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The Role of Taste and Calories in Access-Induced Excessive Sweets Consumption by the Rat

by

Adam Celejewski

Bachelor of Science, University of Toronto, 2007

Thesis

Submitted to the Department of Psychology, Faculty of Science

in partial fulfilment of the requirement for

Master of Science in Psychology

Wilfrid Laurier University

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Abstract

For individuals diagnosed with Binge Eating Disorder (BED) or Bulimia Nervosa (BN) eating is often manifested in intermittent bouts of gorging, a behaviour that is similar to excessive consumption of rewarding drugs in addiction (American Psychiatric Association, 2000; Corwin & Grigson, 2009; Epstein & Shaham, 2010). Our laboratory has found that sucrose solution intake by rats escalates markedly when provided on Discontinuous Access (DisA; 24h once every 3 or 4 days) schedules but is maintained at lower, stable levels with Continuous Access (ConA; *ad lib*) schedules (Hewitt & Eikelboom, 2008). Once DisA/ConA consumption differences are established, they persist even after both access schedules are equalized to alternate day sucrose exposures. To examine whether taste, rather than the postingestive properties of sucrose, drive these access-induced intake changes, saccharin was substituted for sucrose. In Experiment 1, rats with DisA escalated their intake to consume more than ConA rats over a range of saccharin concentrations (1, 0.5, 0.25, and 0.125%). Taste, even without the postingestive properties of sucrose, drove the access consumption effects. Once DisA/ConA saccharin consumption differences were established in Experiment 2, they were maintained for over 50 days of equal access, even when saccharin was replaced with sucrose.

Whereas intermittent access schedules utilizing repeating 1 to 3 day inter-exposure intervals result in gradual and sustained intake increases, a single longer, isolated period of abstinence can result in a Deprivation Effect (DE), a transient increase in post-abstinence intake (Gandelman & Trowill, 1969;

Neznanova, Zvartau & Bespalov, 2002). To explore the influence of access history on DE expression, intake was examined in rats with DisA and ConA 0.25% saccharin (Experiment 2) and 4% sucrose (Experiment 3) experience. In Experiment 2, a robust saccharin DE was observed in all rats but the intake differences induced by initial DisA/ConA were maintained. In Experiment 3, DisA/ConA differences emerged for sucrose but no DE was observed after either 3 or 9 days of sucrose abstinence. Collectively these findings suggest that 1) taste predominantly drives DisA/ConA induced differences, and 2) that this DisA/ConA difference and the DE may be under control of separate factors. These results highlight the importance of taste and postingestive properties in access consumption effects and suggest that not all experiences with access interruptions are the same. This work underscores the role of access factors in excessive sweets consumption which could be involved in BED or BN etiology and may play a similar role in excessive drug intake in addiction.

Abbreviations

BED: Binge Eating Disorder

BN: Bulimia Nervosa

ConA: Continuous Access

DE: Deprivation Effect

DisA: Discontinuous Access

EPM: Elevated Plus Maze

LAb: Long Abstinence

MWF: Mondays, Wednesdays and Friday (diet)

NAb: Non-abstinence

SAb: Short Abstinence

SEM: Standard Error of the Mean

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Introduction

Although occasional overeating is generally not an issue for most, it can become a pathological concern for some. In Binge Eating Disorder (BED) or Bulimia Nervosa (BN), eating often occurs in intermittent, excessive bouts which are characterized by rapacity and a loss of control (American Psychiatric Association, 2000). What factors and conditions govern and contribute to the transition from controlled intake, to uncontrolled and excessive ingestion that characterizes BN and BED are clearly of significant interest.

The pathological obsession with and loss of control over consummatory behaviour as manifested in both eating disorders may parallel the loss of control over drug taking behaviour seen in addiction (Corwin & Grigson, 2009; Davis & Carter, 2009; Epstein & Shaham, 2010). There is in fact a substantial comorbidity between substance dependence, BN and BED (Brewerton *et al.*, 1995; Bulik, Sullivan & Kendler, 2002; Grilo, White & Masheb, 2009; Hudson, Hiripi, Pope & Kessler, 2007; Spitzer *et al.*, 1993). DSM-IV TR diagnostic criteria for BN and BED overlap with criteria for substance dependence. By merely replacing the term "substance" with references to binge eating most subjects diagnosed with BED also met criteria for substance dependence (Cassin & von Ranson, 2007).

Food access may play a role in overeating and contribute to the development of BN and BED (Corwin, 2006; Corwin & Grigson, 2009; Corwin & Hajnal, 2005; Fisher & Birch, 1999; Fisher & Birch, 2000; Huon, 1994). For many people, some food is always available and the concern is access to foods which are over-consumed during binges. These are usually highly palatable, dessert-

type foods, laden with fat and refined sugars (Hadigan, Kissileff & Walsh, 1989; Kales, 1990; Rosen, Leitenberg, Fisher & Khazam, 1986). Access to these foods is often self-restricted (Kales, 1990), but may also be controlled externally by parents for example (Fisher & Birch, 1999; Fisher & Birch, 2000). Whereas human studies are largely epidemiological or correlational and often rely on self-reports, animal models permit experimental manipulation. A number of animal models have explored access to dessert-type foods that are commonly overconsumed during binges (Avena, Rada & Hoebel, 2009; Corwin, 2006; Hagan & Moss, 1997; Hagan *et al.*, 2002; Hewitt & Eikelboom, 2008; Smith, 1989; Van Vort, 1988). Many of these studies utilize access schedules that restrict availability of such fatty or sugary foods permitting the evaluation of consumption patterns for binge-like intake (Avena, Rada & Hoebel, 2008; Corwin, 2006; Hewitt & Eikelboom, 2008). These studies have shown that with some limited (intermittent) access schedules, intake of fatty or sugary foods becomes excessive and resembles binging in humans. However, in addition to access schedules, intake is influenced by properties of the food itself. Two of these, taste and postingestive properties are often although not always interrelated. That is, consumption can be driven by calories in the absence of taste (de Araujo *et al.*, 2008), or by taste in the absence of calories (Smith & Sclafani, 2002). Which properties of the food are responsible for excessive intake is not known.

This thesis explores how consumption of sweet solutions is influenced by their taste, as opposed to the postingestive properties of calories, under different

access schedules. By focusing on saccharin, a non-nutritive sugar substitute that lacks some of the postingestive effects of sugar (Kushner & Mook, 1984; Mook & Cseh, 1981; Smith, 2000) and comparing its intake profile to that of sucrose (Hewitt & Eikelboom, 2008) under continuous and different intermittent access schedules, the role of taste and caloric factors in consumption can be probed. However, it is important to not that saccharin still causes postingestive activity such stomach distension or cephalic phases insulin release (Berthoud *et al.*, 1981). Nevertheless, the physiological effects of saccharin are limited compared to to those of sugar, which has profound impact impact on food intake (Collier & Bolles, 1968), results in a rise in glycemia (Berthoud, Bereiter, Trimble, Siegel & Jeanrenaud, 1981) and can serve as a postingestive reward (de Araujo *et al.*, 2008).

Exploring how access conditions affect intake may be interesting because these factors are also implicated in the development of drug addiction, another disorder of excessive consumption (Ahmed, 2005; Spanagel & Holter, 1999; Wise, 1973). Addiction and pathological overeating have been suggested to share a common etiology (Davis & Carter, 2009; Frascella, Potenza, Brown & Childress, 2010; Holden, 2001; Orford, 2001; Volkow & Wise, 2005). While this thesis will examine factors that lead excessive intake of sweet solutions, the same factors may also be relevant to excessive drug intake.

Access variables that may lead to excessive consumption

In a laboratory setting, factors governing access to a specific food, with or without concurrent restriction of the regular laboratory diet, can be defined

according to the following variables:

1) The duration of the interval between access sessions
2) The duration of an isolated, generally longer access interruption (abstinence) after some experience with a specific food/drink ("abstinence" also refers to withdrawal in the addiction literature)
3) The quality of the food source: amount, nutritive value, energy density and taste quality
4) The cumulative number of individual access sessions (or total duration of access)
5) The duration of an individual access session

These are variables that define how a given food can be accessed and affect how consumption occurs in any experiment. Manipulations of these variables may sometimes lead to intake that is clearly excessive. This thesis examines aspects of the first three variables and explores their role in the development of excessive sweets consumption in a rat model.

Several laboratories, including our own have shown that intermittent, or Discontinuous Access (DisA) to optional fat or sugar, compared to Continuous Access (ConA) or daily access (Variable 1), can lead to a sustained intake escalation (Avena, Rada & Hoebel, 2008; Celejewski & Eikelboom, 2009; Corwin *et al.*, 1998; Hewitt & Eikelboom, 2008; Wojnicki, Stine & Corwin, 2007). Saccharin intake also increases following a single, isolated period of abstinence (Variable 2) but this increase is transient in nature (Dube, Ashton & Trowill, 1970; Gandelman & Trowill, 1969; Neznanova, Zvartau & Bespalov, 2002). Because

consumption of the palatable food in these models can be driven by its taste as well as calories (Sclafani, 2001; Sclafani & Ackroff, 2004), it is not clear if the observed intake escalation with DisA is due to taste or postingestive caloric factors (quality, Variable 3). A simple way to explore this is by providing ingestants with minimal postingestive consequences such as saccharin.

An additional question addressed in this thesis is whether intake increases caused by repeated intermittent access (Variable 1) and by a single longer abstinence period (Variable 2) are under the control of a single factor i.e. any access interruption. Under DisA conditions, the amount consumed during an access session increases with inter-session interval duration. For example, rats will consume more sucrose solution with every fourth day access than with every second day access (Hewitt & Eikelboom, 2008). Similarly, the magnitude of a transient increase in saccharin intake depends on the duration of the saccharin access interruption and increases as abstinence duration is extended at least up to 14 days (Neznanova, Zvartau & Bespalov, 2002; Sukhotina, Malyshkin, Markou & Bespalov, 2003). It is not clear however, if this increase is linked to the sustained consumption increase under DisA conditions.

Intermittent Access Schedules

Intermittent access to an optional, palatable food can lead to very large increases in consumption (Hewitt & Eikelboom, 2008). In addition to the procedure employed in our laboratory, two other intermittent access schedules examining intake of optional palatable foods have been characterized. The cyclic sugar diet model employs daily cycles of 12 h concurrent food and sugar solution

access (10 to 25% glucose or sucrose), provided 4 h into the dark phase, followed by 12 h of sugar solution and food deprivation. (Avena, Rada & Hoebel, 2008; Colantuoni *et al.*, 2002; Colantuoni *et al.*, 2001). In this model, cyclic 12 h access rats are generally compared to *ad lib* sugar and lab chow animals. Because binging in humans is partially defined as consumption of an excessive quantity of food within a short duration (American Psychiatric Association, 2000), and rats maintained on the cyclic sugar diet engage in larger/longer bouts of sugar drinking (Avena, Rada & Hoebel, 2008), it may be argued that they are binging. However, although the rate of sugar intake by the cyclic rats was greater than by *ad lib* access animals, it was comparable in terms of total quantity consumed per day. It therefore appears that cyclic diet rats are simply consuming a "normal" amount of sugar (and likely lab chow as well although never reported) but over shorter duration, particularity as food and sugar availability was delayed by 4 h into the active night cycle. By analogy, eating a larger lunch and dinner after having skipped breakfast probably does not amount to binging.

