reward in the goal box they will almost immediately perform with the same efficiency shown by rats that were rewarded from the beginning (Blodgett, 1929). Although, the results seen with sugar consumption across access conditions are quite different than learning to navigate a maze, the distinction between learning and performance may apply in both scenarios. The classic Blodgett (1929) experiment introduced the idea that for rats learning could occur without any observable changes in performance. The finding that EDA and E3DA rats who showed no difference with 16% sucrose solution immediately demonstrate a consumption difference when given E2DA to 4% sucrose suggest that an EDA-E3DA difference had developed with 16% sucrose but was somehow blocked from presenting. It is possible that the increased caloric value of 16% sucrose prevented a large EDA-E3DA difference from emerging because of a satiety limit on consumption. This possibility is reinforced by the rats preference to consume a fixed portion of its calories from sucrose (Collier

& Bolles, 1968). With 4% sucrose this satiety limit is avoided and, although learned with 16% sucrose, the EDA-E3DA consumption difference is only evident with the weaker solutions.

Access-induced consumption differences may exist, but be masked, at higher sucrose concentrations. The Eikelboom model highlights the importance of considering both value and access conditions when exploring elevated consumption of palatable foods. EDA and E3DA rats may consume similar amounts of highly concentrated sucrose solutions because of their intense palatability. It is unclear how fluid volume or calorie consumption limits and access conditions collectively determine intake.

Commonalities Among All Models

Controlling the intake of rewarding foods with varying access conditions is undoubtedly a complex problem for any animal. It is clear that across all models the periodic presentation and withdrawal of foods promotes elevated total consumption or discrete periods of binging. All models maintain rats on *ad lib* water but only in the Corwin and Eikelboom protocols are rats given unrestricted access to chow. This is a defining feature of these two models because it cannot be argued that the MWF or E3DA rats are consuming out of hunger: rats consume shortening or sugar under these conditions only volitionally. If the analogy is to be made from the intermittent rats to binging humans, energy-replete conditions are imperative as binge eating afflicts nearly 5% of non-food deprived Americans in their lifetime (Hudson, Hiripi, Pope, & Kessler, 2007).

These animal models of binge-eating disorder and sugar, or palatable food, addiction have been investigated to draw correlates to the human condition and explore the existence of nondrug addictions (Avena, Rada, & Hoebel, 2008; Corwin et al., 2011; Corwin & Babbs, 2012;

Corwin & Hajnal, 2005; Parylak et al., 2011). This objective is important and will undoubtedly yield useful information about the etiology of eating disorders and food addiction. However, the simpler question of how the rat regulates intake around changing access conditions and variations in nutritive value remains ill informed. Across three intermittent access protocols rats consume similar amounts of, continuously or periodically presented, highly concentrated sugar solutions but vastly different amounts of shortening or moderate sucrose solutions (Colantuoni et al., 2001; Corwin et al., 1998; Eikelboom et al., Unpublished). Together these findings suggest that access-induced consumption differences are optimally expressed within some moderate range of sucrose concentrations. The Eikelboom protocol provides a tractable methodology for investigating this possibility as manipulations of taste or caloric density can be made to 8 or 16% sucrose solutions in an effort to emulate the reliable 4% EDA-E3DA consumption difference.

The Hedonic Summation of Tastes

Much of the information about the nutritious content of a food is relayed through taste. Humans perceive five taste qualities which correspond to distinct populations of taste receptor cells on the tongue: sweet, bitter, sour, salty, and umami (Bachmanov & Beauchamp, 2007). These tastes are indicative of unique, but occasionally overlapping, postingestive consequences. Sweetness, in the case of natural sugars, is indicative of calorically rich carbohydrate-containing food. Bitterness is associated with toxins or food spoilage potentially causing malaise. Sourness may also indicate spoilage. Saltiness is often associated with the presence of sodium – a mineral that must be carefully regulated to maintain homeostasis (Richter, 1936). Umami refers to the taste of savoury foods rich with amino acids. Germane to the present work are the contrasting consequences predicted by sweets and bitters.

Young & Christensen (1962) determined whether the hedonic properties of distinct flavours would summate in compound solutions. Specifically they recorded rats consumption of simple sucrose and sodium chloride (NaCl) solutions and used this information to predict the consumption of compound sucrose + NaCl solutions. It was noted that NaCl solutions were appetitive at low concentrations and aversive at high concentrations thus defining acceptance and rejection concentration ranges. The adulteration of a simple sucrose solution with an acceptable NaCl concentration increased palatability of the compound solution whereas adulteration with an aversive NaCl concentration lowered palatability. Young & Christensen (1962) concluded that the hedonic intensities of sweet and salty solutions would summate when presented in a compound solution. This investigation was followed up in a similar procedural fashion but with combinations of other distinct flavours. One combination was that of bitterness, with quinine hydrochloride, and sweetness, with sucrose solutions.

Sweetness and bitterness are often thought of as representing pure ends of a palatability spectrum. The tastes of sucrose and quinine elicit almost solely hedonic and aversive reactions, respectively, in human infants and other primates (Steiner, Glaser, Hawilo, & Berridge, 2001). Therefore the adulteration of a sweet solution with a bitter flavour should reduce palatability as a function of the quinine concentration. Kappauf, Burright, & Demarco, (1963) and Young, Burright, & Tromater, (1968) investigated these interactions by exposing rats concurrently to two solutions; a simple sucrose solution and a compound solution of sucrose and quinine during brief choice periods. The concentrations of the sucrose and quinine in the compound solution would vary across choice tests and consumption or licks for each solution were measured. After exposing rats to a number of choice tests the researchers presented their data as isohedonic functions. These functions, which they referred to as isohedons, were presented on graphs with

quinine concentrations on the abscissa and sucrose concentrations on the ordinate. All points on the isohedon correspond to concentrations of sucrose and quinine that are equally acceptable, or isohedonic, to a simple sucrose solution. For clarity, a simple sucrose solution has a unique isohedon that spans a number of sucrose-quinine mixtures. From these investigations detailed information exists regarding, not only isohedonic concentrations of sucrose and sucrose + quinine solutions, but what concentrations of quinine are required to reduce the palatability of a variety of sucrose solutions. These values were used in the current study to choose quinine concentrations that would reduce, but not abolish, sucrose consumption.

Using Quinine to Reduce Caloric Consumption

Rats given E3DA to a 4% sucrose solution consume significantly more of the solution than rats given EDA. Rats with a gastric fistula will consume large amounts of highly concentrated sucrose solutions that would otherwise not be possible (Sclafani & Nissenbaum, 1985). An EDA-E3DA consumption difference presents across a number of saccharin solutions, varying in sweetness, where caloric constraints are irrelevant. All these findings implicate caloric constraints as a factor that may limit sucrose consumption. Thus the absence of an access-induced consumption difference at higher sucrose concentrations may be caused by a caloric maximum on sucrose calories (Collier & Bolles, 1968).

Kappauf, et al. (1963) and Young, et al. (1968) have provided detailed information on how bitter and sweet flavours combine into a single preference in brief choice tests. The data from these experiments suggest that adulterating a sucrose solution with quinine will reduce the palatability of the solution and consequently rats will consume less. Additionally, rats will concentration-dependently reduce sham-feeding of sucrose as the solution is adulterated with

quinine (Weingarten & Watson, 1982). If calories are preventing an EDA-E3DA consumption difference from emerging at higher sucrose concentrations an intake reduction, and consequent alleviation of caloric constraints, should allow for this difference to emerge. Adding bitterness to a concentrated sucrose solution to reduce consumption is a unique strategy to disentangle the roles of caloric density and access in sucrose consumption. Using quinine to reduce consumption will provide useful information with regards to how the rat regulates sucrose consumption based on availability, taste, and caloric density.

Experiment 1

The EDA-E3DA protocol produces large consumption differences with 4% but not 8 or 16% sucrose solutions (Eikelboom et al., Unpublished). At the more concentrated solutions rats typically consume similar amounts regardless of access conditions. However, when rats with a history of EDA or E3DA to a 16% sucrose solution are given E2DA to a 4% sucrose solution a consumption difference immediately emerges. Importantly, the difference emerges between EDA and E3DA rats that previously consumed similar amounts of 16% sucrose in a 24 h period, when they are given equivalent access to a less concentrated 4% sucrose solution. The absence of an EDA-E3DA difference at more concentrated sucrose solutions may be due to the increased caloric value of the solution. Rats with E3DA to the more concentrated sucrose solutions may become sated at consumption levels lower than can be achieved with weaker sucrose solutions.

The aim of experiment 1 was to investigate the potential for quinine adulteration to reduce palatability, consequently intake, and allow for an EDA-E3DA difference to emerge with 8% sucrose. Rats were given 4% or 8% sucrose with or without added .005% quinine (Q) and assigned to EDA or E3DA based on consumption during the initial 24 h exposure. It was expected that the EDA-E3DA consumption difference seen with 4%, and the lack-thereof with

8%, would be reaffirmed here (Eikelboom et al., Unpublished). Consumption levels were predicted to be lower for the quinine-adulterated solutions but more severely so for 4%+Q solution than the 8%+Q. This reduction in consumption was hypothesized to allow for an EDA-E3DA consumption difference to present with the 8%+Q solution. A secondary inquiry of this experiment was to see if an EDA-E3DA difference could emerge, in rats with a history of consuming 8% sucrose, upon the introduction of .005% quinine to their solution. This hypothesis was tested by adding quinine to the rats' initially unadulterated sucrose solutions after twenty common access days. Rats were maintained on their newly adulterated solutions for eight common access days and switched back to their original solutions to ensure that any changes seen during the quinine introduction were not simply a function of time.

Access to solutions in this experiment was actually only 23 h per day; allowing 1 h during which measurements could be taken and cages could be changed. Rats consume the majority of their *ad lib* food, water and any available sugar solutions during the night (Siegel & Stuckey, 1947; Hirsch & Walsh, 1982). To avoid interrupting any nighttime feeding or drinking all measurements were taken 5 h before the onset of the dark cycle.

Methodology

Subjects

Naïve adult male Sprague-Dawley rats (n=64) were ordered from Charles River Canada, St. Constant, Quebec and weighed about 200-225 g upon arrival. The experiment proper began 1 week after arrival. Rats were individually housed in standard plastic shoebox cages (20 x 24 x 45 cm) and maintained on a 12:12 light/dark cycle (lights on at 0900h) in a colony room at 21±2°C. All rats were weighed every three days and had *ad lib* access to Harlan Tek-Lab 8640

Rodent diet pellets (3.11 kcal/g metabolizable energy) and tap water. All experimental procedures in this and following experiments were approved by the Wilfrid Laurier University Animal Care Committee, which follows the policies and guidelines of the Canadian Council on Animal Care.

Materials

The sucrose solutions were made by using tap water and commercially available pure cane sugar, mixed on a weight/weight basis (i.e. grams of solute/grams of water x 100%). Quinine hydrochloride dehydrate ($C_{20}H_{24}N_2O_2 \cdot HCl \cdot 2H_2O$; Sigma MFCD00078498) was dissolved in 60°C water for 20 min and added to solutions to produce a .005% concentration. Solutions were prepared fresh daily approximately 2-3 h before they were given to the rats.

Procedure

Rats were randomly assigned to 4 groups receiving different phase 1 solutions: 4%, 8%, 4%+Q or 8%+Q. Then based on initial consumption rats were further assigned to EDA or E3DA conditions randomly but in a way that equated consumption between the two access conditions on day 1. Therefore after the initial Day 1 exposure half of the rats in each group received their solution again the next day and the other half received their solution again after a two day gap. In phase 1, rats were given twenty 24 h periods of common access to their phase 1 solutions (20 E3DA exposures; 60 EDA exposures). In phase 2 rats were given eight days of common access (8 E3DA exposures; 24 EDA exposures) to their phase 1 sucrose solutions either augmented with or depleted of quinine. That is, rats receiving quinine-adulterated solutions in phase 1 received quinine-free solutions of the same sucrose concentration in phase 2 and the reverse for rats receiving quinine-free solutions in phase 1. In phase 3, rats were given four days of common

access (4 E3DA exposures; 10 EDA exposures) to their phase 1 solutions. All solutions were presented to rats in 500 ml glass bottles that were weighed before and after each cycle. Access was actually ~23 h in duration because solution was not available from 1600 to 1700 h when measurements were taken and cages were cleaned.

Statistics

Sucrose solution consumption was analyzed for days when both EDA and E3DA groups had access to solutions. In all experiments throughout this thesis common access days occurred every three days (1, 4, 7, 10…). In all experiments Day 1 represented the last day for which rats were treated similarly across access conditions; on subsequent days rats were given either EDA or E3DA to the flavoured solutions. As such, Day 1 was analyzed independently in all experiments to highlight overall solution effects before differing access schedules were imposed. A selection of the first and last common access days of every phase of every experiment was analyzed. In some experiments as a result a portion of the days in-between the beginning and end of a phase went unanalyzed. Day 1 consumption was always analyzed in a between group factorial ANOVA. Consumption for a block of common access days was always analyzed in a between-groups repeated-measures ANOVA. Throughout this thesis all results involving the repeated-measures were reported as significant only if also significant with the Greenhouse-Geisser correction.

Results

Day 1 Consumption

On the first day rats were exposed to 4% or 8% sucrose with or without added .005% quinine (Q) for 24 h and assigned to EDA or E3DA to equate consumption during this period. Consumption of 4%, 8%, 4%+Q and 8%+Q for rats in the eight groups on this day is shown in figure 1. Consumption during the initial 24 h exposure to the rats' respective solutions was analyzed in an Access (EDA or E3DA) x Quinine (0% or .005%) x Sucrose (4% or 8%) between groups 3 way factorial ANOVA. The ANOVA revealed an effect of Quinine $F(1, 56)=24.7$, $p<.001$ and a Quinine x Sucrose interaction $F(1, 56) = 10.9$, $p = .002$. Quinine adulteration significantly reduced consumption levels and produced a more severe drop in 4% sucrose (a 114.7 g drop) than 8% sucrose (which only decreased by 23.2 g). This interaction would suggest that the .005% quinine concentration was able to reduce consumption more when added to 4% than 8% sucrose. As rats were assigned to EDA or E3DA from Day 1 onwards to equate consumption there was not an effect of Access or any interaction involving Access (all $F<1$) on Day 1.

The first and last four common access days of Phase 1

Consumption of 4%, 8%, 4%+Q and 8%+Q by rats with EDA or E3DA across the whole experiment is shown in figure 2. In phase 1 rats with EDA and E3DA were given 60 and 20 exposures to their flavoured solutions, respectively. The first (1, 4, 7 &10) and last (49, 52, 55, & 58) four common access days of phase 1 were analyzed in two separate four day blocks to highlight the first the emergence, and later the maintenance, of access and solution effects.

Solution consumption on the first four common access days was analyzed in a four Day (1, 4, 7, & 10) x Access (EDA or E3DA) x Quinine (0% or .005%) x Sucrose (4% or 8%) between-groups repeated-measures ANOVA. The ANOVA revealed significant effects of Day $F(3, 168)=54.7$, $p<.001$, Quinine $F(1, 56)=49.5$, $p<.001$, and a Day x Quinine interaction $F(3, 168)=5.7$, $p=.001$. The analysis also revealed a significant Sucrose x Quinine interaction $F(1, 56)=7.8$, $p=.007$. Figure 1 reveals the effect of Day and the Day x Quinine interaction reflect the increase in consumption over the first four common access days that was more pronounced for the unadulterated sucrose solutions. Quinine adulteration significantly reduced consumption levels as evidenced by the main effect of Quinine. However, this reduction in consumption was greater for 4% sucrose than 8% sucrose, which is reflected by the significant Sucrose x Quinine interaction. This interaction echoes the more salient effect of Quinine adulteration on 4% sucrose consumption seen on Day 1. In these first few days the effects of Access on consumption had not yet reached significance (Access $F<1$).

The analysis of days 49, 52, 55, & 58 was carried out identically to that of the first 4 common days and the data is also shown in figure 2. These days were the last four common access days of phase 1. At this point rats had experienced a long history of their respective access schedules and showed relatively stable consumption profiles. The ANOVA revealed significant main effects of Access $F(1, 56)=21.1$, $p<.001$, Quinine $F(1, 56)=28.2$, $p<.001$, and a significant Day x Sucrose x Quinine interaction $F(3, 168)=4.5$, $p=.004$. Figure 2 suggests this triple interaction is likely the result of decreasing, 4% and 8%+Q consumption, and increasing 8% and 4%+Q consumption. This effect is mostly incidental and a product of daily variability in sucrose consumption. Importantly, at the end of phase 1 rats with E3DA were consuming greater amounts of solution than rats with EDA as evidenced by the main effect of Access. The

Quinine effect remained significant from the beginning of phase 1 and reflects lower consumption of quinine-adulterated solutions than unadulterated sucrose solutions. Surprisingly in this experiment there was no overall significant difference in consumption between the 4 and 8% solutions.

The first and last four common access days of Phase 2

In phase 2 the quinine condition was flipped – rats previously receiving quinine-adulterated solutions were given unadulterated sucrose solutions and the opposite. Phase 2 was carried out for 8 common access days and was analyzed in two blocks of the first (days 61, 64, 67, & 70) and last (73, 76, 79, & 82) four common access days. These two analyses were carried out identically to those of Phase 1 with the exception that the .005% quinine groups were now the 0% quinine groups and the inverse.

The ANOVA of the first four common access days of phase 2 revealed significant main effects of Day $F(3, 168)=6.7$, $p<.001$, Quinine $F(1, 56)=12.4$, $p=.001$, and Access $F(1, 56)=28.0$, $p<.001$. This analysis also revealed significant Day x Quinine $F(3, 168)=6.0$, $p=.001$ and Access x Sucrose $F(1, 56)=6.9$, $p=.01$ interactions. The Day effect and Day x Quinine interaction reflect the changes in consumption that occurred at the beginning of phase 2. Quinine adulteration significantly reduced consumption levels. This reduction was most pronounced at the beginning of phase 2 as the groups that had quinine introduced to their solution increased their consumption gradually over days 61 to 70. In phase 2 rats with E3DA continued to consume significantly more than rats with EDA evident in the significant Access effect. The EDA-E3DA difference seen at the end of phase 1 with 8%+Q was maintained in phase 2 when these rats received pure 8% sucrose (fig 2D). However, the introduction of quinine to rats previously consuming pure

8% sucrose resulted in the emergence of a much larger EDA-E3DA difference (fig 2C). Interestingly, the lower consumption levels seen with quinine-adulterated solutions resulted in a larger EDA-E3DA difference with 8% than 4% sucrose, as evidenced by the significant Access x Sucrose interaction.

The ANOVA of the last four common access days (73, 76, 79, & 82) of phase 2 revealed significant main effects of Access $F(1, 56)=28.1$, $p<.001$, Quinine $F(1, 56)=5.1$, $p=.028$, and a Access x Sucrose interaction $F(1, 56)=8.6$, $p=.005$. The Access x Sucrose interaction remained significant throughout phase 2 and reflects a greater EDA-E3DA consumption difference with the 8% sucrose groups than the 4% sucrose groups. The Quinine effect reflects lower consumption levels for the quinine-adulterated solutions than the unadulterated sucrose solutions which permitted the large consumption difference seen with 8% sucrose to emerge.

The four common access days of Phase 3

In phase 3 rats were returned to their phase 1 solutions for four common access days to ensure that any effects seen in phase 2 were the result of solution manipulations and not time alone. The analysis of days 85, 89, 91, & 94 was carried out identically to that of phase 1. The ANOVA revealed significant effects of Access $F(1, 56)=16.4$, $p<.001$, Quinine $F(1, 56)=37$, $p<.001$ and a significant Day x Access interaction $F(3, 168)=3.2$, $p=.026$). The Day x Access interaction reflects the gradual recovery of phase 1 consumption levels as the groups were switched back to their original solutions; EDA and E3DA rats slightly increased and decreased, respectively. The Access effect reflects greater consumption by E3DA than EDA rats. The Quinine effect reflects lower consumption levels by rats receiving quinine-adulterated solutions

than unadulterated solutions. Comparatively the effects of Quinine and Access were similar before and after phase 2.

Discussion

Experiment 1 was performed with the hypothesis that quinine-adulteration could be used to uncover an EDA-E3DA consumption difference that is normally masked by more concentrated sucrose solutions. The use of quinine as a manipulation of palatability was immediately evident in Day 1 consumption where quinine-adulteration produced a more severe drop in 4% than 8% sucrose consumption (fig 1). This may be taken as evidence that sweeter sucrose solutions are more valuable to the rat, consistent with previous work (Collier & Bolles, 1968; Young & Greene, 1953). Figure 2 shows that rats demonstrate an EDA-E3DA difference when consuming 8% sucrose + .005% Q. It was also evident that introducing .005% Q to rats with a history of EDA or E3DA to pure 8% sucrose produced a much larger EDA-E3DA difference. In a previous report rats with a history of EDA or E3DA to 16% sucrose evidenced a consumption difference when given E2DA to a 4% sucrose solution (Eikelboom et al., Unpublished). The results of this earlier report and experiment 1 suggest that reducing consumption levels, whether it be by introducing a weaker sucrose solution or quinine-adulteration, allows for an EDA-E3DA difference to emerge with more concentrated sucrose solutions.

In the current experiment the 4% sucrose group presented with atypical consumption levels and consequently phase 1 was elongated from the more usual 12 common access days. Usually, with 4% sucrose, an EDA-E3DA consumption difference emerges within the first few days of access where EDA rats stabilize consumption at ~100-150 g and E3DA rats increase

consumption over days into a plateau at ~200-250 g. In this experiment rats with EDA and E3DA both increased consumption sharply over the first few days and then plateaued at an elevated, small, EDA-E3DA difference. Sucrose consumption, particularly at lower concentrations, can be quite variable (Collier & Bolles, 1968). At this point there is no obvious alternative explanation why sucrose consumption in the 4% EDA group was so high.

Although assigning rats to access conditions based on Day 1 consumption has been done repeatedly by researchers it may be a poor strategy for equating groups (Avena & Hoebel, 2003; Corwin & Wojnicki, 2006; Eikelboom et al., Unpublished). In this experiment, both the EDA- and E3DA groups consumed more 4% sucrose than normally seen with the EDA-E3DA protocol. It has been shown that rats split into high and low consumers based on a brief consumption period will respond differently to amphetamine (DeSousa, Bush, & Vaccarino, 2000). Also, Corwin & Wojnicki, (2006) have commented on the difficulty of assigning rats that do not consume during the initial shortening exposure to access conditions. It may be better to give animals several days access to the sucrose solution before assigning them to different groups.

Overall the results of experiment 1 support the hypothesis that quinine can be used to reduce the palatability, and intake, of sucrose solution allowing an EDA-E3DA difference to emerge with 8% sucrose. Unfortunately, this finding was complicated by the atypical 4% sucrose EDA rat results and perhaps suggests that group assignment should be done in a more robust manner. It would seem that the rat regulates the intake of a supplementary sucrose solution based on concentration, availability, and the value of the solutions. The complications with group assignment and the encouraging quinine results were specifically revisited in subsequent experiments.

Experiment 2

The results of experiment 1 supported the initial hypothesis as the reduced consumption seen with 8%+Q sucrose solution allowed an EDA-E3DA consumption difference to emerge. As well the EDA-E3DA differences seen in phase 2 were larger with 8% sucrose than 4% sucrose. However, the reliable EDA-E3DA difference seen with 4% was considerably smaller in this experiment than was previously reported. Although assigning rats to access conditions on the basis of consumption during an initial exposure has been performed previously (Avena & Hoebel, 2003; Corwin & Wojnicki, 2006; Eikelboom et al., Unpublished) the difficulty of how to accommodate rats that refuse to consume or spontaneously alter consumption levels has been mentioned (Corwin & Wojnicki, 2006). In their initial report on sucrose consumption Collier & Bolles (1968) commented that, over the 40 day observation period, rats consuming less concentrated solutions showed considerably more daily-intake variability than more concentrated solutions.

The issue of how to account for these individual differences for group assignment was explored using previously collected data from the Eikelboom lab for the EDA-E3DA protocol. Consumption data for 72 rats with *ad lib* access to 4% sucrose for at least 20 days was compiled. The intake on days 1, 4, 7, & 10 was correlated with consumption on day 20 to assess the quality of a single days intake as a predictor of future consumption (Valyear, Senthinathan, Celejewski, & Eikelboom, 2014). This analysis demonstrated that consumption after a few days access to 4% sucrose was a better predictor of future consumption than Day 1 intake. To ensure that this was not an artefact of later days being temporally closer to Day 20, consumption on days 1, 4, 7, & 10 was correlated with that on days 10, 14, 17, & 20, respectively. In this analysis, Day 1 was still a poorer predictor than Day 4, which accounted for substantially more variance in future

consumption. Days 7 & 10 were only marginally better predictors of future consumption than Day 4. Informing group assignment with Day 1 consumption alone may be complicated by it being the rats' first exposure to the solution. Certain rats may be neophobic towards the solution or may not consume any of the solution in the first 24 h of access.

Atypical results have been reported with the 4% sucrose EDA-E3DA protocol in the past as a result of a single pre-exposure followed by a 'wash out' abstinence period (Valyear, 2013). However, rats with a long history of EDA to 4% sucrose will quickly show an EDA-E3DA consumption difference when half of them are switched to E3DA (Senthinathan, 2013). To successfully argue that changes in consumption are a result of access conditions it is important to ensure that groups show similar baseline intake. In an effort to correct the atypical results seen with 4% sucrose in experiment 1, rats in this second experiment were exposed to 4% sucrose continuously for 6 days to provide a rich dataset on which to base subsequent group assignment.

Experiment 2 is a replication of experiment 1 but with a pre-exposure imposed to reduce individual differences in sucrose consumption having an unexpected impact on group assignment, which likely complicated the results of experiment 1. Immediately following the pre-exposure rats were assigned to consume 4% or 8% sucrose with or without added .005% quinine for 24 h before being given EDA or E3DA. The assignment to access conditions was undertaken to further equate consumption during the pre-exposure as well as the initial 24 h exposure.

In addition to the pre-exposure in this experiment once EDA-E3DA consumption differences were evident hourly measurements of consumption were taken to investigate the temporal profile of EDA-E3DA consumption. Hoebel and colleagues report consumption

differences only in the first hour of access in their 12 h food and sugar deprivation model (Avena et al., 2006). This protocol has been conducted with 25% glucose and 10% sucrose both showing similar results. Hourly measurements were taken to compare the current procedure to the Hoebel protocol specifically with respect to the 8% sucrose group. In both protocols rats consume similar amounts of solution across access conditions but the intermittent group shows elevated first hour consumption in the Hoebel protocol. It was expected that a similar temporal consumption profile would be seen here with the 8% sucrose E3DA group. Hourly measurements were taken for the first six hours of access on the 10th common access day (Day 28) for all rats. For these measurements to be meaningful the timing of sucrose administration each day differed from experiment 1. Where experiment 1 daily measurements were taken and bottles put on in the middle of the day, to leave nighttime feeding and drinking undisturbed, in this experiment bottles were removed 1 h before the lights went out and fresh bottles were placed on cages 1 h into the dark cycle. The rats were weighed and had their cages changed after the bottles were removed which left them undisturbed for at least 1 h before bottles were replaced. Rats typically begin their feeding and drinking 1 h into the dark cycle (Siegel & Stuckey, 1947). Thus the natural initiation of feeding and drinking aligned with bottle replacement, similar to the Hoebel protocol.

Methodology

Subjects

Naïve adult male Sprague-Dawley rats (n=64) weighing about 200-225 g upon arrival were ordered from Charles River Canada, St. Constant, Quebec and were housed and treated

identically to the rats in experiment 1. The only deviation from experiment 1 was the lights on time of 0430 h in this experiment.

Materials

All solution preparations were identical to those in experiment 1.

Procedure

Rats were pre-exposed to a 4% sucrose solution continuously for 6 days (Days -5 to 0). Pre-exposure consumption data was used to assign rats to one of four phase 1 solutions: 4% or 8% sucrose solution with or without .005% added quinine. Rats then received one 24 h exposure of these solutions commencing phase 1(Day 1); consumption on this and pre-exposure days was used to then assign rats to EDA or E3DA. In other words, after this initial phase 1 solution exposure half of the rats received their phase 1 solution again the next day and the other half received their solution again after a two day gap. In phase 1, rats were given twelve 24 h periods of common access to their phase 1 solutions (12 E3DA & 36 EDA flavoured solution exposures; Day 1 to 36)). On the 10^{th} common access day of phase 1 (Day 28: 10^{th} E3DA exposure; 28^{th} EDA exposure) consumption was measured hourly from the time solution was given to the rats (1730 h) for six hours. This was accomplished by having two sets of bottles that could be interchanged in approximately 2-3 min. All solutions were presented to rats in 500 ml glass bottles that were weighed before and after each day. Access was actually only 22 h in duration because solution was not available from 1530 to 1730 h, 1 h before and after the lights went out, when measurements were taken and cages were changed.

Statistics

The statistics for experiment 2 were conducted in the same fashion as in experiment 1 with the only exception being that 6 common access days were analyzed in all the repeated measures ANOVA.

Results

6-day Pre-exposure

In experiment 2 all rats were pre-exposed to 4% sucrose for 6 continuous days and assigned to one of four solution groups and then to EDA or E3DA based on Day 1 and pre-exposure consumption. Consumption during the pre-exposure and the remainder of the experiment is shown in figure 3. Solution consumption (all rats receiving 4% sucrose) during the pre-exposure was analyzed in a 6 Day (Days -5 to 0) x (ultimate) Access (EDA or E3DA) x Quinine (0% or .005%) x Sucrose (4% or 8%) between-groups repeated-measures ANOVA. This analysis revealed a significant effect of Day $F(5, 280)=5.7$, $p<.001$ as consumption tended to increase over the pre-exposure (from ~150 to ~180 g). The factors Access, Sucrose, and Quinine and all interactions were non-significant as group assignment equated pre-exposure consumption across groups. The lack of any Access, Sucrose, or Quinine effects demonstrates that the groups were not different.

Day 1 Consumption

In experiment 2, Day 1 was the last day for which EDA and E3DA rats were treated similarly as well as being the first exposure to 8%, 4%+Q, & 8%+Q solutions. (Note the rats receiving 4% sucrose in the pre-exposure continue to receive this solution in phase 1.) For

reference, the consumption on Day -5, the day all 64 rats were initially exposed to 4% sucrose, or any sugar solution for that matter, is shown in figure 4. Day 1 consumption data, analyzed here, is shown alone in figure 5. Solution consumption on Day 1 was analyzed in an Access (EDA or E3DA) x Quinine (0% or 0.005%) x Sucrose (4% or 8%) ANOVA. Rats were assigned to access conditions to equate Day 1 consumption and as such there was not an effect of Access $F<1$. There was a significant effect of Quinine $F(1, 56)=70.6$, $p<.001$ as rats consumed less of the quinine-adulterated (48.1 g) solutions than the unadulterated solutions (171.2 g) but no significant effect of or interaction involving Sucrose.

The first and last six common access days of Phase 1

Consumption of 4%, 8%, 4%+Q and 8%+Q during phase 1 of experiment 2 are shown in figure 3. In phase 1 (after 4% pre-exposure) rats with EDA and E3DA were given 36 and 12 exposures, respectively. The first (1, 4, 7, 10, 13, & 16) and last (19, 22, 25, 28, 31, & 34) six common access days of phase 1 were analyzed in two separate six day blocks to highlight the first the emergence, and later the maintenance, of access and solution effects.

Sucrose consumption on the first six common access days was analyzed in a Day (1, 4, 7, 10, 13, & 16) x Access (EDA or E3DA) x Quinine (0% or .005%) x Sucrose (4% or 8%) between-groups repeated-measures ANOVA. The ANOVA revealed a significant main effect of Day $F(5, 280)=59.2$, $p<.001$, Quinine $F(1, 56)=20.0$, $p<.001$, and significant Day x Access $F(5, 280)=3.4$, $p=.015$, Day x Sucrose $F(5, 280)=6.2$, $p<.001$, and Day x Quinine $F(5, 280)=18.1$, $p<.001$ interactions. The three-way Day x Sucrose x Access $F(5, 280)=2.7$, $p=.02$ and Day x Sucrose x Quinine $F(5, 280)=4.0$, $p=.002$ interactions were also significant.

The beginning of phase 1 highlights the change in access, solution, and quinine effects

over time (see fig. 3). Rats tended to increase their consumption over the first six common access days, reflected in the significant Day effect. This increase was more pronounced in the E3DA groups than the EDA groups, but the difference in EDA-E3DA consumption was smaller in the 8%, than in the 4%, groups. The gradual emergence of a small EDA-E3DA difference is evidenced by the three-way Day x Sucrose x Access interaction. The Day x Sucrose x Quinine interaction reflects that, collapsed across access conditions, 4% rats show little change in consumption over the first six common access days whereas 4%+Q rats show a marked increase in consumption over this time (from an initial very low consumption level). In contrast the 8% rats showed a gradual increase in consumption over the first six common access days whereas the 8%+Q rats show a pronounced increase in consumption over this time (again from a suppressed initial level). Importantly, the effect of Quinine was significant meaning that these solutions were consumed in lower quantities.

The analysis of the last six common access days (Days 19, 22, 25, 28, 31, & 34) showed stable consumption as the Day effect was non-significant $F<1$. The ANOVA revealed significant effects of Access $F(1, 56)=5.8$, $p=.019$, Quinine $F(1, 56)=5.6$, $p=.021$, and an Access x Sucrose interaction $F(1, 56)=4$, $p=.05$. The Access x Sucrose interaction reflects the clear stable EDA-E3DA difference that can be seen with the 4% sucrose solutions and lack thereof with the 8% sucrose solution where the EDA and E3DA rats showed similar consumption. The Quinine effect was significant as bitter solutions were consumed in lower quantities than unadulterated sucrose solutions. However, unlike in experiment 1, this reduction did not allow for an EDA-E3DA consumption difference to emerge with 8% in experiment 2.