A second intermittent access preparation compares vegetable shortening or sucrose solution intake by rats with 1 or 2 h of access daily, to intake by rats with access on Mondays, Wednesdays and Fridays (MWF) only (Corwin & Wojnicki, 2009; Corwin *et al.*, 1998; Dimitriou, Rice & Corwin, 2000; Wojnicki, Stine & Corwin, 2007; Wojnicki, Stine & Corwin, 2007). This MWF preparation is different from the cyclic sugar diet in two key ways. First, in contrast to the cyclic diet, MWF rats always have *ad lib* access to lab chow. Second, instead of access/abstinence cycles within a 24 h period, all rats in this model have the

same daily access duration, but the interval between daily sessions is varied (every day versus once every two or three days on weekends). Unlike cyclic diet rats, MWF rats consume more sucrose or fat per access session than those with every day access, a true elevation of consumption.

The access paradigm employed in our laboratory incorporates features of both cyclic sugar and MWF models (Celejewski & Eikelboom, 2009; Hewitt & Eikelboom, 2008). Our rats are provided with either ConA to a 4% sucrose solution or 24 h periods of DisA to the same solution once every 2, 3 or 4 days. As with the MWF diet, DisA/ConA model rats are never food deprived, and sucrose solutions are always available in addition to *ad lib* lab chow and water. Similar to the cyclic sugar model, intermittent access (DisA) is compared to *ad lib* access (ConA). This approach has led to a number of findings. First, in agreement with the MWF model, DisA relative to ConA leads to a substantial escalation of sucrose intake. Second, the amount consumed during a 24 h access session increases as the duration of the inter-session interval is lengthened from 1 to 3 days. The sucrose intake escalation under this DisA schedule is much larger, both as a percent difference and in absolute terms than previously reported with MWF access (Wojnicki, Stine & Corwin, 2007). For example, MWF rats were reported to consume approximately 4 ml more (10 ml in total) sucrose solution than their everyday access counterparts. This increase was apparent only during the first 30 min of a 2 hour access session before dissipating. With our preparation, consumption is measured over 24 h sessions instead of 1 or 2 h periods. Compared to the 40 to 50 g water consumed by rats

with no other fluids available, ConA animals consume ~100 g of 4% sucrose. This relatively high level of sucrose solution intake suggests that it is hedonically attractive. Rats with DisA however, increase their intake as the inter-session interval is extended from 1 to 3 days coming to consume about 300 g of solution per 24 h access session. This also underscores the importance of the duration of an individual access session (Variable 5).

Although it might be argued that escalating intake with DisA reflects an increase in sucrose loading to compensate for abstinence days, comparing consumption between access groups under subsequent equal access conditions suggests otherwise. After switching from DisA or ConA access schedules (Phase I) to alternate day exposures (Phase II), the access differences induced in Phase I were maintained. In Phase II, DisA history rats continued to consume more than those with a ConA history for at least 24 days which was as long as followed (Hewitt & Eikelboom, 2008). Therefore, the initial access history can determine future consumption under equivalent access conditions with a history of DisA access leading to chronically higher levels of sucrose intake. For these access-induced differences to persist during the equal access phase, the number of DisA/ConA cycles is important (Variable 4). With eight or twelve every third or fourth day exposures (and 29 or more days of ConA) but not four DisA exposures (or 10 days ConA), access-induced changes persist during the alternate day equal access phase (Hewitt & Eikelboom, 2008). This suggests that a minimum number of DisA exposures is necessary in order to maintain consumption changes. It is unknown whether this escalation is permanent or if it eventually

dissipates.

The Deprivation Effect

In contrast to short, repeating 1 to 3 day abstinence periods as with DisA, which result in a sustained consumption increase, the deprivation effect (DE) is transient intake increase of an optional rewarding food or drink following a longer period abstinence (Neznanova, Zvartau & Bespalov, 2002; Sukhotina, Malyshkin, Markou & Bespalov, 2003). Abstinence in the context of the DE, which is also sometimes referred to as an "elation effect" (Gandelman & Trowill, 1969), refers only to a food or drink that is additional to regularly available lab chow or water.

A DE can be expressed by rats receiving daily access to a palatable saccharin solution. Following a period of abstinence, rats will consume more saccharin on their first post-abstinence session than during pre-abstinence baseline sessions (Ashton & Trowill, 1970; Dube, Ashton & Trowill, 1970; Gandelman & Trowill, 1969). On subsequent daily sessions, saccharin intake decreases towards the pre-abstinence levels. The magnitude of the post-abstinence intake elevation depends on the duration of the access interruption and increases as it is lengthened for at least 14 days (Neznanova, Zvartau & Bespalov, 2002). Although a DE may not be evident after abstinence periods approaching 30 days (Dube, Ashton & Trowill, 1970), the precise time-frame of post-abstinence intake changes is unclear due to methodological differences across studies which may be important. For example, if saccharin containing bottles are replaced with a second water bottle for the duration of abstinence rather than being removed, DE magnitude is diminished (Neznanova, Zvartau &

Bespalov, 2002).

Addiction-like characteristics of excessive food intake

Common neural systems may mediate excessive intake of food and drugs (Davis & Carter, 2009; Epstein & Shaham, 2010; Frascella, Potenza, Brown & Childress, 2010; Holden, 2001; Levine, Kotz & Gosnell, 2003; Lutter & Nestler, 2009; Volkow & Wise, 2005). The cyclic sugar model has examined similarities between excessive eating and drug intake by looking for behaviours and neurobiological changes that are also evident following exposure to rewarding drugs that may be indicative of addiction (Avena, Rada & Hoebel, 2008; Avena, Rada & Hoebel, 2009). For instance, withdrawal symptoms in opiate-treated rats such as somatic symptoms or anxiety-like behaviour, can occur spontaneously after morphine abstinence or can be precipitated by an opiate antagonists like naloxone (Schulteis *et al.*, 1994; Schulteis, Yackey, Risbrough & Koob, 1998). Likewise, rats maintained on the cyclic sugar diet (relative to *ad lib* sugar and chow) display somatic characteristics of opiate withdrawal and anxiety-like behaviour following naloxone injections or a period of food and sucrose deprivation (Avena *et al.*, 2008; Colantuoni *et al.*, 2002).

Psychomotor sensitization refers to an increased drug response, such as elevations in locomotion after repeated administration of stimulant drugs, whereas cross-sensitization is the same effect but elicited by a drug different from the one administered initially (Sanchis-Segura & Spanagel, 2006). Amphetamine-sensitized rats show sugar-induced hyperactivity and consume more sucrose relative to saline controls (Avena & Hoebel, 2003). Conversely,

rats maintained on the cyclic sucrose diet have been reported to increase their locomotor activity in response to a low dose of amphetamine (Avena & Hoebel, 2003) and to consume more alcohol (Avena *et al.*, 2004). Collectively, these findings may suggest cross-sensitization between cyclic sugar access and rewarding drugs.

Finally, in rats maintained on the cyclic diet for periods over a week, a number of neurobiological alterations that resemble changes induced by rewarding drugs have been reported. Notably, these include changes in levels of dopamine receptors, the dopamine transporter, μ-opioid receptors and enkelaphin mRNA (Avena, Rada & Hoebel, 2008; Avena, Rada & Hoebel, 2009).

Research Rationale

This thesis explores two aspects of access variables using the DisA/ConA model. First, the role of taste and postingestive consequences in the excessive consumption of sweet solution was investigated by comparing non-caloric saccharin solution intake under DisA and ConA conditions. Sweet taste is rewarding in the absence of calories and non-nutritive tastants can be consumed in large volumes despite conferring no benefit to the animal (Smith, 2000; Smith & Sclafani, 2002). While the reward value of saccharin has sometimes been questioned (White & Carr, 1985), the fact that rats select a taste of saccharin over cocaine infusions suggests high hedonic capacity (Lenoir & Ahmed, 2007). Conversely, calories can be rewarding in the absence of taste. Taste-blind mice readily learn to prefer sucrose (but not the non-nutritive sweetener sucralose) to water, and its consumption results in neural reward-like activity (de Araujo *et al.*,

2008). Intragastric infusions can produce a conditioned taste preference despite the lack of orosensory stimulation (Puerto, Deutsch, Mólina & Roll, 1976; Sclafani, 2001; Sclafani & Ackroff, 2004). Because sweetness and caloric value of a sucrose solution vary together with concentration (Smith, 2000) sucrose could be consumed due to the hedonic value of taste and calories. Therefore, it is not clear whether escalating consumption in the DisA/ConA model is driven by taste factors, caloric factors or some combination of both. Separating taste and postingestive consequences is not a new problem and is simple to address by replacing sucrose with the artificial sweetener saccharin. While this might suggest that taste is sufficient to induce the DisA/ConA difference, it does not rule out a possible role for positive postingestive factors, in establishing or maintaining the access-driven sucrose intake difference.

In Experiment 1 of this thesis, several saccharin concentrations were made available to rats on either DisA or ConA schedules. Because saccharin intake is driven primarily by taste (Smith & Sclafani, 2002), DisA rats should consume considerably more than ConA rats if taste is predominantly responsible for driving the intermittent access effect. By exploring a variety of saccharin solutions the concentration response function of the effect was explored. In Experiment 2, a single saccharin solution (showing the biggest difference in Experiment 1), was used to determine if any differences in consumption can be maintained after all rats are given alternate day access.

Second, whereas intermittent every 1, 2 or 3 day DisA produces a sustained consumption change, a single longer period of abstinence can result in

a DE that is transient in nature. Experiment 2b examined the impact of longer term abstinence in rats expressing access-induced differences resulting from previous DisA/ConA experience. Because increased intake following the longer abstinence (the DE), did not interact with DisA/ConA-induced differences suggesting that the DE and DisA/ConA consumption differences are under the control of separate factors, Experiment 3 sought to replicate and expand on this finding by returning to the sucrose model. Although previous studies have shown that a DE can be observed with saccharin but not sucrose solutions (Ashton & Trowill, 1970; Ashton, Gandelman & Trowill, 1970), large intake increases have been observed with DisA to sucrose solutions (Hewitt & Eikelboom, 2008). Moreover, a number of operants studies have shown that motivation to obtain sucrose increases over abstinence durations relevant to DE studies (Avena, Long & Hoebel, 2005; Grimm, Shaham & Hope, 2002; Lu, Grimm, Hope & Shaham, 2004; Neznanova, Zvartau & Bespalov, 2002) suggesting that some other factor must account for the lack of sucrose DE. Because a possible explanation for the lack of a DE is an inhibition by the satiating properties of calories, particularly because the concentrations utilized in sucrose DE studies were high (Ashton & Trowill, 1970; Ashton, Gandelman & Trowill, 1970), Experiment 3 further explored the DE in rats with DisA or ConA histories using a relatively weak 4% sucrose solution.

Experiment 1

The objective of Experiment 1 was to determine whether taste factors are primarily responsible for the intake under escelation intermittent access conditions. This was accomplished by providing DisA and ConA to saccharin solutions to circumvent the positive or rewarding postingestive consequences of calorie-laden sucrose solutions (de Araujo *et al.*, 2008; Sclafani, 2001; Sclafani & Ackroff, 2004). If postingestive factors alone underlie the sucrose intake escalation under DisA conditions, the consumption difference between ConA and DisA rats should not be evident with saccharin. If escalating sucrose intake under DisA conditions is also driven by taste, then a similar increase would be expected to occur with DisA to saccharin.

Previous work in our lab has shown that the DisA/ConA intake difference is most robust with a 4% sucrose solution but smaller or absent at lower (1%) and higher (8 and 16%) concentrations (Randell and Eikelboom, Unpublished). A secondary objective of Experiment 1 was to determine whether access-induced intake differences can emerge across a range of saccharin concentrations. Confirming this would suggest that consumption differences for 8 and 16% sucrose solutions were inhibited by limiting satiating postingestive consequences (distinct from rewarding postingestive consequences).

Although the absence of a DisA/ConA difference for the low 1% solution might be attributed to a floor effect, this cannot account for the diminished or absent effect of DisA on the intake of higher sucrose concentrations. Instead, the lack of access-induced differences may be accounted for by the sucrose intake-

concentration function. When the sucrose intake-concentration function is measured over 24 h, the volume consumed increases with concentration, peaks at 8% and declines with further increases in sucrose concentration (Smith, 2002). If sucrose solute consumed, as opposed to solution volume is measured, sucrose solute intake peaks and plateaus with 16% sucrose solutions. However, if postingestive effects are reduced by fitting the animals with a gastric fistula (Sclafani & Nissenbaum, 1987) or circumvented by brief access procedures that largely preclude satiety (Smith & Sclafani, 2002), intake will continue to increase with concentrations beyond 16%. Similarly, preference tests show that the higher of two sucrose concentrations is always preferred (Collier & Bolles, 1968). For sucrose solution volume therefore, the 24 h intake-concentration function takes the shape of an inverted U with a descending arm that is most readily accounted for by the inhibitory, satiating postingestive properties of calories. Thus, if an 8% concentration represents a ceiling for volume and calories consumed over a 24 h period potentially masking DisA/ConA difference, than a 4% solution would still permit further intake increases.

Although both sucrose and saccharin are sweet, they differ in taste characteristics (Dess, 1993). The 24 h saccharin intake-concentration function also takes on an inverted U shape but the descending portion can be accounted for by saccharin's bitter, quinine-like after-taste that increases in salience with concentration (Dess, 1993). For a 24 h period, saccharin solution consumption initially increases with concentration but begins to decreases at higher concentrations regardless of whether rats are real or sham fed (Sclafani &

Nissenbaum, 1985; Smith, 2000). The same intake-concentration function is obtained with short term (Smith & Sclafani, 2002) and preference tests (Smith & Rashotte, 1978). Therefore, unlike sucrose, saccharin intake is regulated primarily by taste. Consequently, a DisA-driven saccharin intake escalation might be expected to emerge across a wider range of concentrations compared to sucrose which would suggest that satiety masks sucrose consumption effects at higher concentrations.

A final caveat regarding the taste characteristics of saccharin vs. sucrose is that although rats drink higher volumes of saccharin compared to other commonly employed sweeteners such as aspartame (Sclafani & Abrams, 1986) or sucralose (Bello & Hajnal, 2005; Sclafani & Clare, 2004), saccharin is not preferred to sucrose except with very dilute sucrose solutions. Therefore, regardless of access conditions less saccharin than sucrose solution is expected to be consumed under similar conditions.

Methods

Subjects

Sixty-four male Sprague Dawley Rats from Charles River Laboratories (St. Constant Quebec), weighing 200–225 g at arrival (approximately 47 days old) were maintained on a 12:12 light/dark cycle (lights on 09:00). Rats were single housed in shoe box cages (21 cm height x 24 cm width x 45 cm length) with *ad lib* access to water and Harlan Teklad Rodent Diet 8460 (3.1 kcal/g metabolizable energy). Due to subjects' high fluid intake, hardwood chip bedding was replaced as needed (more frequently than normal and at least once a week).

All procedures in this and subsequent experiments were approved by the Wilfrid Laurier Animal Care Committee in accordance with Canadian Council on Animal Care policies and guidelines.

Apparatus

Water and one of the 4 saccharin solutions, 0.125, 0.25, 0.5 or 1%, were provided to each rat in glass bottles fitted with rubber stoppers and stainless steel sipper tubes. Throughout the course of the study, water and saccharin bottles were always available on the same side of the cage. Although some spillage of fluids occurred during measurement, between group differences were not affected because bottles were handled similarly for all groups. Daily consumption of saccharin, food and water was reported as the weight difference in grams between two consecutive measurements. Solutions were prepared as needed, four litres at a time from tap water and sodium saccharin (Sigma, Oakville Ontario). All concentrations were reported as weight/volume (w/v) percentages:

$$Percent\ Solute\ Concentration = \left(\frac{grams\ solute}{millilters\ water}\right) \times 100$$

Water and saccharin bottles were replaced after approximately 7 days of use or every three discontinuous exposures.

Procedure

After an initial 7 day period of acclimation to the colony room and daily animal handling, consumption of food, water, and body weight was recorded for

an 8 day baseline period. Next, animals were divided into four weight matched groups (n = 16 each) that were randomly assigned to one of four saccharin concentrations: 0.125, 0.25, 0.5 or 1%. At the end of the first day of saccharin access, animals in each group were assigned to either DisA or ConA conditions (n = 8 each), matched by day 1 saccharin intake and body weight. ConA rats received continuous exposure to their designated saccharin concentration for 34 days whereas DisA rats received 12 every third day saccharin exposures (days 1, 4, 7, ..., 34). Food and water were always available to all rats.

The length of each daily access period was 23 h as food, water, and saccharin were not available for approximately 1 h during which daily measurements were taken. These were conducted 4 h prior to the onset of the dark cycle as to minimize interference with feeding and drinking behaviour. During this time food, water and saccharin (when available), were removed, weighed, and replaced after body weight was recorded. Rats in the DisA groups (n = 32) were weighed first (15:00 to 16:00), followed by rats in ConA groups (n = 32; 16:00 to 17:00). Also during this time, solution and water bottles were refilled to ensure adequate supply for the following day.

To reduce day to day variability, two concurrent replications were conducted with a 1 day difference between them, i.e. half of all rats in each condition started saccharin access 1 day after the first half.

Statistics

Saccharin consumption data were analyzed for common saccharin access days only, that is, for days on which DisA and ConA rats received saccharin

access (days 1, 4, 7, ..., 34). The 12 common saccharin exposures were divided into three blocks consisting of four saccharin exposures each: the first four, the middle four and the last four common exposures which were analyzed using an Access (ConA, DisA) by Concentration (0.125, 0.25, 0.5 and 1%) by Days (four common exposure days) mixed design Analysis of Variance (ANOVA) with repeated measures on Days. Where main effects were significant, REGWQ ($p < 0.05$) post hoc analysis were performed.

Food intake data were averaged over 8 baseline days and three blocks that corresponded to blocks over which saccharin data was analyzed: Block 1 (days 1 to 10), Block 2 (days 11 to 22) and Block 3 (days 23 to 34). Baseline data for mean food, water and last baseline day weight were analyzed in Access by Concentration ANOVAs. For experimental days, food data were analyzed in an Access (DisA, ConA) by Concentration (0.125%, 0.25% 0.5% and 1%) by Block (Blocks 1, 2 and 3) mixed design ANOVA with repeated measures on Block. Weight data were also compared at three time points but for the last day of each block only in an Access by Concentration by Day (days 10, 22, 34) mixed design ANOVA. Water data, which was probably strongly affected by saccharin intake, was not analyzed.

Within-subject effects and interactions were reported as significant only if significance was also met after Greenhouse-Geisser corrections were applied for these and all subsequent analyses. All statistical analyses were performed with SPPS 17.0.

Results

Consumption by DisA and ConA rats is illustrated in Figure 1 by concentration, in Figure 2 by schedule, and averaged across the baseline and three blocks in Table 1. During the first four common exposures (days 1, 4, 7 and 10) there was a main effect of Access [$F(1,56) = 12.57$, $p < 0.001$], and Concentration [$F(3,56) = 21.86$, $p < 0.001$] a within effect of Days [$F(3,168) = 6.80$, $p < 0.001$] and an Access by Days interaction [$F(3,168) = 20.68$, $p < 0.001$]. The main effect of Access was due to greater saccharin intake by DisA relative to ConA animals whereas the effect of Days and Access by Days interaction reflects that this difference was predominantly due to an increase by DisA rats that emerged gradually over these first four common exposures. The main effect of Concentration was due to greater consumption of lower saccharin concentrations regardless of access schedule. Post-hoc testing revealed three homogeneous subsets consisting of the 0.125 and 0.25%, the 0.5%, and the 1% concentrations. Over the middle four common saccharin exposures (days 13, 16, 19 and 22), the main effects of Access [$F(1,56) = 23.77$, $p < 0.001$], and Concentration [$F(3,56) = 14.97$, $p < 0.001$] were maintained. Post-hoc testing revealed three homogeneous subsets consisting of the 0.125 and 0.25%, the 0.25 and 0.5%, and the 1% concentrations. Similarly, over the last four exposures (days 25, 28, 31 and 34), the intake difference between DisA and ConA rats was sustained Access [$F(1,56) = 24.99$, $p < 0.001$] as was the main effect of Concentration [$F(3,56) = 11.14$, $p < 0.001$]. Post-hoc testing revealed two homogeneous subsets consisting of the 0.125, 0.25 and 0.5%, and the 1%

concentrations. The lack of Access by Days interaction during the middle and last four common exposures suggests that consumption levels had stabilized. Over the last eight common exposures, in the order of ascending concentration (1% to 0.25%), DisA rats consumed 54, 81, 62 and 74% more than ConA rats.

All water, food and body weight data are reported in Table 1. There was no effect of Access or Concentration on baseline food and water intake or baseline body weight (note that rats were matched for body weight in their group assignment). For the experimental days, there were also no significant effects of Access or Concentration on food intake or body weight. However, there was an effect of Block for weight [$F(2,112) = 1513.86$, $p < 0.001$] and food intake [$F(2,112) = 52.46$, $p < 0.001$] as rats tended to increase their food consumption with weight gain over the duration of the experiment.

Discussion

These results suggest that taste, in the absence of the positive postingestive consequences of calorie rich sucrose solutions, is predominantly responsible for the DisA-induced intake escalation. While consumption of saccharin solutions varied as expected given the saccharin intake-concentration function (Smith, 2000) across all concentrations, DisA rats consumed more over 24 h periods than ConA animals.

The DisA/ConA differences were observed for different saccharin concentrations in Experiment 1, but not previously with more concentrated sucrose solutions (Hewitt & Eikelboom, 2008). Saccharin lacks the postingestive properties of sucrose and intake differences emerged across the saccharin

concentrations range (taste intensity). Therefore, it is unlikely that taste differences of higher sucrose concentrations could account for the lack of access consumption effects. Instead, because calories increase with sucrose concentration, it is more likely that the postingestive consequences of calories suppressed or masked access-induced differences for more concentrated sucrose solutions.