Hourly consumption on Day 28

On the 10th common access day, Day 28, hourly consumption measurements were taken for the first six hours of solution access. The hourly consumption data for 4%, 8%, 4%+Q, & 8%+Q sucrose solutions are shown in figure 6. Consumption during the first hour was analyzed independently as this day is often the only hour reported in other studies (Avena et al., 2006). Hour 1 consumption was analyzed in a Access (EDA or E3DA) x Quinine (0% or ,005%) x Sucrose (4% or 8%) ANOVA. This analysis, revealed significant effects of Access $F(1, 56)=16.3$, $p<.001$, Quinine $F(1, 56)=14.5$, $p<.001$, and Sucrose $F(1, 56)=10.4$, $p=.002$. Interestingly, rats with E3DA consumed significantly more in the first hour of access than rats with EDA across all solutions, evidenced by the Access effect. In the first hour quinine solutions were consumed in lower quantities than pure sucrose solutions and 8% sucrose intake was greater than 4% sucrose intake. Despite the sucrose and quinine effects, 24 h differences or lack thereof, all solution groups showed an EDA-E3DA difference in the first hour.

Consumption during the remaining 2 to 6 hours was analyzed in a 5 Hour by Access (EDA or E3DA) x Quinine (0% or .005%) x Sucrose (4% or 8%) between-groups repeated-measures ANOVA. This ANOVA revealed significant effects of Hour $F(4, 224)=2.9$, $p=.023$, Quinine $F(1, 56)=5.0$, $p=.029$ and a Access x Sucrose interaction $F(1, 56)=7.9$, $p=.007$. This analysis also revealed a significant four-way Hour x Access x Quinine x Sucrose interaction $F(4, 224)=3.0$, $p=.021$. The Hour x Access x Quinine x Sucrose interaction and Hour effect reflect changes in consumption that occurred over hours 2 to 6. The four-way interaction suggests however that all groups were changing independently. The Access x Sucrose interaction reflects the EDA-E3DA consumption difference that was present for only the 4% sucrose solutions although this difference diminished towards the end of the 6 h period for the 4%+Q group. The

4% sucrose group also presented with the largest 24 h EDA-E3DA difference on Day 28 whereas the 8% groups consumed similarly across access conditions. The quinine effect reflects that quinine solutions were consumed in lower quantities through hours 2 to 6.

Discussion

In experiment 2 a 6-day period of continuous 4% sucrose solution pre-exposure was imposed to inform group assignment. This procedure allowed for the assignment of 64 rats into equivalent solution and access groups. The small EDA-E3DA 4% consumption difference seen in experiment 1 may have been a function of poor group assignment as in experiment 2, where pre-exposure facilitated better group assignment, the 4% sucrose group demonstrated a typical EDA-E3DA consumption difference. Unfortunately, the EDA-E3DA difference seen with 8%+Q in experiment 1 was not reproduced in experiment 2. Unanticipated contrast effects as a result of having the pre-exposure before phase 1 may account for the differing results.

An additional motive for experiment 2 was exploring the hourly consumption data recorded on the 10th common access day. For this data to be collected the solutions were only available for 22 h a day, resulting in a gap beginning an hour before and ending an hour after lights out, throughout the experiment. This gap allowed for the solutions to be made available an hour after lights out when consumption normally would be high, and so the solution would be measured at a time similar to that used by Hobel and associates (Avena et al., 2006). The procedure of taking hourly measurements on the 10th common access day seemed to be rather innocuous as 24 h consumption on this day was unchanged from the preceding and following days. Rada et al. (2005) have reported first hour consumption differences in the Hoebel model of excessive sugar consumption with 10% sucrose. As the current experiment used a marginally

different 8% sucrose concentration, hourly consumption levels were recorded to assess the similarity between the Hoebel model and the current procedure. Generally rats with E3DA to solutions consumed more in the first few hours of access than rats with EDA which coincides with Rada et al. (2005). A further look at the data also suggests that 4% sucrose, which shows a 24 h EDA-E3DA difference, demonstrates a stable EDA-E3DA difference over the first few hours of access whereas EDA-E3DA 8% sucrose consumption is only different in the first hour of access. This data is useful for elucidating the temporal mechanism by which a 24 h consumption difference emerges (see fig 6).

The results of experiment 2 encourage the efficacy of a pre-exposure for creating balanced groups. However, the conflicting 8%+Q results of experiments 1 and 2, a critical concern in this thesis, are problematic. Procedurally, experiments 1 and 2 differ by having a 4% pre-exposure period and the associated contrast effects. As such, experiment 3 aimed to elucidate the effect of pre-exposure experience on future 8%+Q consumption.

Experiment 3

In experiment 2 rats consuming 4% sucrose demonstrated a typical ~100 g EDA-E3DA difference with EDA and E3DA rats consumed ~140 g and ~240 g of solution, respectively. Perplexingly, the 8%+Q EDA-E3DA difference seen in experiment 1 was not seen in the second experiment; 8%+Q EDA and E3DA rats consumed similar amounts of the solution. In particular the 8%+Q EDA group showed an elevated consumption in experiment 2 compared to experiment 1. Imposing a 6-day pre-exposure to a common solution for all rats was done to minimize group differences in sucrose consumption by having enough data to assign the 64 rats to 8 equivalent groups. However, this procedure meant that rats consuming 4%+Q, 8%, and 8%+Q solutions received different solutions in phase 1 from their pre-exposure experience. The contrast between

these solutions may have resulted in the discrepant consumption profiles for the EDA 8%+Q groups in experiments 1 and 2.

To test this hypothesis the effects of various pre-exposure regimens on 8%+Q solution consumption were tested. Rats with EDA to 8%+Q consumed ~100 g in experiment 1 and ~160 g in experiment 2 whereas rats with E3DA to the same solution consumed approximately 200±10 g in both experiments. The discrepant results seem to be a result of large daily consumption differences between experiments 1 and 2 for the EDA 8%+Q group and thus rats received only EDA after pre-exposure in experiment 3. Experiment 3 tested three pre-exposure conditions: a 4% sucrose solution, a vanilla flavoured 4% sucrose solution, or no sugar solution. All groups had water and chow available at all times and were given EDA to 8% sucrose + .005% quinine following the pre-exposure. The no pre-exposure and the 4% sucrose pre-exposure groups replicated the 8%+Q EDA groups from experiments 1 and 2, respectively. The flavoured pre-exposure solution (4% sucrose solution + .5% vanilla extract) was included to control for possible contrast effect resulting from receiving a simple sucrose solution before a compound sucrose + Q solution. If the simple to compound solution contrast effect caused the discrepant 8%+Q EDA results then this flavoured pre-exposure solution group should consume similar amounts to the 8%+Q group in experiment 1.

Methodology

Subjects

Adult female Sprague-Dawley rats (n=30) were obtained from a previous study where all rats were given 40 days of *ad lib* wheel access. The rats were originally ordered from Charles River Canada, St. Constant, Quebec and weighed about 200-225 g upon arrival and 270-360 g

upon initiation of the current experiment. Rats were housed and treated identically to those in experiment 1 with lights on at 0900h.

Materials

All solution preparations were identical to those in experiment 1. The flavoured 4% sucrose solution had a commercially available artificial vanilla extract (Club House™) added to produce a .5% concentration.

Procedure

Rats were randomly assigned to be pre-exposed to a 4% sucrose solution, a 4% sucrose solution flavoured with .5% artificial vanilla extract (4%+V), or no pre-exposure with only water continuously for 6 days. After the 6 pre-exposure days all rats were given access to an 8% sucrose solution adulterated with .005% quinine for 8 days. All solutions were presented to rats in 500 ml glass bottles that were weighed before and after each day. Access was actually only ~23.5h in duration because solution was not available from ~1515h to ~1545h when measurements were taken and cages were changed.

Statsitics

The analysis of experiment 3 was divided into a 6-day pre-exposure which was analyzed as a two group by 6 day mixed ANOVA and then the 8-day 8%+Q exposure analyzed in a three group by 8 day ANOVA. The group pre-exposed to only food and water was not analyzed in the pre-exposure ANOVA.

Results

6-day Pre-exposure

The daily consumption data for experiment 3 is displayed in figure 7. Sucrose consumption during the 6-day pre-exposure was analyzed in a 6 Day x Solution (4% or 4%+V) between-groups repeated-measures ANOVA. This analysis revealed a significant effect of Day $F(5, 90)=9.7$, $p<.001$ as both groups decreased their consumption over the 6 day period. There was also a marginally significant effect of Solution $F(1, 18)=4.3$, $p=.052$ with the 4%+V solution consumed in larger quantities than the pure 4% sucrose solution.

Continuous 8% sucrose + .005% quinine exposure

Consumption during the 8%+Q exposure was analyzed with the intention of identifying any consumption differences evident as a result of the rat's pre-exposure experience. To this end, solution consumption during the 8 day 8%+Q exposure was analyzed in an 8 Day by Pre-exposure (Naïve, 4%, or 4%+V) between-subjects repeated-measures ANOVA. This analysis revealed non-significant effects of Day, Pre-exposure, and their interaction (all $F<1$). Consumption levels were stable across the 8-day exposure and similar for all three groups. The lack of any between-group effects suggests that neither the imposition of a pre-exposure nor the solutions used impacted future 8%+Q consumption.

Discussion

In experiment 3 rats consumed similar amounts of 8%+Q solution regardless of their pre-exposure condition. This result would seem to weaken the possibility that a contrast effect produced discrepant experiment 1 and 2 results.

One difference in experiment 3, when compared to experiments 1 and 2, was the use of female rats. The only sex differences in sugar consumption that have been reported suggest that female rats tend to prefer sweeter solutions than males. That is, female rats will consume more of a .25% saccharin + 3% glucose solution than a .125% saccharin + 3% glucose solution whereas male rats will prefer the .125% saccharin + 3% glucose solution (Valenstein, Cox, & Kakolewski, 1967). Furthermore, female rats tend to show a preference for saccharin solutions to glucose solutions whereas male rats prefer the glucose solution Valenstein et al. (1967). These differences remain when female and male rats with similar body weights or similar ages are compared.

In experiment 2 when rats were switched from 4% sucrose during the pre-exposure to 8%+Q in phase 1 consumption decreased quite dramatically. In experiment 3 only the rats receiving a flavoured pre-exposure solution decreased their consumption when given 8%+Q; the pure 4% sucrose pre-exposure group consumed similar amounts of 4% sucrose and 8%+Q. It is unclear why a drop in consumption from the pre-exposure to phase 1, for the 4% to 8%+Q group, was seen in experiment 2 but not in experiment 3. One possible explanation is that female rats are more sensitive to quinine than male rats. This might explain the sex difference in saccharin (which has a bitter aftertaste) + glucose solution consumption reported earlier. This may reflect a sex difference that has not previously been investigated in rats.

Experiment 4

A number of studies have investigated taste perception of flavours, including bitters, in humans (Falconer, 1947; Fischer, Griffin, England, & Garn, 1961; Fischer & Griffin, 1963; Hansen, Reed, Wright, Martin, & Breslin, 2006; Harris & Kalmus, 1949; Hartvig, Hausner, Wendin, & Bredie, 2014) rats (Tordoff, Alarcon, & Lawler, 2008) and mice (Bachmanov, Reed,

Tordoff, Price, & Beauchamp, 1996). A commonality among all the human studies is considerable individual variability in bitterness sensitivity. In humans bitterness sensitivity can be explained by a number of genetic factors (Reed et al., 2010; Shi, Zhang, Yang, & Zhang, 2003; Soranzo et al., 2005) including a quinine-specific variant which accounts for 12% of the variance in quinine sensitivity (Hansen et al., 2006).

The human studies of individual differences in quinine sensitivity have focused on detection thresholds. One methodology has been to classify people as tasters (sensitive) or non-tasters (insensitive) based on the bimodal distribution of detection thresholds (Falconer, 1947; Fischer & Griffin, 1963). The nature of detection thresholds require experimenters to use quinine concentrations that are salient enough to be detected by some, but not all, participants as individual differences are most pronounced at moderate intensities. This point is further evidenced in animal studies looking at strain differences in flavour preferences. Across 14 rat breeds, strain differences were most pronounced at a .001% quinine concentration, but not a lower .0004% solution, while a higher .04% solution was unanimously rejected (Tordoff et al., 2008). As incidental controls in two other studies comparing 7 and 3 rat strains higher quinine concentrations (.25%) were consumed in amounts small enough to be dismissed as spillage (Goodwin, Bergeron, & Amit, 2000; Overstreet et al., 1993). Collectively the human studies of detection thresholds and the rat studies of strain differences suggest that individual differences in quinine sensitivity are most evident at moderate concentrations.

In experiment 3 rats consumed virtually identical amounts of 8%+Q regardless of whether they were pre-exposed to water and chow alone or with an additional solution of 4% sucrose or 4% sucrose + vanilla. The null result of experiment 3 and the evidence of variability in quinine sensitivity suggest that the discordant results for the 8%+Q groups in experiments 1

and 2 may be caused by differences in bitterness perception. Specifically, the bitterness may have been more salient to the rats with EDA to 8%+Q in experiment 1 as they consumed ~60 g less than the same group in experiment 2. Despite this variability, quinine adulteration reduced consumption in both experiments. The .005% quinine concentration used in these two studies may be moderately intense and thus promote the likelihood of individual differences. To circumvent this issue in experiment 4 rats were exposed to 8% sucrose adulterated with .0025%, .005%, .01%, or .02% quinine to cover the concentration range used in previous studies.

As in experiment 2, hourly measurements of consumption were taken on the 10th common access day (Day 28). Given the practicality of the procedure used in experiment 2 the same procedure was used in this fourth experiment. That is, a daily 2 h gap in solution access was used throughout the experiment. This gap began an hour before and ended an hour after lights out. As rats feed and drink mostly at night, this two hour gap allowed the first hour of consumption measurement to coincide with the natural initiation of feeding and drinking.

Methodology

Subjects

Naïve adult male Sprague-Dawley rats (n=64) were ordered from Charles River Canada, St. Constant, Quebec weighing about 200-225 g upon arrival and were housed and treated identically to the rats in experiment 1 with lights on at 0400 h.

Materials

All solution preparations were identical to those in experiment 1. The quinine was added to solutions to produce a .0025%, .005%, .01%, or .02% concentration.

Procedure

Rats were randomly assigned to 4 groups. Each of the four groups was assigned to their phase 1 solution: 8% Sucrose + .0025% Q, 8% Sucrose + .005% Q, 8% Sucrose + .01% Q or 8% Sucrose + .02% Q. Rats received their solution for three pre-exposure days, and then on Day 1, commencing phase 1; consumption during the pre-exposure days was used to assign rats to EDA or E3DA. After Day 1 exposure to one of the 4 solutions half of the rats in each group received their solution the next day and the other half received their solution after two days. In phase 1 rats were given twelve 24 h periods of common access to their phase 1 solutions (12 E3DA exposures; 36 EDA exposures). On the tenth common access day of phase 1 (Day 28: 10^{th} E3DA exposure; 28^{th} EDA exposure) consumption was measured hourly from the time solution was given to the rats (1700 h) for six hours. This was accomplished by having two sets of bottles that could be interchanged in approximately 2-3 min. In phase 2 rats were given four days of common access (4 E3DA exposures; 10 EDA exposures) to an 8% sucrose + 0.02% Q solution; rats receiving this solution in phase 1 were maintained on the same solution across phases. All solutions were presented to rats in 500 ml glass bottles that were weighed before and after each day. Access was only 22 h in duration because solutions were not available from 1500 to 1700h, 1h before and after the lights went out, when measurements were taken and cages were changed.

Statistics

The statistics for experiment 4 were conducted in the same fashion as in experiments 1 & 2. The pre-exposure in experiment 4 was three days in duration and was analyzed in one repeated-measures ANOVA. Phases 1 and 2 could be divided into blocks of four common access days. The first and last four common access days of phase 1 and the four common access

days of phase 2 were all analyzed in repeated measures four Group x two Access ANOVAs. One rat with EDA and another with E3DA to 8%+.005% Q died, for unknown reasons, during the course of this study. All their data was excluded from all analyses.

Results

3-day Pre-exposure

In experiment 4 rats were pre-exposed to 8% sucrose + .0025%, .005%, .01%, or .02% quinine for 3 continuous days and assigned to EDA or E3DA based on Day 1 and pre-exposure consumption. Consumption during the pre-exposure and the remainder of the experiment is shown in figure 8. Solution consumption during the 3 day pre-exposure was analyzed in a 3 Day (-2, 1, & 0) x (ultimate) Access (EDA or E3DA) x Quinine (.0025%, .005%, .01%, or .02%) between-groups repeated measures ANOVA. The ANOVA revealed a significant effect of Day $F(2, 108)=22.3$, $p<.001$, Quinine $F(3, 54)=9.8$, $p<.001$ and a significant Day x Quinine interaction $F(6, 108)=2.5$, $p=.027$ ($p=.05$ with Greenhouse-Geisser correction). The significant Day effect reflects a general increase in consumption over the pre-exposure. However, this increase was more pronounced for rats consuming moderate quinine concentrations (.005% & .01%) than the extreme concentrations (.0025% & .02). The Quinine effect was significant here as more concentrated quinine solutions were consumed in lower quantities. There was not an effect of Access during the pre-exposure as assignment to EDA and E3DA was performed to equate consumption.