That sucrose calories may play an inhibitory role under conditions which otherwise lead to excessive intake is also corroborated by the absence of a large intake increase with the cyclic sugar diet which employed relativity energy dense (10 or 20% w/v) sucrose or glucose concentrations (Avena, Rada & Hoebel, 2008; Avena, Rada & Hoebel, 2009; Colantuoni *et al.*, 2001). Moreover, rats subject to MWF access were found to consume slightly more sucrose solution over 2 h access sessions relative to their daily access counterparts for 3.2% and 10% but not 32% concentrations (Wojnicki, Stine & Corwin, 2007).

Although access-induced differences were observed with saccharin, the intake difference between DisA and ConA rats was smaller than previously reported for a 4% sucrose solution, even compared to the most consumed saccharin concentrations (0.25 and 0.125%). DisA rats consumed an average of 62 to 81% more saccharin than those with ConA. In comparison, under similar access conditions, DisA animals consumed two to three times as much of a 4% sucrose solution as animals with ConA. Although the smaller magnitude of the DisA/ConA difference and lower overall intake of saccharin relative to sucrose may have occurred due to a positive postingestive component for sucrose,

saccharin's less attractive taste was probably the main contributing factor (Smith & Sclafani, 2002). The present data however, do not rule out the impact that postingestive factors may have had on the DisA/ConA difference. This could be ascertained in future studies by employing taste blind animals such as trpm5$^{-/-}$ knockout mice which lack functional transient receptor channel M5 required for sweet, bitter and umami taste signalling (de Araujo *et al.*, 2008) and therefore consume sucrose only for its postingestive consequences. Intragastric infusion can also be utilized to bypass taste consequences.

Finally, the 1% saccharin solution was consumed at lower levels than all other concentrations (Figure 2). This was probably due to the bitter component of the saccharin flavour which would have been most salient at the highest, 1% concentration (Dess, 1993). Even a relatively palatable 0.1% saccharin solution can result in both positive ingestive and negative aversive responses in a taste reactivity procedure (Parker & Lopez, 1990) suggesting both attractive and unpleasant taste components.

In conclusion, Experiment 1 suggests that taste is predominantly responsible for the emergence of access-induced differences. Second, although postingestive feedback can be rewarding and stimulate intake under certain conditions (de Araujo *et al.*, 2008; Sclafani, 2001; Sclafani & Ackroff, 2004), it might also inhibit access-induced intake increases at higher sucrose concentrations under DisA/ConA conditions.

Experiment 2

Experiment 1 established that taste is predominantly responsible for access-driven consumption changes. The main objectives of Experiment 2 were first, to replicate this finding and second to determine whether saccharin access history can affect consumption after access conditions are equalized. As described in the introduction, access-induced differences in sucrose intake have been shown to persist under equal access conditions (Hewitt & Eikelboom, 2008) suggesting that access history has long term effects on consumption. As sucrose and its consumption differs from saccharin in a number of ways, it is not clear if saccharin DisA/ConA consumption changes could be maintained under similar equal access conditions. Because the first part of this experiment (Experiment 2a) shows that access-induced changes do indeed persist under equal access conditions, a number of other potentially important factors are also explored.

Elevated drug intake in addiction has been hypothesized to be driven by a negative affective or anxiety-like state (Koob & Le Moal, 2001). In order to examine if a similar affective state is associated with escalated levels of saccharin intake, behavioural measures of anxiety were assessed during Phase II of 2a. Details of these procedures and results can be found in Appendix A as they are not central to the main thrust of this thesis and showed no significant differences between access conditions.

Although intermittent access to every third or fourth day sucrose or saccharin can result in large and sustained intake increases, isolated longer periods of saccharin abstinence can result in a DE - a transient saccharin intake

increase (Gandelman & Trowill, 1969; Neznanova, Zvartau & Bespalov, 2002). Experiment 2b examined the expression of the saccharin DE in animals with a preceding history of DisA or ConA experience to determine if these two access driven consumption increases are related.

Finally, because saccharin and sucrose have different taste characteristics, to determine whether access-induced changes in saccharin consumption are flavour specific or generalize between different sweet solutions, in Experiment 2c saccharin was replaced with a sucrose solution.

A consequence of these multiple tests, is the relatively long equal access period (55 days) that followed initial differential access in Phase I during which rats were provided with same solution on the same access schedule. This allowed the effects of DisA/ConA histories to be followed over a longer duration. The complete time-line of Experiment 2 is summarized in Figure 3 and Table 2. Because in Experiment 1 weight or food consumption was not affected by the saccharin access schedule, these data were not analyzed.

General Methods

Subjects

Thirty-two male Sprague Dawley Rats were obtained from Charles River Laboratories, St. Constant Quebec. All specifications and conditions were identical to Experiment 1.

Apparatus

Administration and preparation of 0.25% saccharin (Sigma 1002) solution was described for Experiment 1. Water and solution bottles were replaced after

approximately 7 days of use or every two discontinuous cycles of exposures.

Procedures

Rats were initially acclimatized to the colony room for a 7 day period during which they were handled daily. Next food, water, saccharin, sucrose solutions (when available) and body weight were followed daily for 8 baseline days and 101 experimental days as summarized in Table 2 and Figure 3. Measurements were recorded in the manner described for Experiment 1 except that food, water and saccharin were removed only for the time required to record all values for each subject individually. Therefore, all animals received 24 h (minus 1 or 2 minutes) of access to food, water, and when available, saccharin or sucrose solutions.

Experiment 2a

Changes in sucrose solution intake induced by DisA and ConA can persist even after access schedules are equalized (Hewitt & Eikelboom, 2008). However, as with the emergence of these consumption effects, consequences of access history may be due to postingestive or taste properties of sucrose. Therefore, the main objective of Experiment 2a was to determine whether a DisA/ConA saccharin solution intake difference can be maintained after access schedules for all animals are switched to equal access conditions, as in sucrose studies (Hewitt & Eikelboom, 2008). Switching the saccharin access schedule to alternate day exposures (Phase II) for rats that initially received DisA or ConA (Phase I) permits a direct comparison of intake between animals with different access histories. In order to ensure maximum consumption differences, the

interval between access sessions was increased from 2 to 3 days for a 0.25% saccharin solution which had produced the greatest DisA/ConA intake difference in Experiment 1.

Methods

Procedure. Following an 8 day baseline period, on Day 1 of Phase I, 0.25% saccharin was provided for 24 h. After the first saccharin day, rats were assigned to either DisA or ConA conditions (n = 16 each), matched for saccharin intake and body weight. The DisA condition rats received saccharin for 24 h once every 4 days whereas ConA rats received constant saccharin access. After 12 DisA exposures and 45 days of ConA, both access schedules were changed to alternate day access for 12 24 hour exposures (Phase II). Therefore, between days 46 and 69, saccharin was available every other day for all rats.

Two anxiety tests, elevated plus maze (EPM) and light/dark emergence, were conducted on day 56 to explore whether anxiety-like affective states are linked to changes in consumption. See Appendix A for details.

Statistics. Saccharin consumption data were analyzed on common saccharin exposure days only (days 1, 5, 9, ..., 45). That is, on days during which rats in ConA and DisA groups received access to saccharin. Both Phase I and II saccharin intake was compared across three blocks, each consisting of four common saccharin exposures. The transition between Phases I and II was also compared across one block of four common access days (days 41, 45, 47 and 45). Blocks were analyzed in Access (ConA, DisA) by Days (four common access days) mixed design ANOVAs with repeated measures on Days.

Results

Saccharin intake by DisA and ConA rats is illustrated in Figure 4. Over the first four common saccharin exposures (days 1, 5, 9 and 13), there was a main effect of Access [$F(1,30) = 12.7$, $p < 0.001$], an effect of Days [$F(3,90) = 5.4$, $p < 0.01$] and an Access by Days interaction [$F(3,90) = 12.7$, $p < 0.001$] as intake by DisA rats increased gradually while consumption by ConA rats decreased slightly. As in Experiment 1, the main effect of Access was maintained over the middle four exposures (days 17, 21, 25 and 29) [$F(1,30) = 38.09$, $p = 0.001$] and the last four exposures (days 33, 37, 41 and 45) [$F(1,30) = 42.29$, $p < 0.001$]. The lack of Access by Days interaction during the middle and last four common exposures suggests that consumption levels stabilized. The differences in consumption over the last eight common exposures expressed as a percent increase of DisA over ConA was 116% (158 g DisA, 72 g ConA).

At the beginning of Phase II on day 46, saccharin access conditions were equalized for all rats to 24 h periods of alternate day access. Over the transition between Phase I and II (saccharin days 41, 45, 47 and 49), there was a main effect of Access [$F(1,30) = 26.1$, $p < 0.001$] and an Access by Days interaction [$F(3,90) = 21.4$, $p < 0.001$]. The interaction appeared to be due to a decrease in saccharin consumption by DisA rats, and an increase in consumption by ConA rats as access conditions were changed (see Figure 4).

Over the course of Phase II, the main effect of Access [$F(1, 30) = 12.56$, $p < 0.001$] was maintained during the first four saccharin exposures (days 47, 49, 51 and 53) and there was an effect of Days [$F(3,90) = 5.04$, $p < 0.01$], most likely

due to an unexplained intake increase on day 49 and decrease on day 51. Over the middle four saccharin exposures (days 55, 57, 59 and 61) there was only a main effect of Access [$F(1, 30) = 15.5$, $p < 0.001$]. Finally, over the last four saccharin four exposures (days 63, 65, 67 and 69) there was a main effect of Access [$F(1, 30) = 12.56$, $p < 0.01$], as well as an effect of Days [$F(3,90) = 5.67$, $p < 0.01$] apparently due to an intake increase for all rats on day 67. Once again, a lack of Days by Access interactions along with the maintained effect of Access suggests that access-induced differences remained stable over the course of Phase II. The mean percent increase maintained during the 12 saccharin exposures of Phase II by DisA over ConA animals dropped to 61% (129 g DisA, 80 g ConA) from 116% in Phase I. All data collected are summarized in Table 3.

Discussion

In agreement with Experiment 1, solution intake by DisA rats escalated over the initial four exposures to significantly exceed ConA consumption. The magnitude of the intake difference between DisA and ConA rats was greater than in Experiment 1 for the equivalent concentration. With every fourth day DisA in Experiment 2, rats drank a little over twice as much as those with ConA, approximately 40 g more than rats with every third day DisA to the same solution in Experiment 1. ConA rats consumed similar amounts in Experiments 1 and 2. The difference in DisA consumption in Experiments 1 and 2 may be accounted for by the extension of the inter-exposure interval from 2 to 3 days underscoring its importance in intermittent access schedules.

In Phase II, when access conditions were equalized, rats with a DisA

history continued to consume more than rats with a ConA history. This difference was robust and persisted over the 12 alternate day access sessions (24 days) of Phase II replicating previous sucrose work (Hewitt & Eikelboom, 2008). This suggests that taste factors are predominantly responsible for the persistence of DisA/ConA differences after access is equalized.

Experiment 2b

Previous studies have investigated consumption effects of chronic intermittent access, as with the DisA schedule, or of individual, longer abstinence periods. Whereas intermittent access schedules utilizing repeating 1 to 3 day inter-exposure intervals result in gradual and sustained intake increases (Avena, Rada & Hoebel, 2008; Corwin, 2006; Hewitt & Eikelboom, 2008) a single, isolated period of abstinence results in a transient post-abstinence consumption increase – a DE (Gandelman & Trowill, 1969; Neznanova, Zvartau & Bespalov, 2002; Sinclair & Senter, 1968). The impact of different access schedules (DisA/ConA) on DE expression is unknown. Experiment 2b examined the impact of a single longer saccharin access interruption in rats with different access histories by following saccharin consumption after an 8 day abstinence period after the end of Phase II. This abstinence duration is consistent with previous studies investigating the DE (Gandelman & Trowill, 1969; Neznanova, Zvartau & Bespalov, 2002; Zakharova *et al.*, 2004).

Methods

Procedure. Following the removal of saccharin bottles on the last alternate exposure day of Phase II (day 69), rats began an 8 day saccharin

abstinence period which was followed by 4 alternate day saccharin exposures (days 78 to 84).

Statistics. Saccharin DE data were analyzed comparing the last pre-abstinence saccharin day (day 69), with the first post-abstinence day (day 78) in an Access (DisA, ConA) by Days (last pre-abstinence day, first post-abstinence day) mixed-design ANOVA with repeated measures on Days. Saccharin intake during the 4 alternate day post-abstinence exposures was compared in an Access (DisA, ConA) by Days (four common access days) mixed-design ANOVA with repeated measures on Days.

Results

Following 8 days of saccharin abstinence, access was restored on day 78 for four alternate day saccharin exposures illustrated in Figure 5. Over the last pre- and first post-abstinence days there was a main effect of Access [$F(1,30) = 9.03$, $p < 0.001$] an effect of Days [$F(3,90) = 145.01$, $p < 0.001$] but no Access by Days interaction. All rats increased their intake after abstinence but the access-induced intake difference was maintained. Over the course of the four post-abstinence alternate day saccharin exposures, there was a main effects of Access [$F(1,30) = 10.36$, $p < 0.003$], an effect of [Days [$F(3,90) = 56.46$, $p < 0.001$], but no Access by Days interaction. The effect of Days was due to decreasing consumption by rats in both groups after the first post-abstinence saccharin exposure and the effect of Access was due to the maintained access-induced consumption difference. All data collected are summarized in Table 3.

Discussion

Rats in both groups displayed a robust DE after saccharin was restored following 8 days of abstinence. This agrees with previous studies reporting a saccharin DE after similar periods of abstinence (Gandelman & Trowill, 1969; Neznanova, Zvartau & Bespalov, 2002). The expression of DE did not interact with the access schedule history: the DE was similar for rats in both groups. When access was restored after abstinence, both DisA and ConA rats increased their consumption from their pre-abstinence Phase II baselines by similar amounts. On days subsequent to the first post-abstinence day saccharin intake by all rats decreased while the access-induced difference was maintained. The lack of interaction between access history and DE expression suggests that the increase caused by the 8 day access interruption was not related to increased saccharin intake induced by DisA and that separate mechanisms may underlie DE and DisA/ConA consumption differences.

Experiment 2c

Because saccharin and sucrose differ in terms of taste (Dess, 1993), it is not clear whether access-induced changes in saccharin intake could generalize to other sweet substances. To answer this question, saccharin can be replaced with another sweet substance such as sucrose. Observing a maintained access-induced difference after a switch to a sucrose solution would indicate that access-induced intake differences can generalize from one sweet flavour to another rather than being taste specific. In Experiment 2c a 4% sucrose solution was provided to rats displaying saccharin access-induced consumption changes under equivalent access schedules.

Methods

Procedure. On Day 86, the saccharin solution was substituted with a 4% (w/v; Redpath Sugar) sucrose solution for 8 alternate day exposures (days 86, 88, 90, ..., 100). The sucrose solution was prepared in the manner previously described for saccharin solutions (Experiment 1).

Statistics. Sucrose solution intake data were analyzed over two subsequent Blocks consisting of four exposures each in Access (ConA, DisA) by Days (four common sucrose days) mixed-design ANOVAs with repeated measures on Days.

Results

Sucrose consumption over the 8 alternate day exposures is illustrated in Figure 6. Over the first 4 alternate day sucrose exposures (days 86, 88, 90, 92), there was a main effect of Access [$F(1,30) = 8.06$, $p < 0.01$], an effect of Days [$F(3,90) = 31.53$, $p < 0.001$] and an Access by Days interaction [$F(3,90) = 5.73$, $p < 0.001$]. The main effect of Access was due to the maintenance of saccharin access-induced consumption differences with sucrose. The Days effect appears due to gradually increasing consumption by rats in both groups during this period while the interaction effects appears due to the larger increase in sucrose consumption by DisA than ConA rats. Over the second 4 alternate day sucrose exposures, consumption stabilized and there was only a main effect of Access [$F(1,30) = 7.73$, $p < 0.01$]. All data collected is summarized in Table 3.

Discussion

Experiment 2c results show that access-induced saccharin intake

differences are not flavour specific and generalized to sucrose. These consumption differences were initially induced during Phase I (days 1 – 45) whereas sucrose was presented on day 86, after 41 days of equivalent access (see Figure 3). During this long equal-access period, all rats received the same exposure to the same solutions. Over the course of the 8 alternate day sucrose exposures, all rats increased their intake over the initial sucrose exposures, but the DisA rats did so more rapidly and to a greater extent than those with ConA. Intake stabilized over the last four exposures as access-induced differences were maintained. It is possible that the gradual increase over the first four exposures was due to learning about the more attractive nature of sucrose relative to saccharin as evidenced by its higher consumption.

In addition to demonstrating that access-induced differences generalize to sucrose, their persistence throughout Experiment 2c indicates that access-induced changes are remarkably durable. In total they persisted over 55 days of equal access (32 saccharin and 15 sucrose). Thus, even in the absence of postingestive consequences of caloric solutions, changes induced by DisA to sweet solutions are relativity permanent.

Experiment 3

In Experiment 2b, the post-abstinence consumption increase added linearly to the apparently permanent consumption differences induced by previous DisA or ConA. This suggests that a single isolated period of abstinence may have an effect on consumption that is different from intermittent DisA, that is, not all experiences with abstinence are the same. The primary objective of Experiment 3 was to replicate this finding. Because the DE is a time-dependent phenomenon and short repeating abstinence periods may be different from a single long abstinence period, in Experiment 3, consumption by rats with DisA/ConA experience was examined after 3 or 9 abstinence days and compared to animals maintained on an alternate day accesses schedules.

A second objective of Experiment 3 was to examine DE expression with sucrose. This is because previous studies which measured sucrose solution intake following abstinence did not observe a sucrose DE (Ashton & Trowill, 1970; Ashton, Gandelman & Trowill, 1970) despite the fact that large intake increases have been observed in DisA to sucrose (Hewitt & Eikelboom, 2008). The failure to observe a sucrose DE may have been due to several aspects of the procedure employed. First, in these studies, bottles containing sucrose solutions were replaced with water bottles (rather than removing them during abstinence), which has been shown to significantly reduce, although not entirely abolish subsequent saccharin DE expression (Neznanova, Zvartau & Bespalov, 2002). Second, the sucrose solutions utilized in the sucrose DE studies were relatively energy dense 8 and 16% concentrations. It is therefore possible that

unconditioned or learned satiety effects may have suppressed DE expression (Davis, Smith, Singh & McCann, 1999; Smith & Sclafani, 2002; Weingarten & Kulikovsky, 1989). As discussed, postingestive factors may inhibit further intake increases for more energy dense sucrose solutions during DisA. Finally, both studies exploring the sucrose DE utilized relatively short abstinence intervals (2 or 3 days), whereas saccharin studies have shown that the saccharin DE continues to increase for at least 14 days and was only significant after 3 days of abstinence (Neznanova, Zvartau & Bespalov, 2002). If these concerns are addressed, a sucrose DE may be observed.

Under the protocol employed in our laboratory, solution bottles are removed rather than replaced with water bottles. Second, because large consumption increases have been observed with DisA to 4% sucrose solution, a satiety ceiling effect that could mask a sucrose DE might be avoided with this concentration. Finally, a longer abstinence duration that reliably produces a saccharin DE may also result in a sucrose DE.

Other studies also suggest that a sucrose DE may be expressed under the appropriate conditions. For example, operant responding for 25% glucose solutions increases after a 14-day abstinence period (Avena, Long & Hoebel, 2005). Cue induced reinstatement of sucrose-seeking behaviour following extinction also increases as the duration of abstinence is lengthened (Grimm, Fyall & Osincup, 2005; Lu, Grimm, Dempsey & Shaham, 2004). Taken together, these results suggest that the motivation to consume sucrose increases with abstinence duration.

Finally, because eating disorders are substantially more prevalent in women (Hudson, Hiripi, Pope & Kessler, 2007), to enhance model validity, female rats were employed in Experiment 3.

Methods

Subjects

Forty-two female Sprague Dawley Rats, bred on site and derived from rats provided by Charles River Canada, were paired housed until approximately 70 days of age before being separated and single housed. All other specifications and conditions were identical to Experiment 1.

Apparatus

Sucrose solution (4% w/v; Redpath Sugar), food and water intake, as well as body weight were measured every day beginning on baseline day -7 through to day 102 in a manner identical to Experiment 2.

Procedure

As in Experiments 1 and 2, after 7 days of initial acclimatization to the colony room and to individual housing accompanied by daily handling, food and water consumption as well as body weight was followed for an 8 day baseline period. Next, a 4% sucrose solution was provided for 24 h. Rats were then assigned to two groups (n = 21 each), matched for weight and initial sucrose solution consumption and designated as either ConA or DisA. As in Experiment 2, the DisA sucrose inter-exposure interval was 3 days whereas ConA rats received constant sucrose access. After 12 DisA exposures or 45 days of ConA to sucrose, both access schedules were changed to alternate day access for 4

alternate day exposures (days 47, 49, 51, and 53).

On day 53, after sucrose intake was measured, both DisA and ConA group were subdivided into three groups (n = 7 each), matched by weight and average Phase II sucrose consumption. Each of these three groups with a prior history of ConA or DisA access was randomly assigned to one of the following conditions: Long Abstinence (LAb), Short Abstinence (SAb) and Non-Abstinence control (NAb). Sucrose bottles were not available for a period of 9 days for the LAb rats, 3 days for the SAb rats, whereas NAb rats continued to receive alternate day sucrose access. After sucrose access was restored for LAb and SAb rats, consumption was followed for at least 12 more alternate day exposures (days 57 to 85 for SAb rats and 63 to 85 for LAb rats). The experimental design is summarized in Table 4.

Statistics

Sucrose solution intake data were analyzed on common sucrose exposure days only (1, 5, 9, ..., 45 Phase 1 and days 47, 49, 51 and 53 for Phase II). Phase I was partitioned into three consecutive blocks as for Experiments 1 and 2. Intake during each block as well as for the transition between Phases I and II (days 41, 45, 47 and 49) was analyzed in Access (ConA, DisA) by Group (LAb , SAb, NAb) by Days (four common access days) mixed design ANOVAs with repeated measures on Days.