Day 1 Consumption

In experiment 4, as well as experiments 1 & 2, Day 1 was the last day for which EDA and E3DA rats were treated similarly. Unique to experiment 4 however, the solutions received during the pre-exposure and phase 1 were identical. For reference, consumption on Day -2, the day rats were naively exposed to their respective solutions, is shown in figure 9. Day 1 consumption data, analyzed here, is shown in figure 10. Day 1 solution consumption was analyzed with an Access (EDA or E3DA) x Quinine (.0025%, 0.005%, .01%, & .02%) ANOVA. This analysis revealed an effect of Quinine $F(3, 54)=7.7$, $p<.001$ as the more concentrated quinine solutions were consumed in lower amounts (fig 10). Rats were assigned to access conditions to equate Day 1 consumption and as such there was not an effect of Access $F<1$.

The first and last four common access days of Phase 1

Consumption of 8% sucrose + .0025%, .005%, .01%, or .02% quinine during phase 1 and the remaining phases of experiment 2 are shown in figure 8. In phase 1 rats with EDA and E3DA were given 36 and 12 exposures, respectively. The first (1, 4, 7, & 10) and last (25, 28, 31, & 34) four common access days of phase 1 were analyzed in two separate four day blocks to highlight, first the emergence, and later the maintenance, of access and solution effects.

Solution consumption on the first four common access days was analyzed in a four Day (1, 4, 7, & 10) by Access (EDA or E3DA) x Quinine (.0025%, .005%, .01%, or .02%) between-groups repeated-measures ANOVA. The ANOVA revealed a significant main effect of Day $F(3, 162)=31.2$, $p<.001$, Quinine $F(3, 54)=9.5$, $p<.001$ and a Day x Access $F(3, 162)=18.1$, $p<.001$ interaction. The Day effect and Day x Access interaction reflect that consumption levels were changing over the first four common access days. Specifically, the E3DA groups were

increasing their consumption and the EDA groups were plateauing. Quinine adulteration concentration-dependently reduced consumption levels but at this point in phase 1 a significant Access effect had not yet emerged.

The analysis of the last four common access days showed stable consumption as the Day effect was nonsignificant $F<1$. The ANOVA revealed significant effects of Access $F(1, 54)=28$, $p<.001$ and Quinine $F(3, 54)=7.5$, $p<.001$. The Access effect reflects the emergence of a stable EDA-E3DA consumption difference. The Quinine effect remained significant from the first four common access days of phase 1 and reflects lower consumption of more concentrated quinine solutions.

The four common access days of Phase 2

In phase 2 all rats were given the same 8%+.02% Q solution for four common access days. The analysis of days 37, 40, 43, & 46 was carried out identically to that of phase 1 with the exception that the Quinine factor referred to previous solution exposure. Consumption was stable throughout phase 2 as the effect of Day was non-significant $F<1$. The ANOVA revealed a significant effect of Access $F(1, 54)=71$, $p<.001$ as the E3DA groups consumed significantly more 8%+.02% Q than the EDA groups. An appreciable EDA-E3DA consumption difference can be seen for phase 2 in figure 8. There was not an effect of Quinine; phase 1 solution exposure did not affect consumption during phase 2. Thus the EDA-E3DA consumption difference in phase 2 was stable and indifferent to previous solution exposure.

Hourly consumption on Day 28

On the 10th common access day, Day 28, hourly consumption measurements were taken for the first six hours of solution access. The first hour of access was analyzed independently as

was done in experiment 2. The hourly consumption data for 8% sucrose + .0025%, .005%, .01%, or .02% quinine solutions is shown in figure 11. Hour 1 consumption was analyzed in an Access (EDA or E3DA) x Quinine (.0025%, .005%, .01%, or .02%) between-groups ANOVA. The ANOVA revealed significant effects of Access $F(1, 54)=40.0$, $p<.001$ and Quinine $F(3, 54)=5.8$, $p=.002$. The Access effect shows that there was a significant EDA-E3DA consumption difference in the first hour of access. The Quinine effect reflects that more concentrated solutions were consumed in lower quantities than less concentrated solutions.

Consumption during the remaining 2 to 6 hours was analyzed in a 5 Hour x Access (EDA or E3DA) x Quinine (.0025%, .005%, .01%, or .02%) between-groups repeated-measures ANOVA. The ANOVA revealed significant effects of Hour $F(4, 216)=6.9$, $p<.001$, Access $F(1, 54)=22.5$, $p<.001$, Quinine $F(3, 54)=7.6$, $p<.001$ and an Hour x Access interaction $F(4, 216)=3.5$, $p=.008$. The Hour effect and Hour x Access interaction reflect that consumption was changing over the five hours of access. Specifically, E3DA rats tended to consume more in the first hours of access than the hours following. The E3DA groups decreased their consumption over the five hour period whereas EDA consumption remained stable. The Access effect reflects greater consumption throughout the five hour period by the E3DA groups than the EDA groups. The Quinine effect reflects that more concentrated solutions were consumed in lower quantities than less concentrated solutions.

Discussion

In experiment 4 rats presented with an EDA-E3DA consumption difference with quinine-adulterated 8% sucrose solutions. Two main procedural adjustments were implemented: 1) rats were given access to the same solution from the pre-exposure to phase 1 as to eliminate possible

solution contrast effects and 2) a range of quinine concentrations were used to explore variable bitterness sensitivities. With these implementations in place it was clear that added bitterness reduced 24 h consumption levels allowing for an EDA-E3DA difference to emerge with 8% sucrose. It appeared that the EDA-E3DA difference became larger with the increasing quinine concentration. In phase 2 when all rats received 8% sucrose + .02% Q the EDA-E3DA difference was equivalent in magnitude regardless of previous solution exposure. The hourly data collected on the 10th common access day (Day 28) also show that consumption seems to wane for the E3DA groups and remain stable for the EDA groups. It seems that large 24 h EDA-E3DA differences require E3DA consumption to wane more slowly.

General Discussion

Concerns about sugar consumption have become a public interest and a research topic in recent years. Much of this attention has fallen around the term 'food addiction' and, as pointed out by Salamone & Correa (2013), addiction has been used hastily as a suffix in this context (and others). In spite of this interest, some basic questions about the regulation of volitional feeding remain. The current investigation was conducted by performing simple manipulations of access, nutritive value, and palatability with the intention of exploring how sugar intake is controlled. The specific hypothesis of this thesis was that a volitional intake reduction, evoked by added bitterness, would allow rats with EDA and E3DA to 8% sucrose, that usually consume similar amounts, to demonstrate the access-induced consumption difference seen with 4% solutions. In a more general sense, I argue that the rat regulates its intake of a supplementary sugar source around nutritive value and availability in an integrated fashion.

In experiment 1 a striking, near immediate, consumption difference emerged between rats receiving EDA or E3DA to an 8% sucrose solution adulterated with .005% quinine. In contrast,

rats with EDA or E3DA to pure 8% sucrose consumed similar amounts of solution with only a small EDA-E3DA difference emerging after a relatively long history of access. In phase 2 of experiment 1, when the quinine condition was flipped, a striking EDA-E3DA difference became immediately apparent in rats previously consuming pure 8% sucrose. Despite consuming similar amounts before phase 2 rats with EDA more severely reduced their consumption than rats with E3DA. This result suggested that the rats had learned their respective access schedules but did not demonstrate consumption differences while consuming large quantities of pure 8% sucrose. When the value of the solution was reduced, by introducing quinine, a previously masked EDA-E3DA difference was able to emerge. The EDA-E3DA consumption difference seen in phase 1 with 8%+Q and in phase 2 when quinine was introduced to rats previously consuming pure 8% sucrose supported the original hypothesis of this thesis.

In experiment 1, 4% sucrose consumption showed an unexpected EDA-E3DA feeding profile with only a small difference in consumption and introduced the question of how rats should be assigned to groups in the EDA-E3DA protocol. A search of the literature suggested that this was not an uncommon problem (Corwin & Wojnicki, 2006) and it appeared that 4% sucrose consumption was more variable than that seen with more concentrated solutions (Collier & Bolles, 1968). Prompted by this literature and the findings in experiment 1, an analysis of EDA 4% sucrose consumption across six different studies, including experiment 1, was conducted (Valyear et al., 2014). The results of this analysis suggested that consumption in a 24 h period following a few days access to a solution was a better predictor of future consumption than the first 24 h consumption. Thus, the strange 4% sucrose results in experiment 1 were attributed to poor group assignment and several day pre-exposure periods were used in subsequent experiments.

In experiment 2 all rats were pre-exposed for six continuous days to 4% sucrose before being assigned to receive EDA or E3DA to 4% or 8% sucrose with or without .005% quinine. This pre-exposure period provided a rich dataset on which to base group assignment and aided the creation of eight equivalent groups of rats. The 4% sucrose EDA-E3DA difference presented more conventionally in this experiment where rats with EDA slowly decreased their consumption over time and E3DA rats increased and maintained higher consumption levels. The pure 8% sucrose group consumed similar amounts of solution across access conditions as in experiment 1. Unexpectedly, and contrary to experiment 1, the 8%+Q group in this experiment failed to show an EDA-E3DA consumption difference. This result was inconsistent with the original hypothesis of this thesis.

The discrepancy in 8%+Q consumption between experiments 1 and 2 was puzzling. Procedurally these two experiments differed by the absence or presence of a pre-exposure period, and the resulting contrast effects for groups that experienced a solution change from the pre-exposure period to phase 1. Experiment 3 was conducted to test the hypothesis that the 8%+Q consumption discrepancy seen between experiments 1 and 2 was the result of pre-exposure differences. This seemed not to be the case as rats consumed identical amounts of 8%+Q regardless of whether they were pre-exposed to pure 4% sucrose, flavoured 4% sucrose, or only water and chow.

Just as individual differences in sucrose consumption seemed to be an issue in experiment 1, variable bitterness sensitivity may have complicated the results with .005% quinine. As noted in the introduction to experiment 4 many studies have looked at the variability in detection thresholds for bitter compounds. As such, a series of quinine concentrations were used to expand the previously used .005% concentration. Experiment 4 was a culmination of the

information about pre-exposure and bitterness sensitivity learned in previous experiments. Although experiment 3 yielded a null result, solutions were kept constant from the pre-exposure to phase 1 in experiment 4 to make the pre-exposure as innocuous as possible while still permitting accurate group assignment. In this experiment an EDA-E3DA consumption difference emerged with 8% sucrose as quinine was always added and seemed to be larger and develop more quickly with the more concentrated quinine solutions. After phase 1 consumption had stabilized all rats were given access to 8% sucrose + .02% quinine. This was the most bitter of the solutions used in experiment 4 and demonstrated the EDA-E3DA difference most quickly. In phase 2 all rats presented with a stable EDA-E3DA consumption difference regardless of their phase 1 solution.

Together these results speak to the efficacy of added bitterness to reduced sucrose consumption and allow for an EDA-E3DA difference to be demonstrated. In previous work it was shown that rats with a history of EDA or E3DA to more concentrated sucrose solutions demonstrated an access-induced consumption difference when given equivalent E2DA to 4% sucrose (Eikelboom et al., Unpublished). This result sets a framework for thinking about the EDA-E3DA difference as being present, but masked, with more concentrated sucrose solutions. As reported by Collier & Bolles (1968b) rats will preferentially consume 60% of their daily calories from a sucrose solution but this proportion cannot be achieved with weaker sucrose solutions. In the current thesis when 8% sucrose consumption was substantially reduced by quinine adulteration an EDA-E3DA difference emerged similar to that usually seen with 4% sucrose. As well, in phase 2 EDA rats reduced their consumption more drastically than E3DA rats when bitterness was introduced, or intensified, in their solution.

Further support for the idea that EDA-E3DA differences do develop with a more concentrated sucrose solution, but only manifest when consumption is reduced, comes from the results seen with hourly intake data. In experiments 2 and 4 consumption was measured hourly for the first six hours of solution availability on Day 28. These measurements were taken as a preliminary investigation to explore the temporal presentation of EDA-E3DA consumption profiles and to compare the current protocol to other work where access-induced consumption differences occur only in the first hour of solution availability (Avena et al., 2006). In experiment 2, rats showing a 24 h EDA-E3DA consumption difference with 4% sucrose did so by stably consuming different amounts across access schedules throughout the first six hours. Also, EDA-E3DA consumption differences were seen in the first hour of access with 8% sucrose, despite similar 24 h intake. These rats later showed a large 24 h EDA-E3DA difference when quinine was introduced to their solution in phase 2. The temporal consumption profile for rats receiving EDA or E3DA reflects a difference between these groups that fades in the 24 h period for 8% sucrose. In experiment 4 EDA-E3DA differences were present in the first few hours of access but faded quickly for the weaker quinine concentrations. Ultimately the hourly data provide preliminary data for two interesting findings: 1) that 24 h EDA-E3DA consumption differences are the product of temporally disparate consumption profiles and 2) that rats consuming similar amounts of solution in a 24 h period may show short-lived EDA-E3DA differences at the beginning of the access period. It should be stressed that the hourly measurements in the current thesis were taken at low resolution, for a brief time period, and require more sophisticated investigation. Despite the crude hourly investigation conducted here Hoebel and associates have established that rats with different access schedules, and similar 24 h intake, can show stark neurochemical and motivational differences in response to sugar. Rats

with a history of intermittent access to 10% sucrose in the Hoebel protocol show a greater dopamine response in the nucleus accumbens shell than continuous access controls (Rada et al., 2005). This intermittent group, with access to 25% glucose, has shown greater operant responding for sugar after a period of abstinence compared to rats with continuous access (Avena et al., 2005).

The greatest complication in this thesis was variable quinine sensitivity. Initially the hypothesis that an intake reduction would uncover an EDA-E3DA consumption difference for more concentrated sucrose solutions was approached by giving rats EDA or E3DA to 4% or 8% sucrose with or without added quinine. It had been shown previously that the same concentration of quinine was more salient when added to weaker sugar solutions than for sweeter solutions (Kappauf et al., 1963; Young et al., 1968). Thus the .005% quinine concentration was chosen because it was expected to reduce 8% sucrose consumption but not be so strong as to obliterate 4% sucrose consumption. In hindsight choosing a higher quinine concentration may have reduced the individual differences in quinine sensitivity and clarified the results of experiments 1 and 2. In experiment 4 this issue was addressed by using a series of quinine concentrations. The EDA-E3DA difference with 8% sucrose was somewhat graded, becoming larger as the quinine concentration increased suggesting subsequent work should use a higher quinine concentration.

In future studies it would be important to establish an effective, unambiguous, bitter additive to reduce consumption. The genetic basis of quinine sensitivity is poorly understood compared to other compounds (Wooding, 2006) and there is certainly no shortage of bitter compounds (Wiener, Shudler, Levit, & Niv, 2012). Although, quinine has commonly been used for its aversive taste the concentration used, and possibly other bitters, should be considered.

Another factor worth considering for future work would be the concentration of sucrose used. A number of studies suggest that 8% sucrose, when offered continuously in addition to chow and water, represents a peak in voluminous sucrose solution consumption (Richter & Campbell, 1940; Sclafani & Nissenbaum, 1987; Smith & Sclafani, 2002; Spector & Smith, 1984; Young, 1948). The current studies built on previous work in the lab done mostly with a 4% sucrose solution. The 8% sucrose solution being more concentrated, eliciting a different EDA-E3DA profile than 4%, and usually being consumed in greater quantities than 4% seemed like a logical choice. Solutions more concentrated than 8% sucrose show lower volume intake, but greater calorie consumption. The 8% sucrose solution was unique in that by adding quinine to this solution voluminous intake could be reduced to the level of pure 4% sucrose. However, using more concentrated sucrose solutions would have yielded less between-rat consumption variability, cleaner effects, and possibly lessened the group assignment issues.

As mentioned in the introduction, and demonstrated here, rats with intermittent access to a supplementary food source typically consume more than rats with continuous access. This feeding behaviour has been observed with mild sucrose solutions (3.2 & 4%), pure fat, and mixtures of the two (Corwin et al., 1998; Eikelboom et al., Unpublished; Lardeux et al., 2013; Wojnicki et al., 2007). However, concentrated sucrose solutions (8%, 16%, & 32%) seem to be resistant to the presentation of access effects as these solutions are consumed in similar amounts regardless of availability (Eikelboom et al., Unpublished; Wojnicki et al., 2007). In the current thesis when adding quinine markedly reduced sucrose consumption an EDA-E3DA consumption difference became apparent. This was shown with 8% sucrose in the EDA-E3DA protocol but could also be tested in the MWF protocol. Wojnicki et al. (2007) showed that rats with 2 h of access to 3.2% or 10% sucrose on MWF consumed more, in that period, than rats with daily 2 h

access. This was not the case with 32% sucrose where rats consumed similar amounts of solution across MWF and daily access conditions. It would be interesting to see if quinine adulteration could bring out an access-induced consumption difference with 32% sucrose in this protocol as it has with 8% sucrose in the EDA-E3DA protocol.