Sucrose DE data for the LAb and SAb groups were analyzed by comparing the last pre- and first post-abstinence days in Access (ConA, DisA) by Days (last pre-abstinence day, first post-abstinence) mixed design ANOVAs with

repeated measures on Days for each abstinence group.

Post-abstinence consumption data were compared to NAb rats (always across the same common exposure days) for each abstinence group. The analysis was conducted for three consecutive blocks of four common exposures comparing each abstinence group to the NAb group. The SAb and LAb groups were each compared to the NAb group in Access (ConA, DisA) by Group (SAb or LAb, NAb) by Days (four common exposure days) mixed design ANOVAs with repeated measures on Days. The three additional sucrose days for the SAb group rats were not analyzed.

Results

Phases I & II

Sucrose solution intake by DisA/ConA LAb, SAb and NAb rats during Phases I and II is illustrated in Figure 7. During the first four common sucrose exposures (days 1, 5, 9, and 13), as in previous experiments there was main effect of Access [$F(1,36) = 20.95$, $p < 0.001$], within effect of Day [$F(3,108) = 4.31$ $p < 0.01$] and an Access by Day interaction [$F(3,108) = 23.23$, $p < 0.0001$]. This was due to a large increase of sucrose intake by DisA rats and a small decrease by ConA rats. The main effect of Access was maintained over the middle four exposures (days, 17, 21, 25 and 29) [$F(1,36) = 36.32$ $p < 0.001$]. Over the last four exposures (days 33, 37, 41, and 45), there was also a main effect of Access [$F(1,36) = 46.78$, $p < 0.001$] and an Access By Days Interaction [$F(3,108) = 3.66$, $p < 0.05$]. The effect of Access during the middle and last four common exposures suggests that consumption differences were reasonably stable. The

Access by Day interaction effect for the last block appeared to be due to a slight intake decrease by DisA rats and a slight increase by ConA rats over these days. During these last eight exposures of Phase I, DisA rats consumed 114% percent more than ConA rats. At no point did abstinence group assignment impact intake levels as there was a complete lack of Group effects.

At the beginning of Phase II, ConA and DisA access schedules were changed to alternate day exposures. During the transition between Phases I and II (days 41, 45, 47 and 49), the main effect of Access was maintained, [$F(1,36) = 30.98$, $p < 0.001$] and there was an Access by Days interaction [$F(3,108) = 6.43$, $p < 0.001$]. The interaction appeared to be due to an intake increase by ConA rats, and a small decrease by DisA rats at the beginning of Phase II. The main effect of Access was due to maintained access-induced difference. Over the four alternate access days of Phase II (days 47, 49, 51 and 53), the main effect of Access persisted [$F(1,36) = 22.66$, $p < 0.0001$]. The lack of Access by Days interaction suggests intake quickly stabilized after the transition to Phase II. During Phase II, DisA rats consumed on average 68% more solution than ConA rats. Once again, the lack of Group effects indicates that group assignment had no impact on sugar intake. All collected data are summarized are Table 5.

Deprivation Effect

At the end of Phase II, sucrose access was withdrawn and restored after a period of 3 (SAb) or 9 (LAb) abstinence days, illustrated in Figure 8. The two NAb groups continued to receive alternate day access. Comparing sucrose solution consumption during the last pre- and first post-abstinence day in the SAb groups

(days 53 and 57), there was an effect of Access [$F(1,12) = 5.85$, $p < 0.05$], but not Days, [$F(1,24) = 0.001$, $p > 0.05$]. The interaction effect approached significance, [$F(1,12) = 4.726$, $p = 0.05$]. This interaction appeared to reflect a small intake decrease by DisA rats and a small increase by ConA rats over this period. The same analysis for the LAb group (days 53 and 63) yielded an effect of Access [$F(1,12) = 6.70$, $p < 0.05$] but not Days [$F(1,24) = 0.029$, $p > 0.05$]. The lack of Days effect indicates an absence of a sucrose DE for both LAb and SAb conditions. The significant Access effect indicates that access-induced differences were maintained after both short and long abstinence periods.

For the SAb group which was compared with the NAb group on common saccharin days, over the first four post-abstinence sucrose days (days 57, 59, 61 and 63) there was a main effect of Access [$F(1,24) = 8.08$, $p < 0.001$] but not Group [$F(1,24) = 0.35$, $p > 0.05$] or Access by Group interaction [$F(1,24) = 0.24$, $p > 0.05$]. Similarly, over the middle four post-abstinence exposures (days 65, 67, 69 and 71), there was only a main effect of Access [$F(1,24) = 5.55$, $p < 0.05$]. Over the last four exposures (days 73, 75, 77 and 79), there again was a only main effect of Access [$F(1,24) = 7.03$, $p < 0.05$]. Thus although SAb ConA rats may have consumed slightly more sucrose during the post-abstinence period than NAb ConA rats, this effect was not significant.

The situation was similar for the LAb group and during the first four post-abstinence sucrose days (days 63, 65, 67 and 69) there was only a main effect of Access [$F(1,24) = 10.86$, $p < 0.01$]. There was however, a Days by Group interaction [$F(3,72) = 5.08$, $p < 0.01$] which appeared to be due to increasing

consumption by both LAb DisA and LAb ConA rats after abstinence relative to the NAb groups. It was explored further in a Group (LgA, NcR) by Access ANOVA for the first post-abstinence day (day 63). There was only an effect of Access [$F(1,24) = 13.57$ $p < 0.001$] indicating that the Days by Group interaction was not due to a DE on the first post abstinence day. Over the middle four post abstinence days (days 71, 73, 75 and 77) there was only a main effect of Access [$F(1,24) = 13.909$, $p < 0.001$]. Finally, over the last four post-abstinence exposures, there was again only a main effect of Access [$F(1,24) = 15.05$, $p < 0.05$]. Overall, although there appeared to be a trend of increased consumption by SAb ConA and LAb DisA rats in Figure 8, these effects were not large and never reached significance. The effect of previous access history was maintained throughout the duration of the post-abstinence period. All collected data are summarized are Table 5.

Discussion

Phase I and II are in agreement with previous findings from our laboratory using male rats (Hewitt & Eikelboom, 2008). Once every fourth day DisA to 4% sucrose resulted in significantly higher solution intake than ConA. In Phase II, under equivalent access conditions, access-induced changes were maintained. Therefore, female rats readily express the DisA/ConA consumption effects previously described for males.

Post-abstinence sucrose solution consumption was not affected by either short or long periods of abstinence. This is in contrast to elevated post-abstinence consumption following 8 days of saccharin abstinence by both ConA

and DisA history rats in Experiment 2b as well as previous reports describing a saccharin DE (Gandelman & Trowill, 1969; Neznanova, Zvartau & Bespalov, 2002) but consistent with a lack of evidence for a similar consumption increase following sucrose abstinence (Ashton & Trowill, 1970; Ashton, Gandelman & Trowill, 1970).These findings are discussed further in the general discussion.

General Discussion

In addition to replicating previous results, several new findings are documented in this thesis. First, Experiments 1 and 2 demonstrated that DisA, relative to ConA to an optional sweet solution results in intake increases even when that solution is non-nutritive saccharin. Therefore, access-induced consumption changes appear to be driven predominantly by taste in the absence of the positive reinforcing postingestive factors of sucrose (de Araujo *et al.*, 2008; Sclafani & Ackroff, 2004). Experiment 2 showed that even with saccharin, once the DisA/ConA difference is established, it can persist after both access schedules are switched to a common alternate day access. More saccharin in Experiment 2 (and sucrose in Experiment 3) was consumed by DisA than ConA rats during the extended equivalent access period. Further underscoring the importance of taste, Experiment 2c showed that access-induced differences could transfer from saccharin to sucrose solutions. Experiment 2c also extended the duration over which access history consumption effects are known to persist; even after 55 days of equal access rats that had initially received DisA consumed more sucrose solution than rats with a ConA history.

Experiments 2b and 3 examined the influence on consumption of a single longer abstinence period in groups with a DisA or ConA sweet solution history. In Experiment 2b, 8 days of saccharin abstinence resulted in a robust but transient saccharin intake elevation, i.e. a DE. The preservation of earlier access-induced intake differences following abstinence suggests that the effect of a single abstinence period on consumption is not an extension of DisA/ConA induced

differences. In contrast, Experiment 3 failed to find a DE with 4% sucrose after either 3 or 9 abstinence days. That saccharin and sucrose support a DisA/ConA difference but only saccharin supports DE expression provides further evidence for a difference between the two consumption effects. Because sucrose, but not saccharin intake is limited by calories (Smith & Sclafani, 2002) the sucrose DE may have been suppressed by postingestive satiety factors.

This work shows that access variables play an important role in consumption. Repeated intermittent abstinence (Variable 1) can lead to sustained and long term intake increases (p. 11). On the other hand, a single long abstinence period (Variable 2) may cause a transient intake increase that is different from that induced by Variable 1. The quality of the ingestant (Variable 3) appears to interact with both variables. Whereas sweet taste is sufficient for access difference to emerge with Variable 1, postingestive consequences may have had a largely inhibitory effect on Variable 2. This is largely consistent with the idea that orosensory properties stimulate intake of palatable foods whereas postingestive factors often act to limit their consumption (Sclafani & Ackroff, 2004; Smith, 2000).

Relation to previous work

Our laboratory has been examining the influence of different access schedules on liquid sucrose intake. The finding which provided the impetus the present thesis was that consumption of sucrose solutions increases dramatically with DisA relative to ConA (Hewitt & Eikelboom, 2008). This difference was found to persist when rats with different access histories were switched to equivalent,

alternate day access schedules. The first prediction tested in Experiments 1 and 2a was that DisA/ConA access-induced differences could be induced and maintained by sweet taste alone in the absence of reinforcing postingestive factors. Both Experiments 1 and 2 confirm that DisA versus ConA to saccharin solutions induce large intake differences. Experiment 2a showed that as for sucrose (Hewitt & Eikelboom, 2008), once saccharin consumption differences are established, they can be maintained under equivalent access schedules. Moreover, in Experiment 2c consumption changes induced by DisA/ConA to one sweet taste, saccharin, could generalize to another sweet taste, sucrose. Collectively, these findings demonstrate that access history can significantly affect current and future consumption by the influence of sweet taste alone.

These finding also conform to early studies that examined saccharin during intermittent access. First, Pinel and Huang (1976) reported that repeating alternate day 24 h saccharin access resulted in an increased saccharin preference (relative to water which was always available in a second bottle) for a high saccharin concentration (1.5%). Second, Wayner et al. (1972) noted that four rats presented with a low concentration of saccharin (0.05%) for 2 days on and 1 day off tended to increase their saccharin intake but their results were not supported by statistics.

A second finding reported by our lab was that the DisA/ConA effect was most robust for a 4% sucrose solution but less apparent or absent at higher (8 and 16%) and lower (1%) concentrations (Hewitt & Eikelboom, 2008). The lack of access-induced difference for the 1% solution is probably due to it being less

attractive than a 4% solution (Smith, 2000; Young & Greeene, 1953). Because saccharin is generally less preferred than sucrose (Collier & Novell, 1967; Young & Madsen, 1963), small or non-existent DisA/ConA with low sucrose concentrations are also consistent with smaller saccharin intake differences in Experiments 1 and 2.

Although in choice tests more concentrated sucrose solutions are always preferred, the amount of solution that can be ingested over a period of time is limited by its postingestive effects which increase with concentration (Collier & Bolles, 1968; Smith & Sclafani, 2002). Therefore, more satiating postingestive consequences of more concentrated solutions may have masked DisA/ConA differences for 8 and 16% sucrose solutions. This agrees with findings showing that 2 hour MWF relative to 2 hour daily access results in increased intake of 3.2% and 10% but not 32% sucrose solutions (Wojnicki, Stine & Corwin, 2007) and 3.2% and 10% but not 32% sucrose/fat mixtures (Wong, Wojnicki & Corwin, 2009).

For saccharin, both preference and intake decrease at higher concentrations but this appears to be determined primarily by increasing salience of its bitter, quinine-like aftertaste (Dess, 1993; Smith & Sclafani, 2002). Therefore, a second prediction tested in Experiment 1 was that the DisA/ConA intake effect would be evident across a range of saccharin concentrations due to its relative lack of post-ingestive satiating consequences. That this was confirmed adds to the idea that with higher sucrose concentrations postingestive inhibitory factors may mask access-induced differences.

Although DisA/ConA consumption differences may not be evident with higher sucrose concentrations, previous work in our laboratory has shown that this may only mask rather than abolish the effect of access (Adams and Eikelboom, Unpublished). When rats were given DisA/ConA to a 16% sucrose solution, intake differences were small or absent. However, when all rats were subsequently switched to alternate day exposures and the concentration dropped from 16 to 4%, consumption differences readily emerged with DisA history rats consuming more than those with a ConA history. Therefore, although the effect of DisA access may have been masked with more calorie-laden solutions, it was revealed by switching to a lower concentration.

Experiment 2 also showed that the impact of access history persists over a relatively long duration. After 45 days of initial ConA/DisA in Phase I, access history effects did not dissipate even after 55 days of equal access. That is, even though all rats were provided with access to the same solutions on the same schedule, rats with a DisA history continued to consume more than those with ConA history.

Taken together, these results show that taste can drive access-induced consumption changes that are long-term and durable. This is important because like taste, postingestive consequences alone can be rewarding (de Araujo *et al.*, 2008; Sclafani, 2001; Sclafani & Ackroff, 2004). However, these findings do not exclude a potential role for postingestive processes in inducing access differences which may function independently of taste. Further studies might address this issue by employing intragastric infusions bypassing orosensory

stimulation or by utilizing taste-blind animals.

Experiments 2b and 3 explored consumption effects of a longer isolated period of abstinence relative to the short repeating periods as in the DisA schedule. In agreement with previous work (Gandelman & Trowill, 1969; Neznanova, Zvartau & Bespalov, 2002), both access history groups displayed a robust saccharin DE after 8 days of saccharin abstinence. The size of the post-abstinence intake increase relative to the last pre-abstinence day was similar for both DisA and ConA rats while the access-induced difference evident during the alternate day access phase was maintained. This DisA/ConA difference was also maintained on subsequent alternate day access exposures as saccharin intake decreased to pre-abstinence levels. In other words, while access-induced changes persisted after access to saccharin was restored, the access history did not impact the relative size of the saccharin DE.

Experiment 3 was designed to expand this finding to sucrose, but contrary to the predicted outcome and in contrast to Experiment 2b, a DE was not observed when 4% sucrose was utilized and there was no evidence for increased consumption after either 3 or 9 days of abstinence. This was surprising because a 4% solution is significantly more attractive than a 0.25% saccharin solution.

Several possible explanations for the failure to find a sucrose DE can be ruled out. First, although the alternate day equal access phase of Experiment 3 (8 days) which preceded the longer abstinence was shorter than for Experiment 2 (24 days), in both cases solution intake was stable. Second, although female

rats were used in Experiment 3 and males for Experiment 2, previous work has shown that both male and female rats express a saccharin but not a sucrose DE (Ashton, Gandelman & Trowill, 1970; Dube, Ashton & Trowill, 1970). Third, although a ceiling effect might explain the lack of DE for DisA rats, it does not account for its absence in ConA animals which consumed less sucrose solution overall and could have consumed more as evidenced by DisA rats consumption. Fourth, because the DE was only observed with saccharin it is possible that motivation to obtain saccharin but not sucrose increases after abstinence. However, there are a number of operant studies that suggest increased motivation for sucrose after periods of abstinence. Using a standard operant procedure, rats were found to increase responding for sucrose after being returned to operant chambers following 14 days of sucrose abstinence (Avena, Long & Hoebel, 2005) suggesting a higher motivation for sucrose. Moreover, the reinstatement model which is often employed to study craving (Shaham *et al.*, 2003) has also provided evidence for increasing motivation to obtain sucrose during abstinence (Grimm, Fyall & Osincup, 2005; Grimm *et al.*, 2003; Grimm, Shaham & Hope, 2002; Lu, Grimm, Hope & Shaham, 2004). In these studies, initial lever presses resulted in 10% liquid sucrose delivery paired with a tone-light stimulus. Following a period of abstinence, responding was measured again during an extinction test (no stimulus or sucrose) and a subsequent reinstatement test (reinforced by the tone-light stimulus only). Responding during both extinction and reinstatement tests was found to increase with abstinence duration, peaking after approximately one month (Grimm, Fyall & Osincup, 2005;

Lu, Grimm, Hope & Shaham, 2004). This increasing responding was taken to index increasing craving. Therefore these findings can be taken to suggest that sucrose craving "incubates" during abstinence in a manner that may be similar to a saccharin DE. However, because these operant procedures may be different from measuring 24 h *ad lib* intake, it is not clear how DEs and the incubation of craving are related.

Only one study compared post-abstinence extinction and reinstatement responding with post-abstinence *ad lib* intake (Grimm, Fyall & Osincup, 2005). Rats consumed more 10% sucrose solution in their home cage after 7 days (relative to 1 or 30 days) of sucrose absence suggesting a possible sucrose DE. However, these results may be problematic for several reasons. First, the saccharin DE has been shown to be dependent on context, and is not expressed when the post-abstinence environment is distinct from the pre-abstinence environment (Neznanova, Zvartau & Bespalov, 2002). In the case of Grimm et al. (2005), rats were trained to respond for sucrose in an operant chamber, whereas *ad lib* access following abstinence occurred in the animal's home cage. Second, groups were not divided on the basis of sucrose consumption which is highly variable between rats. Taking these issues into consideration along with the fact that a sucrose DE was not observed in Experiment 3 after a comparable abstinence duration, nor by others (Ashton & Trowill, 1970; Ashton, Gandelman & Trowill, 1970) suggests that findings reported by Grimim et al. (2005) were anomalous. Therefore, although evidence suggests that sucrose craving increases with abstinence it does not necessarily translate to increased post-

abstinence consumption and other factors must account for the discrepancy between Experiments 2b and 3.

All studies suggesting increasing motivation for sucrose with abstinence utilized operant procedures during which sucrose access was limited (Avena, Long & Hoebel, 2005), or not available (Grimm, Fyall & Osincup, 2005; Grimm *et al.*, 2003; Grimm, Shaham & Hope, 2002; Lu, Grimm, Hope & Shaham, 2004). On the other hand, studies utilizing non-operant *ad lib* access, as in Experiment 3, have consistently failed to observe a sucrose DE (Ashton & Trowill, 1970; Ashton, Gandelman & Trowill, 1970). This is despite the fact that a saccharin DE was observed in Experiment 2b and in previous studies (Gandelman & Trowill, 1969; Neznanova, Zvartau & Bespalov, 2002; Sinclair & Li, 1989). As operant procedures limit or eliminate sucrose intake, they also limit the postingestive feedback which inhibits sucrose intake, i.e. satiety. Inhibitory postingestive feedback exerts stronger limiting control over sucrose than saccharin consumption (Smith, 2000; Smith & Sclafani, 2002) so differences in satiety induced by the two solutions could account for the discrepant outcomes of Experiments 2b and 3.

If this is indeed the case, further support would be provided for a distinction between DE and DisA/ConA consumption effects suggesting that the former is more liable to suppressing postingestive influences. Whereas the shortest isolated access interruption reported to result in a saccharin DE intake increase was 3 days (Neznanova, Zvartau & Bespalov, 2002), no consumption increases were observed with sucrose after 3 or 9 days in Experiment 3. On the

other hand, experiments reported here and by others (Hewitt & Eikelboom, 2008; Pinel & Huang, 1976) showed that repeated 1, 2 or 3 day abstinence periods produce robust intake increases of both saccharin and sucrose solutions.

Suppression of sucrose DE by satiety is also consistent with its limiting effect on consumption of higher sucrose concentrations despite their being preferred to lower ones (Collier & Bolles, 1968; Smith & Sclafani, 2002) and that DisA/ConA differences for sucrose solutions above 4% were suppressed by satiety as proposed earlier (p. 54). Inhibitory postingestive feedback appears to be mediated by both stomach distension and by nutritive information presumably transduced by chemoreceptors in the intestine (Powley & Phillips, 2004). Vagal afferents innervating the stomach and intestine project to the nucleus of the solitary tract which also receives orosensory input. This nucleus can serve as a site that could integrate orosensory and postingestive feedback to control feeding (Smith, 2000).

The influence of satiety on the DE can be addressed in future studies. For example, if a 4% sucrose solution is too satiating to produce a DE over 24 h, then a weaker, less calorie-dense solution may not be. In addition, taking finer resolution measurements of consumption, comparing individual licks or intake over a shorter time frame following onset of post-abstinence access may reveal an initial DE that is masked over the course of 24 hours. Alternatively, postingestive feedback could be limited by exploring the DE in sham feeding preparation.

Role of access variables

The introduction identified access variables that influence how food is consumed (p. 11). The current data underscores that intermittent access schedules can lead to a sustained intake escalation (Variable 1). The results are consistent with previous findings showing that intermittent access to optional fat or sugar can lead to elevated levels of consumption (Avena, Rada & Hoebel, 2008; Corwin, 2006; Hewitt & Eikelboom, 2008; Wojnicki, Stine & Corwin, 2007). It also agrees with previous findings showing that intermittent access history can significantly impact consumption even after access schedules are made equivalent (Hewitt & Eikelboom, 2008).

Isolated periods of abstinence (Variable 2) appear to produce transient consumption increases which Experiment 2b and 3 suggest may be different from access-induced consumption changes induced by DisA/ConA. Although increasing duration of abstinence probably results in increased craving (Grimm, Fyall & Osincup, 2005; Spanagel & Holter, 1999) Experiment 3 shows that this does not always result in increased intake. This suggests that postingestive and taste factors (quality; Variable 3) are a determining properties which interact with Variables 1 and 2 to determine how much or little is consumed. Not only does it appear that taste is predominantly responsible for access-induced consumption changes with Variable 1, but that they can also be maintained by taste in the absence of calories. Postingestive effects on the other hand may suppress access-induced changes by Variables 1 and 2, although isolated periods of abstinence appear to be more liable.

Previous work has examined the number of access sessions (Variable 4)

that are required for establishing a persistent DisA/ConA difference. With eight or twelve DisA exposures every third or fourth day and 29 days of ConA or more to sucrose, differences induced by access persist during alternate day access exposures, but not after only four DisA exposures relative to 10 days of ConA (Hewitt & Eikelboom, 2008).