Together the results of this thesis suggest that the rat regulates supplementary sugar intake around nutritive value and availability in a collective analysis. This is evidenced by the finding that quinine-adulteration can be used to degrade the value, and intake, of 8% sucrose solution and allow for an EDA-E3DA difference to emerge. Although rats with EDA or E3DA consume similar amounts of concentrated sucrose solution on common access days this should not be taken as evidence that EDA-E3DA difference is absent. In all experiments where 8% consumption was substantially reduced an EDA-E3DA consumption difference became readily apparent. It is then that a limit on consumption, possibly the rats' tendency to assume 60% of its calories from sugar, prevents access induced consumption differences from emerging at highly concentrated sucrose solutions.

References

Amit, Z., Stern, M. H., & Wise, R. A. (1970). Alcohol preference in the laboratory rat induced by hypothalamic stimulation. *Psychopharmacologia*, *17*(5), 367–77. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/5098930

Avena, N. M., & Hoebel, B. G. (2003). A diet promoting sugar dependency causes behavioral cross-sensitization to a low dose of amphetamine. *Neuroscience*, *122*(1), 17–20. doi:10.1016/S0306-4522(03)00502-5

Avena, N. M., Long, K. A., & Hoebel, B. G. (2005). Sugar-dependent rats show enhanced responding for sugar after abstinence: evidence of a sugar deprivation effect. *Physiology & Behavior*, *84*(3), 359–62. doi:10.1016/j.physbeh.2004.12.016

Avena, N. M., Rada, P., & Hoebel, B. G. (2006). Sugar bingeing in rats. *Current Protocols in Neuroscience*, *Chapter 9*, Unit 9.23C. doi:10.1002/0471142301.ns0923cs36

Avena, N. M., Rada, P., & Hoebel, B. G. (2008). Evidence for sugar addiction: Behavioral and neurochemical effects of intermittent, excessive sugar intake. *Neuroscience & Biobehavioral Reviews*, *32*(1), 20–39. doi:10.1016/j.neubiorev.2007.04.019

Bachmanov, A. A., & Beauchamp, G. K. (2007). Taste Receptor Genes. *Annual Review of Nutrition*, (170), 389–414. doi:10.1146/annurev.nutr.26.061505.111329.Taste

Bachmanov, A. A., Reed, D. R., Tordoff, M. G., Price, R. A., & Beauchamp, G. K. (1996). Intake of ethanol, sodium chloride, sucrose, citric acid, and quinine hydrochloride solutions by mice: a genetic analysis. *Behavior Genetics*, *26*(6), 563–73. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3661408&tool=pmcentrez&rendertype=abstract

Blodgett, H. C. (1929). The effect of the introduction of reward upon the maze performance of rats. *University of California Publications in Psychology*, *4*, 113–134.

Boggiano, M. M., & Chandler, P. C. (2006). Binge eating in rats produced by combining diet with stress. *Current Protocols in Neuroscience*, *Chapter 9*, Unit 9.23A.

Bolles, R. C. (1961). The interaction of hunger and thrst in the rat. *Journal of Comparative and Physiological Psychology*, *54*(5), 580–584.

Bolles, R. C., Hayward, L., & Crandall, C. (1981). Conditioned taste preferences based on caloric density. *Journal of Experimental Psychology. Animal Behavior Processes*, *7*(1), 59–69. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7229574

Caprio, S. (2012). Calories from soft drinks--do they matter? *The New England Journal of Medicine*, *367*(15), 1462–3. doi:10.1056/NEJMe1209884

Celejewski, A. (2011). *The role of taste and calories in access-induced excessive sweets consumption by the rat*. (Unpublished masters thesis). Wilfrid Laurier University, Waterloo, ON.

Colantuoni, C., Schwenker, J., McCarthy, J., Rada, P., Ladenheim, B., Cadet, J. L., … Hoebel, B. G. (2001). Excessive sugar intake alters binding to dopamine and mu-opioid receptors in the brain. *Neuroreport*, *12*(16), 3549–52. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11733709

Collier, G., & Bolles, R. C. (1968). Some determinants of intake of sucrose solutions. *Journal of Comparative & Physiological Psychology*, *65*(3), 379–83. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/5667378

Collier, G., Hirsch, E., & Hamlin, P. H. (1972). The ecological determinants of reinforcement in the rat. *Physiology & Behavior*, *9*(5), 705–16. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/4655163

Corwin, R. L. W., Avena, N. M., & Boggiano, M. M. (2011). Feeding and reward: Perspectives from three rat models of binge eating. *Physiology & Behavior*, *104*(1), 87–97. doi:10.1016/j.physbeh.2011.04.041

Corwin, R. L. W., & Babbs, R. K. (2012). Rodent models of binge eating: are they models of addiction? *Institute for Laboratory Animal Research Journal*, *53*(1), 23–34. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/23520597

Corwin, R. L. W., & Hajnal, A. (2005). Too much of a good thing: neurobiology of non-homeostatic eating and drug abuse. *Physiology & Behavior*, *86*(1-2), 5–8. doi:10.1016/j.physbeh.2005.06.021

Corwin, R. L. W., & Wojnicki, F. H. E. (2006). Binge Eating in Rats with Limited Access to Vegetable Shortening. *Current Protocols in Neuroscience*, *Chapter 9*(Unit 9.23B), 1–11.

Corwin, R. L. W., Wojnicki, F. H. E., Fisher, J. O., Dimitriou, S. G., Rice, H. B., & Young, M. A. (1998). Limited access to a dietary fat option affects ingestive behavior but not body composition in male rats. *Physiology & Behavior*, *65*(3), 545–53. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9877422

DeSousa, N. J., Bush, D. E., & Vaccarino, F. J. (2000). Self-administration of intravenous amphetamine is predicted by individual differences in sucrose feeding in rats. *Psychopharmacology*, *148*(1), 52–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10663417

Eikelboom, R., Hewitt, R., & Adams, K. L. (n.d.). *Sucrose addiction: Absence makes the heart (and brain) grow fonder* (pp. 1–41). Waterloo, ON.

Falconer, D. S. (1947). Sensory thresholds for solution sof phenyl-thio-carbamide. *Annals of Eugenics*, *13*, 211–22.

Falk, J. L. (1961). Production of polydipsia in normal rats by an intermittent food schedule. *Science*, *133*(3447), 195–196.

Fischer, R., & Griffin, F. (1963). Quinine dimorphism: A cardinal determinant of taste sensitivity. *Nature*, *200*, 343–47.

Fischer, R., Griffin, F., England, S., & Garn, S. M. (1961). Taste thresholds and food dislikes. *Nature*, *191*, 1328.

Goodwin, F. L., Bergeron, N., & Amit, Z. (2000). Differences in the consumption of ethanol and flavored solutions in three strains of rats. *Pharmacology, Biochemistry, & Behavior*, *65*(3), 357–62. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10683473

Hagan, M. M., Wauford, P. K., Chandler, P. C., Jarrett, L. a, Rybak, R. J., & Blackburn, K. (2002). A new animal model of binge eating: key synergistic role of past caloric restriction and stress. *Physiology & Behavior*, *77*(1), 45–54. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12213501

Hansen, J. L., Reed, D. R., Wright, M. J., Martin, N. G., & Breslin, P. a S. (2006). Heritability and genetic covariation of sensitivity to PROP, SOA, quinine HCl, and caffeine. *Chemical Senses*, *31*(5), 403–13. doi:10.1093/chemse/bjj044

Harris, H., & Kalmus, H. (1949). The measurement of taste sensitivity to phenylthiourea (PTC). *Annals of Eugenics*, *15*(1), 24–31.

Hartvig, D., Hausner, H., Wendin, K., & Bredie, W. L. P. (2014). Quinine sensitivity influences the acceptance of sea-buckthorn and grapefruit juices in 9- to 11-year-old children. *Appetite*, *74*, 70–8. doi:10.1016/j.appet.2013.11.015

Hirsch, E., & Walsh, M. (1982). Effect of limited access to sucrose on overeating and patterns of feeding. *Physiology & Behavior*, *29*(1), 129–34. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7122719

Hoebel, B. G., & Teitelbaum, P. (1962). Hypothalamic control of feeding and self-stimulation. *Science*, *135*(3501), 375–377.

Hudson, J. I., Hiripi, E., Pope, H. G., & Kessler, R. C. (2007). The prevalence and correlates of eating disorders in the National Comorbidity Survey Replication. *Biological Psychiatry*, *61*(3), 348–58. doi:10.1016/j.biopsych.2006.03.040

Kappauf, W. E., Burright, R. G., & Demarco, W. (1963). Sucrose-quinine mixtures which are isohedonic for the rat. *Journal of Comparative & Physiological Psychology*, *56*(1), 138–143.

Lardeux, S., Kim, J. J., & Nicola, S. M. (2013). Intermittent access to sweet high-fat liquid induces increased palatability and motivation to consume in a rat model of binge consumption. *Physiology & Behavior*, *114-115*, 21–31. doi:10.1016/j.physbeh.2013.03.005

Lees, J. (1999). Incidence of weight loss in head and neck cancer patients on commencing radiotherapy treatment at a regional oncology centre. *European Journal of Cancer Care*, *8*(3), 133–6. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10763643

LeMagnen, J. (1960). Etude de quelques facteurs associés à des modifications de la consummation spontanée d'alcohol éthylique. *Journal of Physiology*, *52*, 873–884.

Martin, J., & Timofeeva, E. (2010). Intermittent access to sucrose increases sucrose-licking activity and attenuates restraint stress-induced activation of the lateral septum. *American Journal of Physiology Regulatory Integrrative & Comparative Physiology*, *298*(13), 1383–98. doi:10.1152/ajpregu.00371.2009.

Nissenbaum, J. W., & Sclafani, A. (1987). Sham-Feeding response of rats to polycose and sucrose. *Neuroscience & Biobehavioral Reviews*, *I*, 215–222.

Overstreet, D. H., Kampov-Polevoy, a B., Rezvani, a H., Murrelle, L., Halikas, J. a, & Janowsky, D. S. (1993). Saccharin intake predicts ethanol intake in genetically heterogeneous rats as well as different rat strains. *Alcoholism, Clinical & Experimental Research*, *17*(2), 366–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8488981

Owen, J. (1980). *Feeding Strategy*. (K. Goldie-Morrison, L. Gamlin, & D. Burnie, Eds.). Lomdon: The University of Chicago Press.

Parylak, S. L., Koob, G. F., & Zorrilla, E. P. (2011). The dark side of food addiction. *Physiology & Behavior*, *104*(1), 149–56. doi:10.1016/j.physbeh.2011.04.063

Pinel, J. P., & Huang, E. (1976). Effects of periodic withdrawal on ethanol and saccharin selection in rats. *Physiology & Behavior*, *16*(6), 693–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/981364

Rada, P., Avena, N. M., & Hoebel, B. G. (2005). Daily bingeing on sugar repeatedly releases dopamine in the accumbens shell. *Neuroscience*, *134*(3), 737–44. doi:10.1016/j.neuroscience.2005.04.043

Redpath. (n.d.). Redpath Sugar Label.

Reed, D. R., Zhu, G., Breslin, P. a S., Duke, F. F., Henders, A. K., Campbell, M. J., … Wright, M. J. (2010). The perception of quinine taste intensity is associated with common genetic variants in a bitter receptor cluster on chromosome 12. *Human Molecular Genetics*, *19*(21), 4278–85. doi:10.1093/hmg/ddq324

Richter, C. P. (1936). Increased salt appetite in adrenalectomized rats. *American Journal of Physiology*, *115*, 155–161.

Richter, C. P., & Campbell, K. H. (1940). Taste thresholds and taste preferences of rats for five common sugars. *Journal of Nutrition*, (1859).

Salamone, J. D., & Correa, M. (2013). Dopamine and food addiction: lexicon badly needed. *Biological Psychiatry*, *73*(9), e15–24. doi:10.1016/j.biopsych.2012.09.027

Sclafani, A., & Ackroff, K. (2003). Reinforcement value of sucrose measured by progressive ratio operant licking in the rat. *Physiology & Behavior*, *79*, 663–670. doi:10.1016/S0031-9384(03)00143-4

Sclafani, A., & Nissenbaum, J. W. (1985). On the role of the mouth and gut in the control of saccharin and sugar intake: a reexamination of the sham-feeding preparation. *Brain Research Bulletin*, *14*(6), 569–76. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/4027696

Sclafani, A., & Nissenbaum, J. W. (1987). Taste preference thresholds for Polycose, maltose, and sucrose in rats. *Neuroscience & Biobehavioral Reviews*, *11*(2), 181–5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3614784

Sclafani, A., & Springer, D. (1976). Dietary induced obesity in adult rats: Similarities to hypothalamic and human obesity syndromes. *Physiology & Behavior*, *17*, 461–471.

Senthinathan, G. (2013). *Developmental profile of access induced sucrose consumption and response to access change in male rats*. (Unpublished masters thesis). Wilfrid Laurier University, Waterloo, ON.

Shi, P., Zhang, J., Yang, H., & Zhang, Y.-P. (2003). Adaptive diversification of bitter taste receptor genes in Mammalian evolution. *Molecular Biology and Evolution*, *20*(5), 805–14. doi:10.1093/molbev/msg083

Siegel, P. S., & Stuckey, H. L. (1947). The diurnal course of water and food intake in the normal mature rat. *Journal of Comparative & Physiological Psychology*, *40*(5), 365–70. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/20267828

Skinner, B. F. (1930). On the conditions of elicitation of certain eating reflexes. *Proceedings of the National Academy of Sciences of the United States of America*, *16*, 433–438.

Skinner, B. F. (1932). On the rate of formation of a conditioned reflex. *The Journal of General Psychology*, *7*, 274–286.

Skinner, B. F. (1938). *The behaviour of organisms*. (R. M. Elliott, Ed.). New York: Appleton-Century-Crofts INC.

Smith, J. C., & Sclafani, A. (2002). Saccharin as a sugar surrogate revisited. *Appetite*, *38*(2), 155–60. doi:10.1006/appe.2001.0467

Smith, J. C., & Wilson, L. S. (1989). Study of a lifetime of sucrose intake by the Fischer-344 rat. *Annals of the New York Academy of Sciences*, *561*, 291–306. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2735686

Smith, M., & Duffy, M. (1957). Evidence for a dual reinforcing effect of sugar. *Journal of Comparative & Physiological Psychology*, *50*(3), 242–247.

Soranzo, N., Bufe, B., Sabeti, P. C., Wilson, J. F., Weale, M. E., Marguerie, R., … Goldstein, D. B. (2005). Positive selection on a high-sensitivity allele of the human bitter-taste receptor TAS2R16. *Current Biology*, *15*(14), 1257–65. doi:10.1016/j.cub.2005.06.042

Spector, A. C., & Smith, J. C. (1984). A detailed analysis of sucrose drinking in the rat. *Physiology & Behavior*, *33*(1), 127–36. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6505048

Steiner, J. E., Glaser, D., Hawilo, M. E., & Berridge, K. C. (2001). Comparative expression of hedonic impact: affective reactions to taste by human infants and other primates. *Neuroscience & Biobehavioral Reviews*, *25*(1), 53–74. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11166078

Thaw, A. K., & Smith, J. C. (1992). Conditioned suppression as a method of detecting taste thresholds in the rat. *Chemical Senses*, *17*(2), 211–223. doi:10.1093/chemse/17.2.211

Tordoff, M. G., Alarcon, L. K., & Lawler, M. P. (2008). Preferences of 14 rat strains for 17 taste compounds. *Physiology & Behavior*, *95*(3), 308–32. doi:10.1016/j.physbeh.2008.06.010

Valenstein, E. S., Cox, V. C., & Kakolewski, J. W. (1967). Polydipsia Elicited by the Synergistic Action of a Saccharin and Glucose Solution. *Science*, *157*(3788), 552–554.

Valenstein, E. S., Kakolewski, J. W., & Cox, V. C. (1967). Sex Differences in Taste Preference for Glucose and Saccharin Solutions. *Science*, *156*(3777), 942–943.

Valyear, M. (2013). *The effects of access conditions and quinine adulteration on sucrose consumption*. (Unpublished undergraduate thesis). Wilfrid Laurier University, Waterloo, ON.

Valyear, M., Senthinathan, G., Celejewski, A., & Eikelboom, R. (2014). Predicting future sucrose consumption. Guelph, ON: 8th Annual Guelph Neuroscienc Research Day.

Wayner, M. J., & Fraley, S. (1972). Enhancement of the consumption of acclimated sapid solutions following periodic and prolonged withdrawal. *Physiology & Behavior*, *9*(3), 463–74. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/4658591

Wayner, M. J., Greenberg, I., Tartaglione, R., Nolley, D., Fraley, S., & Cott, A. (1972). A new factor affecting the consumption of ethyl alcohol and other sapid fluids. *Physiology & Behavior*, *8*(2), 345–62. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/4665346

Weingarten, H. P., & Watson, S. D. (1982). Sham feeding as a procedure for assessing the influence of diet palatability on food intake. *Physiology & Behavior*, *28*(3), 401–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7079355

Wiener, A., Shudler, M., Levit, A., & Niv, M. Y. (2012). BitterDB: a database of bitter compounds. *Physiology & Behavior*, *40*(Database issue), D413–9. doi:10.1093/nar/gkr755

Wise, R. A. (1973). Voluntary ethanol intake in rats following exposure to ethanol on various schedules. *Psychopharmacologia*, *29*(3), 203–10. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/4702273

Wojnicki, F. H. E., Babbs, R. K., & Corwin, R. L. W. (2010). Reinforcing efficacy of fat, as assessed by progressive ratio responding, depends upon availability not amount consumed. *Physiology & Behavior*, *100*(4), 316–21. doi:10.1016/j.physbeh.2010.03.004

Wojnicki, F. H. E., Stine, J. G., & Corwin, R. L. W. (2007). Liquid sucrose bingeing in rats depends on the access schedule, concentration and delivery system. *Physiology & Behavior*, *92*(4), 566–74. doi:10.1016/j.physbeh.2007.05.002

Wong, K. J., Wojnicki, F. H. W., & Corwin, R. L. W. (2009). Baclofen, raclopride, and naltrexone differentially affect intake of fat/sucrose mixtures under limited access conditions. *Pharmacology, Biochemistry, & Behavior*, *92*(3), 528–36. doi:10.1016/j.pbb.2009.02.002

Wooding, S. (2006). Phenylthiocarbamide: A 75-year adventure in genetics and natural selection. *Genetics*, *172*(4), 2015–23. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1448785&tool=pmcentrez&rendertype=abstract

Woods, S. C. (1991). The eating paradox : How we tolerate food. *Psychological Review*, *98*(4), 488–505.

Young, P. T. (1948). Studies of food preference, appetite and dietary habit VIII. Food-seeking drives, palatability and the law of effect. *Journal of Comparative & Physiological Psychology*, *41*(4), 269–300.

Young, P. T., Burright, R. G., & Tromater, L. J. (1968). Preferences of the white rat for solutions of sucrose and quinine hydrochloride. *American Journal of Psychology*, *76*(2), 205–217.

Young, P. T., & Christensen, K. R. (1962). Algebraic summation of hedonic processes. *Journal of Comparative & Physiological Psychology*, *55*(3), 332–6. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14009362

Young, P. T., & Greene, J. T. (1953). Quantity of food ingested as a measure of relative acceptability. *Journal of Comparative & Physiological Psychology*, *46*, 288–294.

Figure Captions

Figure 1

Mean (±SEM) experiment 1 Day 1 intake for 4%, 8%, 4%+Q, & 8%+Q sucrose solutions. This was the rats' first exposure to any sucrose solutions. Day 1 was the last day for which EDA and E3DA rats were treated similarly as access conditions were imposed following this day. Quinine produced a reduction in consumption that was more pronounced with 4% than 8% sucrose.

Figure 2

Mean (±SEM) experiment 1 daily intake for 4% (A), 4%+Q (B), 8% (C), & 8%+Q (D) sucrose solutions during phase 1 (days 1 to 60), phase 2 (days 61 to 84), and phase 3 (days 85 to 94). Rats received EDA or E3DA to their respective solutions in phase 1. In phase 2 the quinine condition was flipped as quinine was added to or removed from the rat's phase 1 solution. In phase 3 all rats received their phase 1 solutions. Rats consuming 8%+Q presented with an EDA-E3DA consumption difference that is normally only present with 4% sucrose. In phase 2 the introduction of quinine to the 8% sucrose solution allowed an EDA-E3DA consumption difference to emerge.

Figure 3

Mean (±SEM) experiment 2 daily intake for 4% (A), 4%+Q (B), 8% (C), & 8%+Q (D) sucrose solutions during the 4% sucrose pre-exposure (days -5 to 0) and phase 1 (days 1 to 36). All rats received 4% sucrose continuously during the pre-exposure. Rats were then given EDA or E3DA to their respective solutions in phase 1. In experiment 2 an EDA-E3DA consumption difference was only seen with the 4% sucrose solutions.

Figure 4

Mean (±SEM) experiment 2 Day -5 intake of 4% sucrose in experiment 2 displayed in the groups that would become their conditions in phase 1. This was the rats' first exposure to any sucrose solution and all 64 rats were given 4% sucrose.

Figure 5

Mean (±SEM) experiment 2 Day 1 intake for 4%, 8%, 4%+Q, & 8%+Q sucrose solutions. This was the rats' first exposure to their phase 1 solutions as the rats received a 6-day 4% sucrose pre-exposure prior to Day 1. Day 1 was the last day for which EDA and E3DA rats were treated similarly as access conditions were imposed following this day. Quinine-adulterated solutions were consumed in lower amounts than unadulterated sucrose solutions.

Figure 6

Mean (±SEM) hourly intake of 4% (A), 4%+Q (B), 8% (C), & 8%+Q (D) during the first six hours of access on Day 28 of experiment 2.

Figure 7

Experiment 3 mean (±SEM) daily intake of sucrose solutions during a 6-day pre-exposure to; chow and water alone, or to a 4% sucrose, or a 4% sucrose + .5% vanilla solution and then when all rats received an 8-day exposure to 8% sucrose + .005% Q. Rats consumed similar amounts of 8%+Q regardless of what they consumed during the pre-exposure.

Figure 8

Mean (±SEM) experiment 4 daily intake for 8% sucrose adulterated with .0025% (A), .005% (B), .01% (C), or .02% (D) quinine during the pre-exposure (days -3 to 0), phase 1 (days 1

to 36), phase 2 (days 37 to 46). Rats were given EDA or E3DA to their respective solutions in phase 1 and all rats received 8% sucrose + .02% Q in phase 2. Quinine adulteration concentration-dependently reduced intake and allowed for an EDA-E3DA consumption difference to emerge with 8% sucrose. A similar EDA-E3DA difference was observed in phase 2 regardless of phase 1 solution exposure.

Figure 9

Mean (±SEM) experiment 4 Day -2 intake of 8% sucrose adulterated with .0025%, .005%, .01%, or .02% quinine. This was the rats' first exposure to their respective solutions. Quinine concentration-dependently reduced consumption of 8% sucrose.

Figure 10

Mean (±SEM) experiment 4 Day 1 intake of 8% sucrose adulterated with .0025%, .005%, .01%, or .02% quinine. This was the rats' fourth exposure to their respective solutions as solutions remained constant from the pre-exposure to phase 1 in experiment 4. Day 1 was the last day for which EDA and E3DA rats were treated similarly as access conditions were imposed following this day. Quinine concentration-dependently reduced consumption of 8% sucrose.

Figure 11

Mean (±SEM) hourly intake of 8% sucrose adulterated with .0025% (A), .005% (B), .01% (C), or .02% (D) quinine during the first six hours of access on Day 28 of experiment 4. Hourly EDA-E3DA consumption differences were most pronounced in the first hour of access and became smaller throughout the six-hour period.

Figures

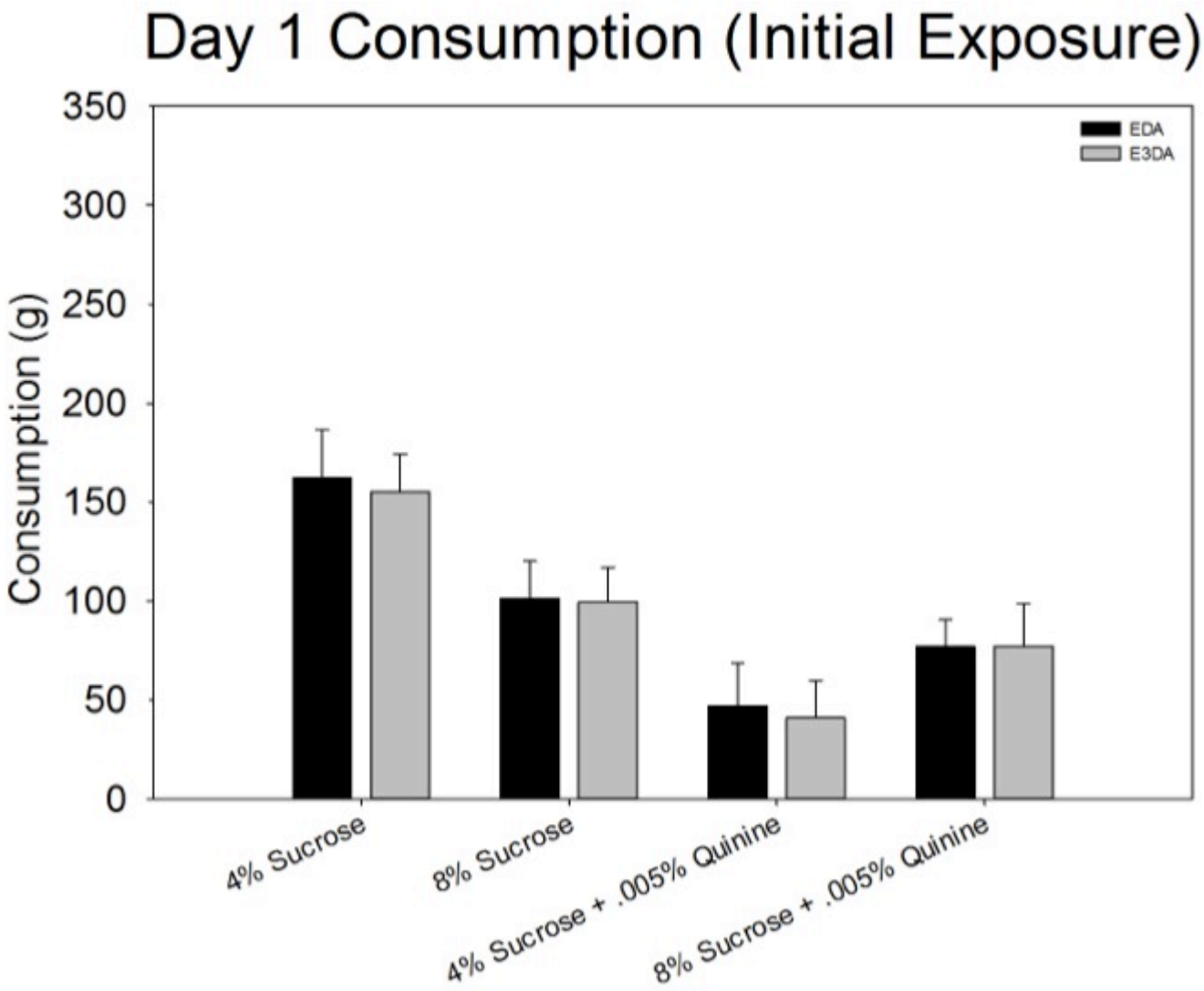


Figure 1: Mean (±SEM) Day 1 intake for Experiment 1

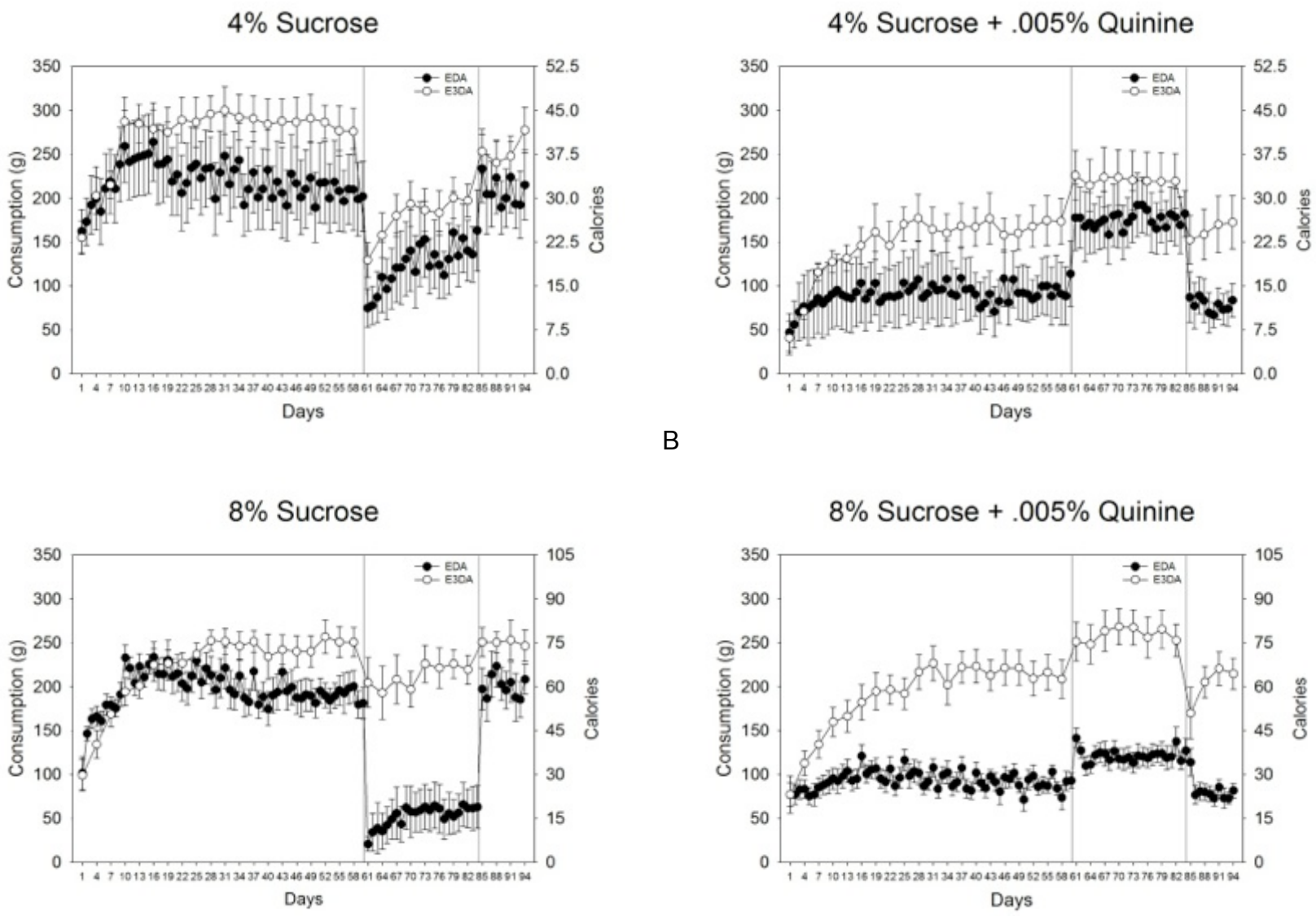


Figure 2: Mean (±SEM) daily consumption for Experiment 1

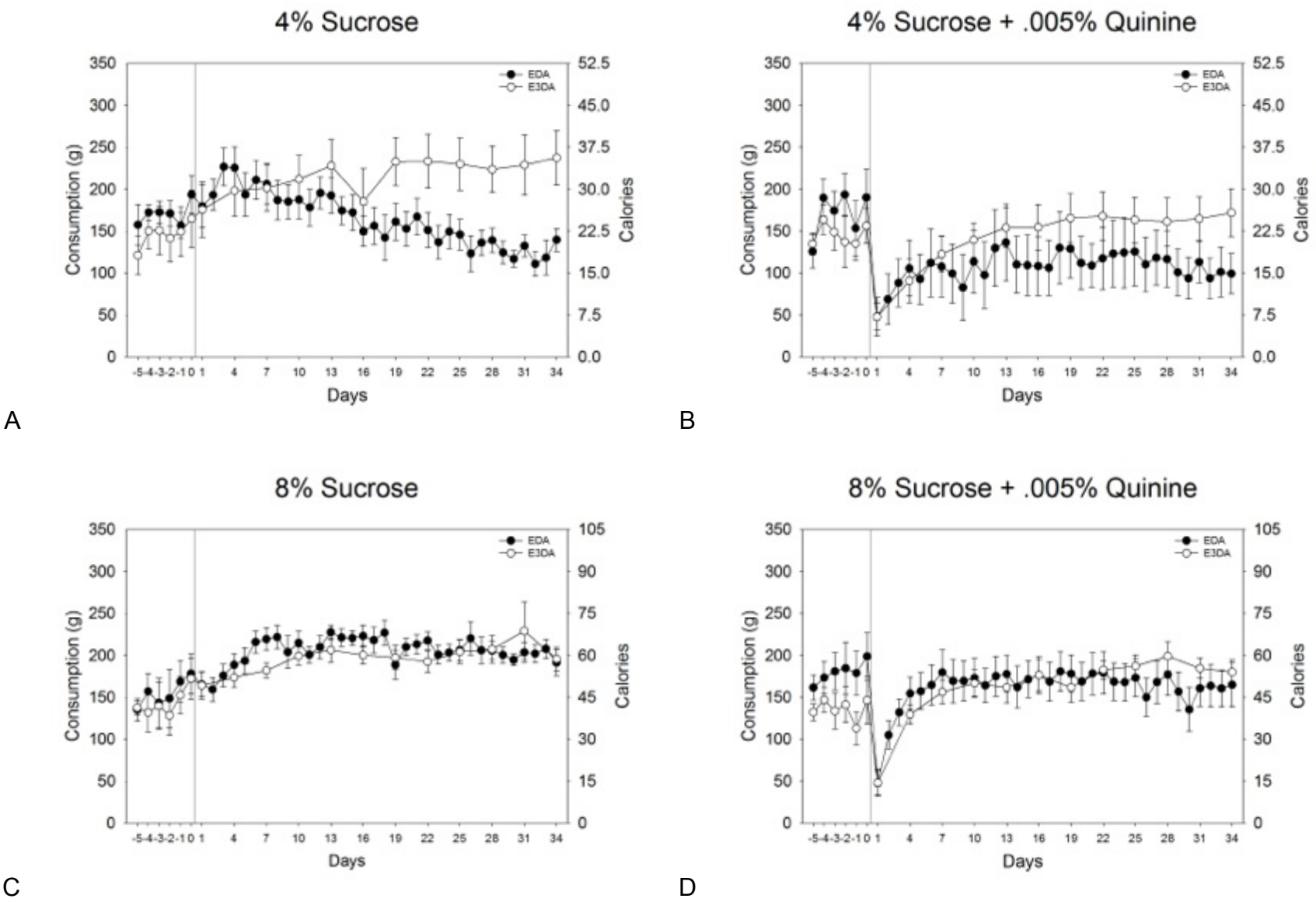


Figure 3: Mean (±SEM) daily consumption for Experiment 2

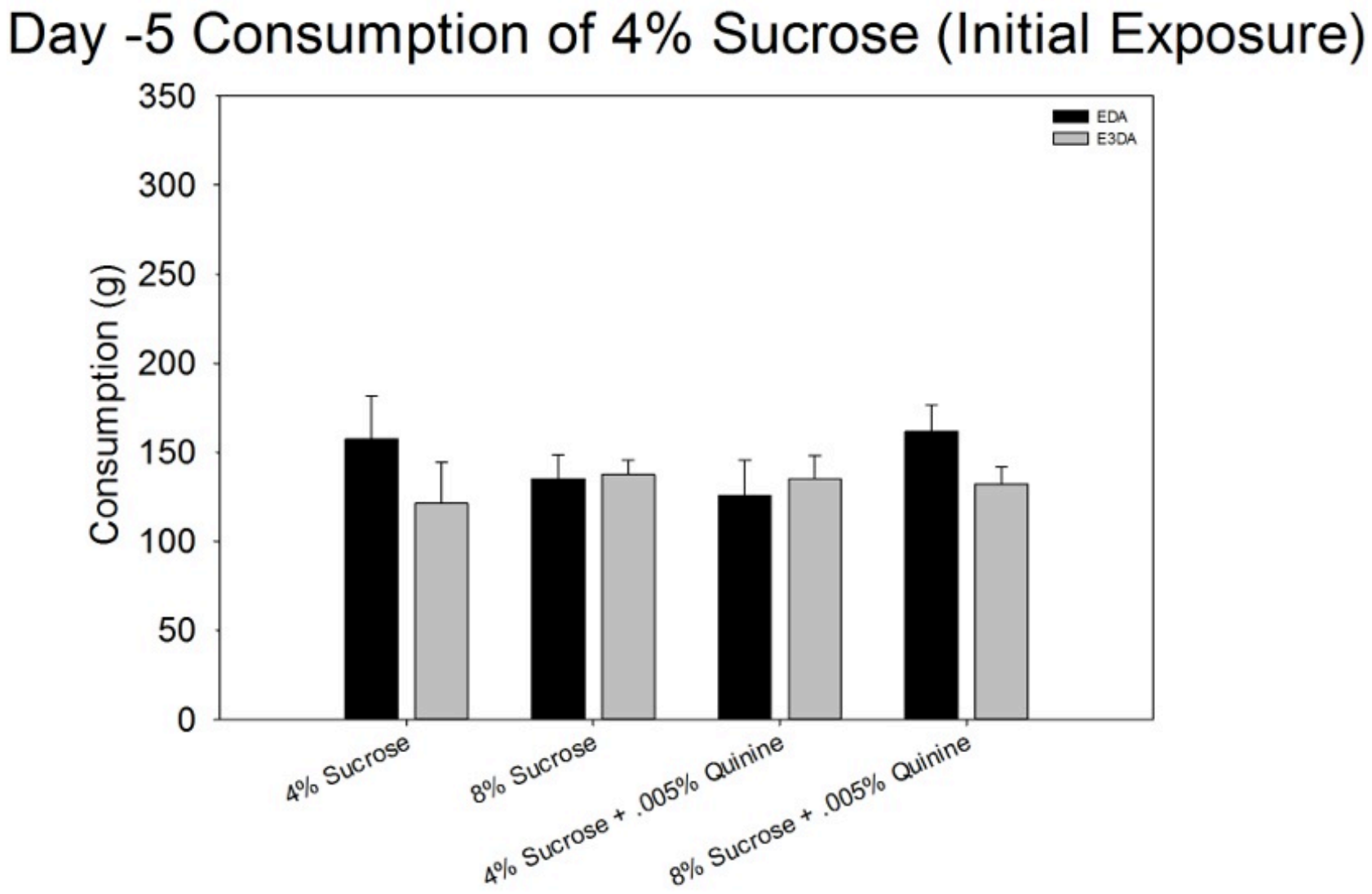


Figure 4: Mean (±SEM) Day -5 intake for Experiment 2

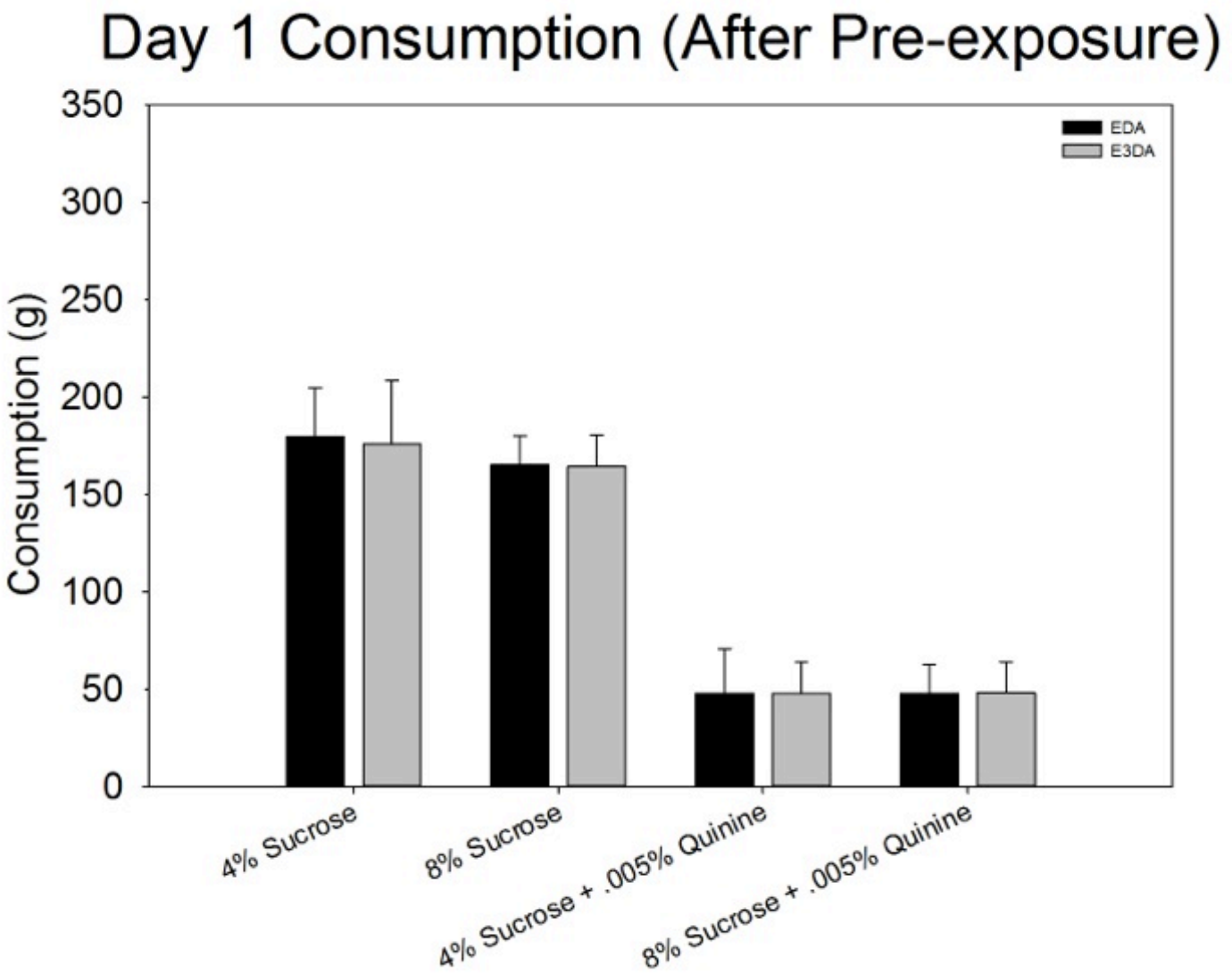
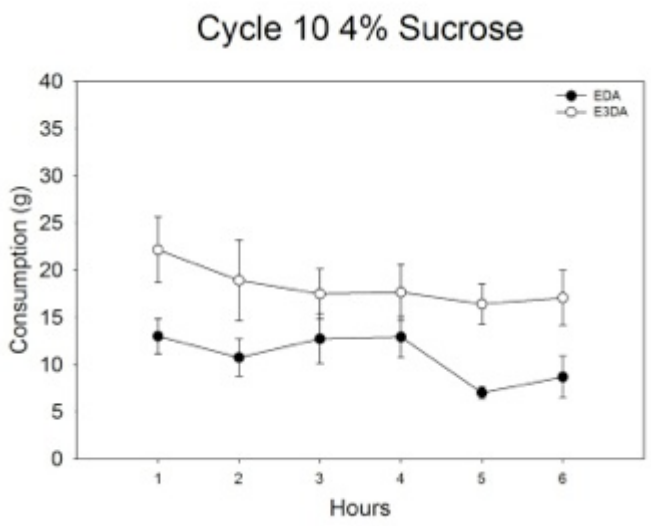
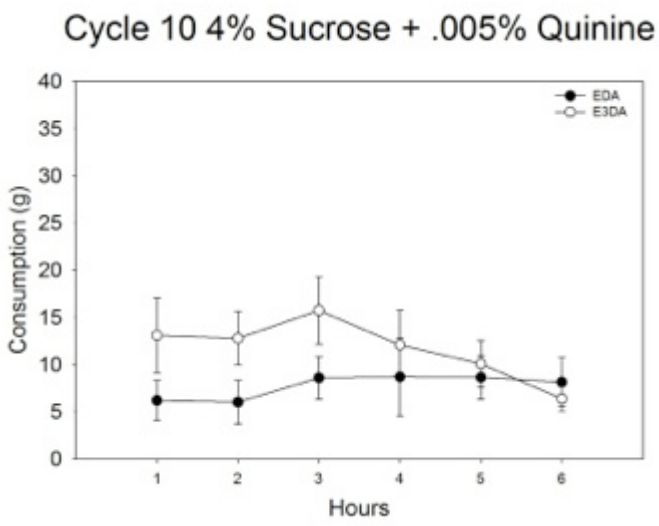


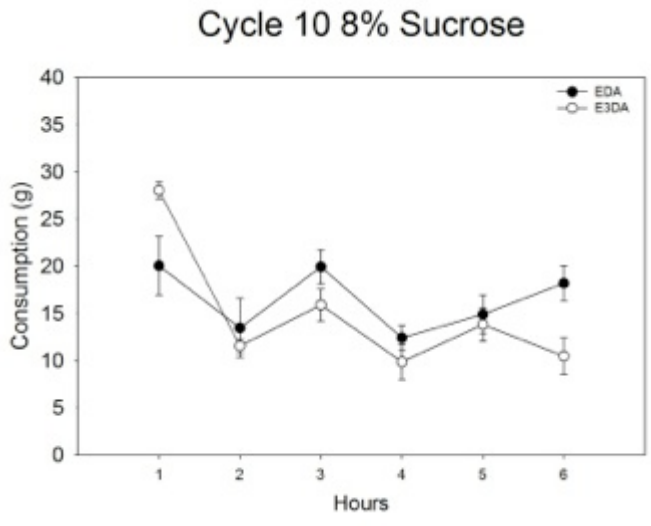
Figure 5: Mean (±SEM) Day 1 intake for Experiment 2



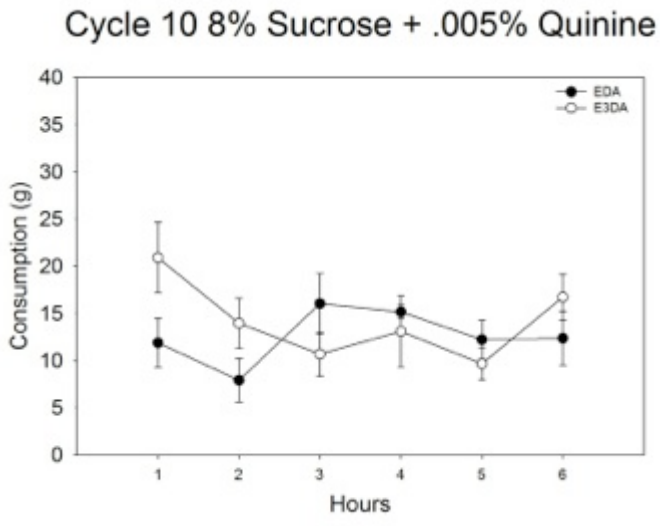
A



B



C



D

Figure 6: Mean (±SEM) hourly intake on Day 28 for Experiment 2

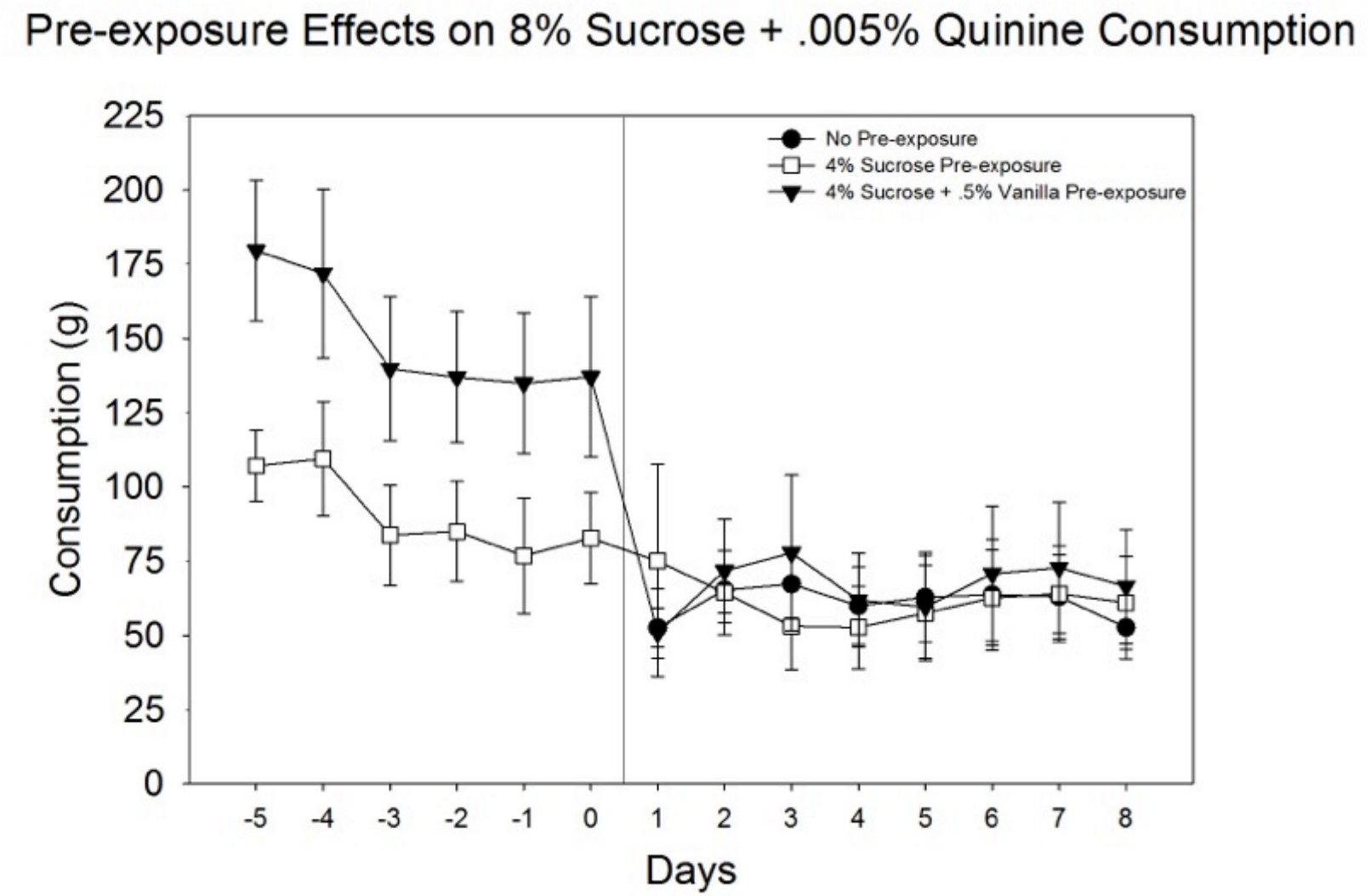
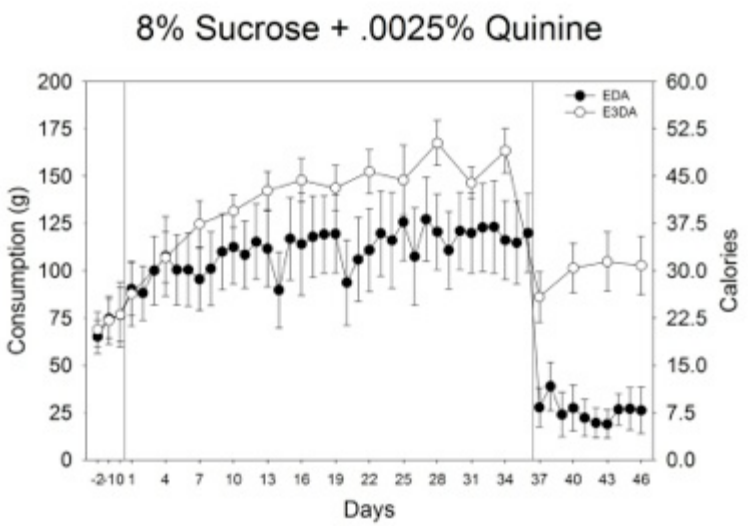
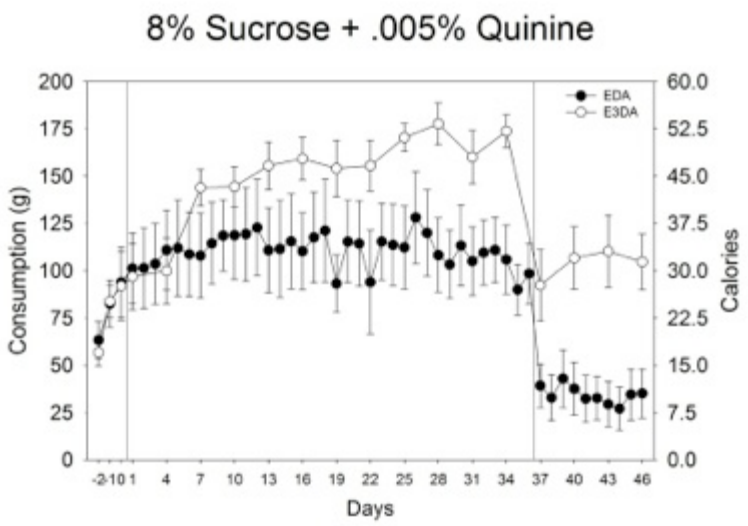


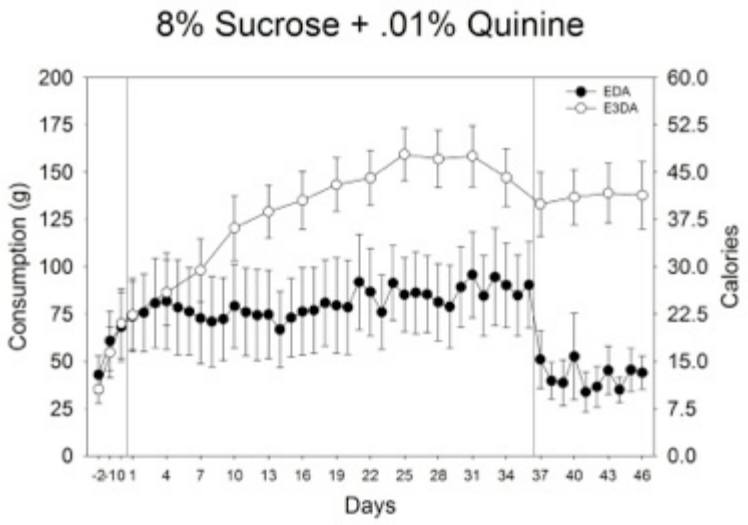
Figure 7: Mean (±SEM) daily consumption for Experiment 3



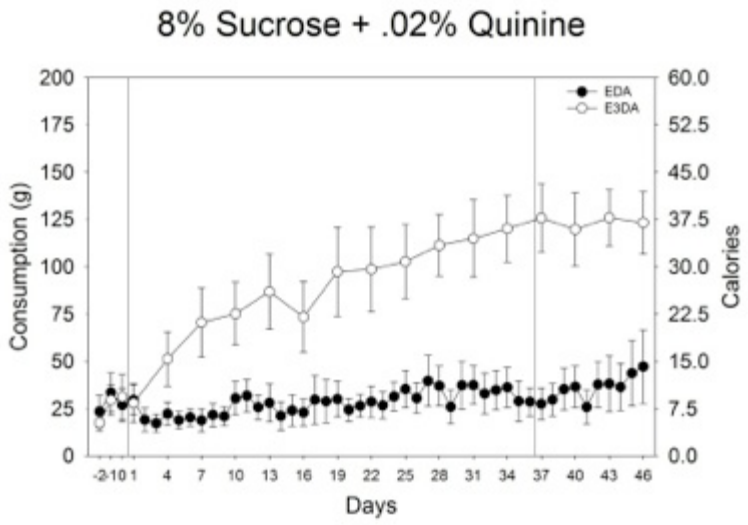
A



B



C



D

Figure 8: Mean (±SEM) daily consumption for Experiment 4

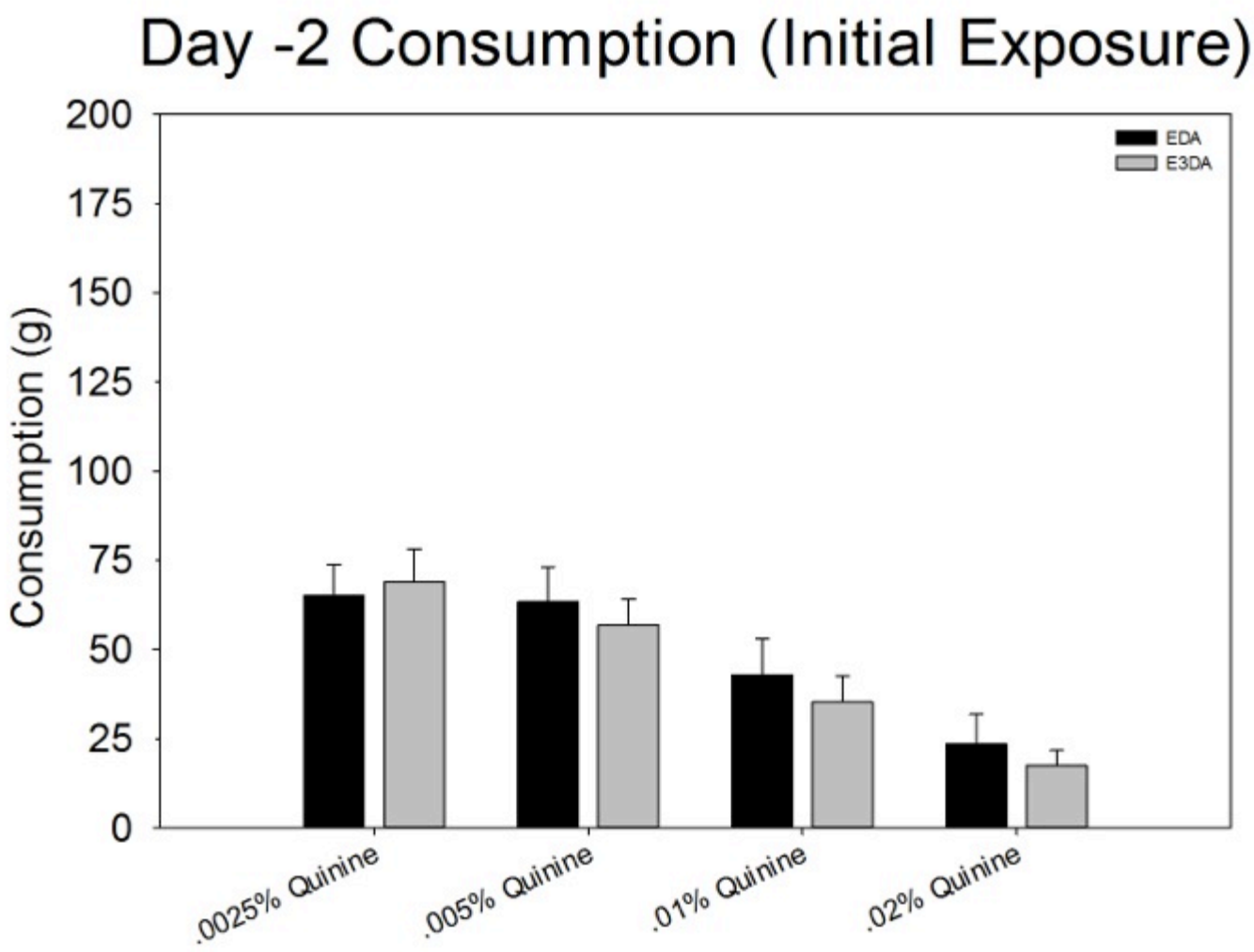


Figure 9: Mean (±SEM) Day -2 intake for Experiment 4

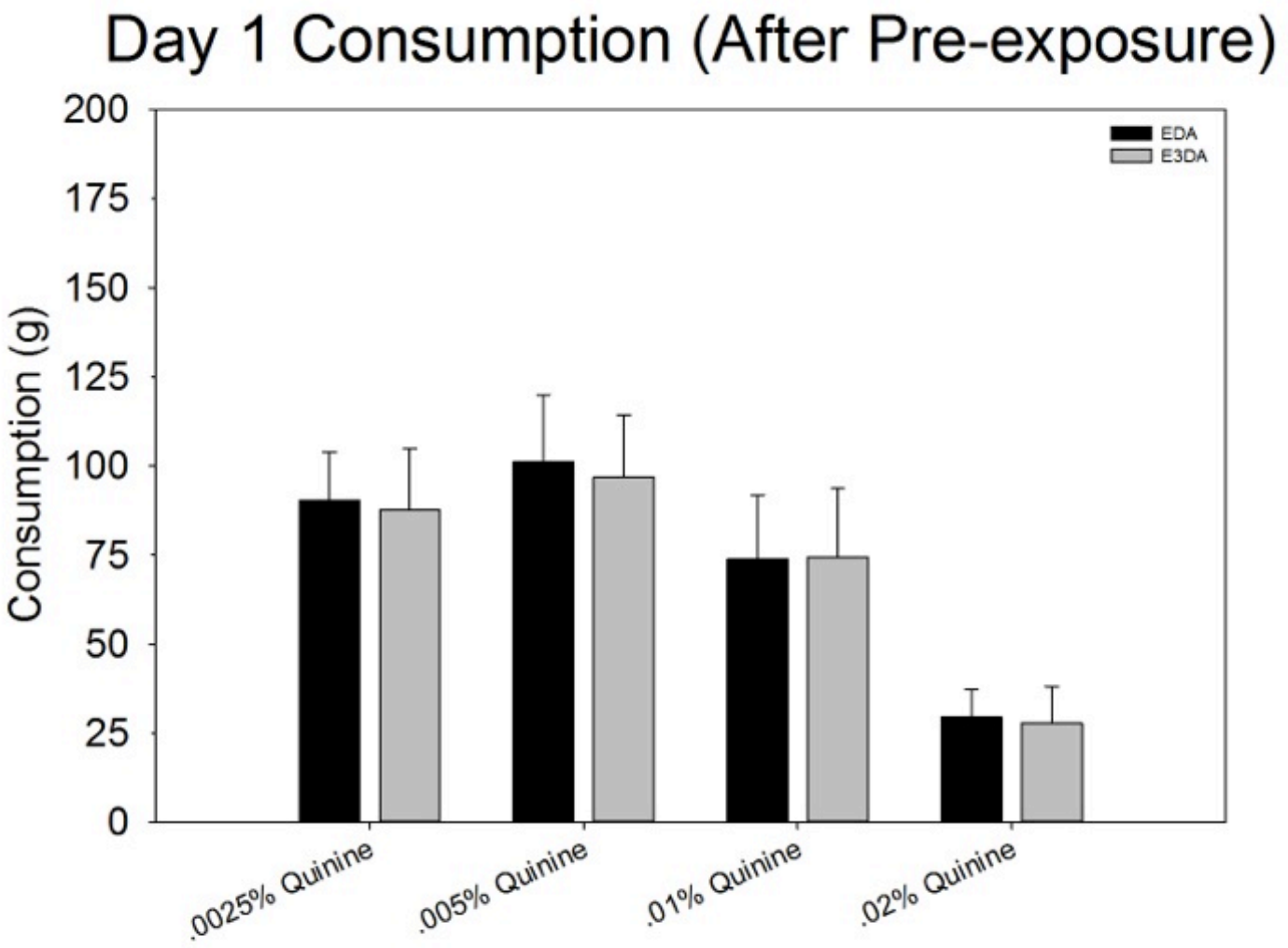
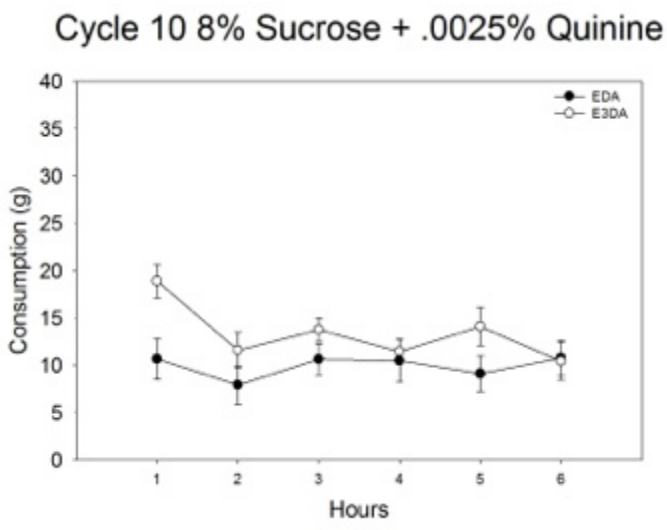
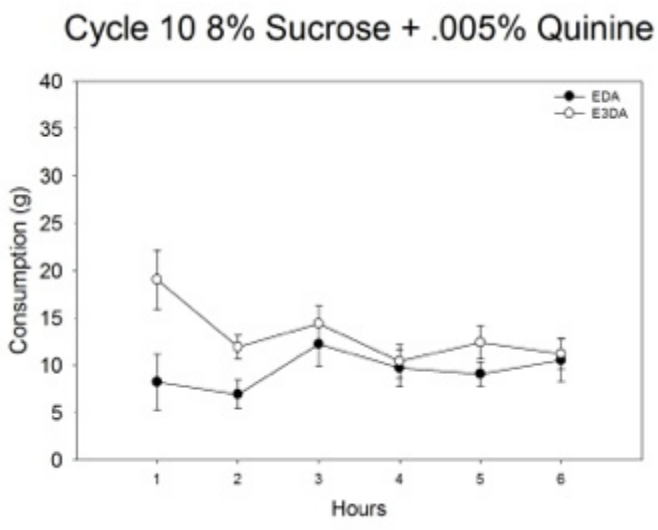


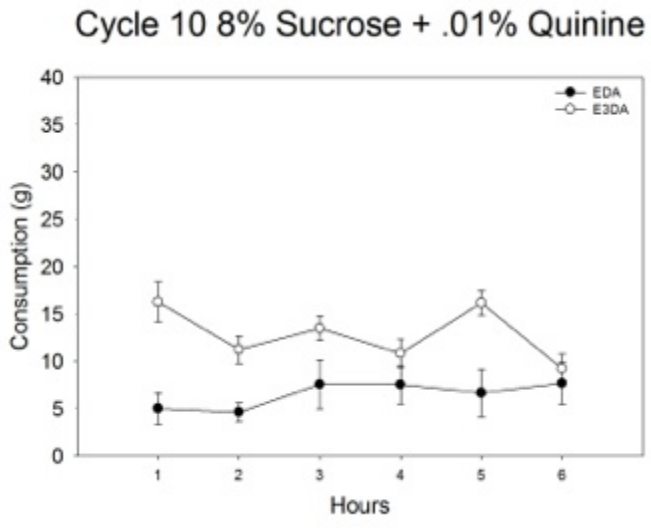
Figure 10: Mean (±SEM) Day 1 intake for Experiment 4



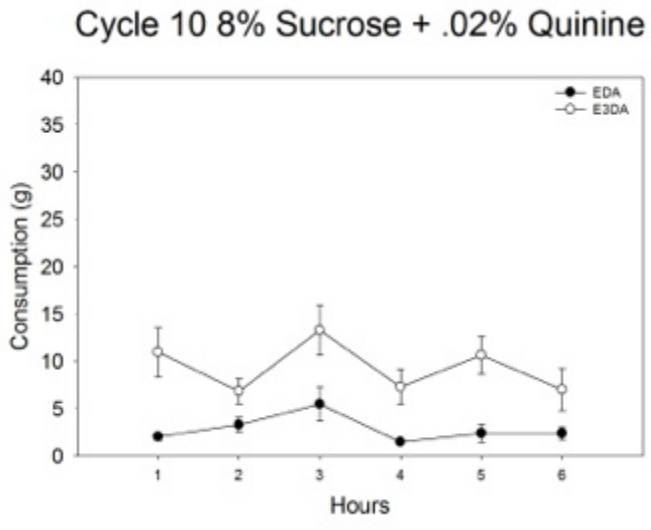
A



B



C



D

Figure 11: Mean (±SEM) hourly intake on Day 28 for Experiment 4