Combined, this work shows that relatively simple manipulations can have long term effects on consumption. Intake differences after 45 days of ConA/DisA to saccharin persisted over 55 days of equal access without any indication of dissipating. That these variables affect intake is interesting because previous research has shown that similar manipulations may contribute to escalating drug intake and the development of drug addiction.

Access Variables and Excessive Intake of Food and Drugs

The outlined access variables may also exert similar control over drug self-administration. Alcohol studies methodologically similar to Experiments 1-3 and previous sucrose work (Hewitt & Eikelboom, 2008) have also yielded similar results (Pinel & Huang, 1976; Sinclair & Senter, 1967; Wise, 1973). Moreover, escalating intake of rewarding drugs is concomitant with certain behaviours characteristic of addiction and similar behaviours may emerge with escalating intake of palatable foods. This suggests that common phenomena are involved in excessive food and drug intake and implies overlapping neural substrates.

Access variables and alcohol intake

A number of studies have found that, as for sweet solutions, intermittent ethanol access can lead to a large and sustained intake increases (Pinel &

Huang, 1976; Simms, Bito-Onon, Chatterjee & Bartlett, 2010; Simms *et al.*, 2008; Spanagel *et al.*, 1996; Wayner *et al.*, 1972; Wise, 1973). Alternate day 24 hour periods of 20% ethanol access result in escalating intake whereas continuous access to the same solution results in low, stable levels of intake (Wise, 1973). Such large intake increases with DisA are particularly striking as it is notoriously difficult to induce ethanol drinking at appreciable levels in rats without the aid of additional methods such as sucrose fading (Samson, 1986).

Escalating ethanol intake with intermittent access may be influenced by the inter-session interval which appears to interact with the concentration of the solution. Whereas intake of 20% ethanol increased with the duration of the inter-exposure interval for up to 5 days (Holloway, Bird & Devenport, 1984) with lower 10 or 7% concentrations, the increase became asymptotic with 1 day inter-session intervals (Sinclair & Bender, 1979). Interestingly, this suggests that longer inter-session intervals could yield larger consumption changes for higher sucrose (>4%) or saccharin concentrations.

The alcohol DE is a transient intake increase that follows isolated access interruptions (Heyser, Schulteis & Koob, 1997; Holter *et al.*, 1998; LeMagnen, 1960; Spanagel & Holter, 1999). As with saccharin, the alcohol DE increase as a function of abstinence duration (Neznanova, Zvartau & Bespalov, 2002; Sinclair, Walker & Jordan, 1973) and has proved so robust that a simple mathematical equation has been proposed to model it (Sinclair, 1979; Sinclair & Li, 1989).

Finally long term access (Variable 4) has been argued to lead to loss of control over drug taking (Wolffgramm & Heyne, 1995; Wolffgramm, Galli, Thimm

& Heyne, 2000). For example, drinking of ethanol solutions is less inhibited by quinine adulteration after long but not short exposure (Wolffgramm & Heyne, 1991). Similarly, adulterating ethanol with quinine suppresses an alcohol DE in rats with two but not eight months of ethanol experience (Spanagel, Holter, Allingham, Landgraf & Zieglgansberger, 1996), suggesting that with extended access consumption becomes inflexible.

Importantly, the aforementioned alcohol studies utilized procedures very similar to those described in Experiments 1-3 in that alcohol was delivered in a non-operant manner in standard drinking bottles attached to the animal's homes cage. However, merely showing that both alcohol and sweets consumption increases under similar conditions is a relatively crude measure of behaviour. Evidence from a number of self-administration studies suggests that escalating intake may be accompanied by a number of behavioural changes argued to reflect addiction, some of which may also be evident with palatable foods.

Escalating intake and addiction-like behaviour. Rats with intermittent DisA to a palatable food or rewarding drug escalate their intake to unusually high levels. An escalation from low to high levels of drug intake has been argued to reflect the transition from moderate and controlled, to excessive and compulsive consumption (Koob & Le Moal, 2001; Wolffgramm, Galli, Thimm & Heyne, 2000) and has been shown to be concomitant with a number of behaviours thought to be characteristic of addiction (Ahmed, 2005; Wolffgramm & Heyne, 1995; Wolffgramm, Galli, Thimm & Heyne, 2000). Intake of rewarding drugs such as cocaine escalates when it is available intermittently for 6 hour sessions (Ahmed

& Koob, 1998; Ahmed, Walker & Koob, 2000). Although in these studies intermittent access was not the manipulation of interest, daily access sessions were sometimes skipped to combat weight loss (Ahmed & Koob, 1998; Ahmed & Koob, 1999), effectively resulting in a DisA schedule. For instance, in the study by Ahmed and Koob (1999) escalating intake was observed over 48 access sessions spaced over 100 days.

Rats that escalate cocaine self-administration also display increased motivation for the drug as reflected by higher breakpoints on a progressive ratio schedule (Paterson & Markou, 2003), are more resistant to extinction, more prone to reinstatement of drug seeking behaviour (Ahmed & Cador, 2006; Ahmed, Walker & Koob, 2000) and persist in drug-seeking in the face of aversive consequences (Vanderschuren & Everitt, 2004). These are behaviours thought to parallel characteristics of human drug addiction (Deroche-Gamonet, Belin & Piazza, 2004). Similar behavioural changes have been found to emerge with escalating intermittent intake of palatable foods. Rats with MWF access had higher breakpoints for vegetable shortening relative to animals with everyday access (Wojnicki, Babbs & Corwin, 2010). Additionally, rats with DisA, relative to ConA sucrose solution experience preferred their sucrose solution more in a preference test (relative to sweet, Kool-Aid flavoured solutions; Adams and Eikelboom, Unpublished). Although these findings suggest that escalating food intake with intermittent access is concomitant with an increased motivation for food, evidence is limited and more studies examining these changes would be useful.

Relevance to BN and BED

BN and BED are both characterized by intermittent bouts of gorging, or binging on highly palatable foods, but BN is also accompanied by inappropriate purging behaviour (American Psychiatric Association, 2000). A binge is defined as "eating in a discrete time period, an amount of food that is definitely larger than what most people would eat in a similar time period under similar circumstances" (American Psychiatric Association, 2000). The forbidden food hypothesis suggests that foods consumed during binges tend to be designated as "forbidden", ones to which the patient has self-restricted their access (Kales, 1990). In the DisA/ConA, MWF, and cyclic access models, rats which receive limited access to fats or sweets engage in longer/larger bouts of eating/drinking and consume more during a discrete time period than animals with less limited or *ad lib* ConA (Avena, Rada & Hoebel, 2008; Corwin *et al.*, 1998; Hewitt & Eikelboom, 2008; Wojnicki, Stine & Corwin, 2007). This repetitive overeating corresponds to the definition of binging in humans diagnosed with BN or BED. Although in humans consumption is often self-restricted, children who's parents were rated more restrictive with dessert/snack type foods consumed more when given free access to similar foods in an experimental setting (Fisher & Birch, 1999). Therefore, intermittent access protocols may model aspects of binge-like eating, possibly implicating cycles of intermittent gorging and restriction in the development of BN and BED. This is interesting because although binging is associated with dieting there is disagreement about whether dieting causes binging (Grilo & Masheb, 2000). Intermittent access protocols, which like dieting,

restrict access suggest that restrictive eating habits may precede excessive eating.

Criteria for BN and BED also overlap significantly with criteria for addiction (American Psychiatric Association, Diagnostic and statistical manual of mental disorders (4th ed., text rev.)., 2000; Corwin & Grigson, 2009; Epstein & Shaham, 2010) and these disorders are frequently co-expressed (Brewerton *et al.*, 1995; Bulik, Sullivan & Kendler, 2002; Bushnell *et al.*, 1994; Herzog *et al.*, 1992). It is therefore particularly interesting that manipulations that lead to excessive intake of food in rats can also lead to escalating drug consumption, and suggests that common mechanisms lead to excess in both cases. It may therefore be warranted to further explore the relationship between eating disorders and drug abuse.

Concluding Comments

This thesis shows that relatively simple access manipulations can have remarkably large and long term consumption effects. Because the access parameters leading to excessive food or drug intake are similar, a common mechanism may be involved. This is not a radical proposal especially given that both are examples of a behaviour that can be expressed casually and in controlled manner but in certain situations, and for some individuals, can becomes excessive and uncontrolled (American Psychiatric Association, 2000). Additionally, the extensive overlap in neurobiology involved in addiction and eating behaviour supports such a possibility (Figlewicz *et al.*, 2003; Kelley & Berridge, 2002; Lutter & Nestler, 2009; Zheng, Lenard, Shin & Berthoud, 2009).

According to one account, addiction is a result of repeating drug withdrawal that causes a chronic increase in the threshold for activation of reward circuits (Koob & Le Moal, 1997). This elevated threshold leads to increased drug intake as a compensatory measure. Opiate systems are known to be involved in sugar intake (Levine, Kotz & Gosnell, 2003) and precipitated and spontaneous opiate-like withdrawal symptoms have been reported in rats with cyclic sugar experience (Avena, Bocarsly, Rada, Kim & Hoebel, 2008; Colantuoni *et al.*, 2002). This is similar to depression or anxiety like behaviours that are reflective of drug withdrawal but dissipate over 2-6 days (Barr & Markou, 2005). Alternating access between highly palpable chow for 2 days of the week and less palatable standard lab chow on the other 5 may cause increased release of corticotropin releasing factor (Cottone *et al.*, 2009), the same neuropeptide suggested to be involved in the chronic reward deficiency (Koob & Le Moal, 2001). Therefore, one possibility is that repeating DisA to sweet solutions produces intermittent withdrawal that leads to alterations in the reward threshold leading to increased intake similar to those seen with drug consumption. Because a behavioural criterion of BED in humans is feelings of depression or disgust that follow an episode of overeating (American Psychiatric Association, 2000), it may be useful to evaluate behavioural measures that suggest withdrawal after intermittent access to palatable foods (Appendix A).

That taste alone might have such profound effects on consumption patterns is not surprising given that the capacity to distinguish food sources high in energy is essential to survival. Animals may have evolved mechanisms that

encourage consumption of infrequently available, high quality resources. On the other hand, environments in which highly palatable food sources are constantly available may not require increased intake. A potential consequence of this for humans is that rich foods coupled with access restrictions (possibly ones that are self-imposed), may lead to aberrant eating patterns such as those manifested in BED or BN.

Appendix A

In humans diagnosed with BN or BED, an episode of binging may be followed by distress or guilt or other aversive affective states (American Psychiatric Association, 2000) and these disorders are often comorbid with depressive and anxiety disorders (Brewerton *et al.*, 1995; Hudson, Hiripi, Pope & Kessler, 2007). According to one account of addiction, drug withdrawal reduces in brain reward area activation and the recruitment of brain stress systems that becomes chronic with frequent drug use (Koob & Le Moal, 2001). The resulting aversive affective state may then drive drug consumption. It is possible that rats binging on food may display behaviours reflecting aversive affective states that are known to follow drug use, such as anxiety (Barr & Markou, 2005).

For rats, a validated operational measures of anxiety is a greater proportion of time spent in closed relative to open arms of an EPM (Pellow, Chopin, File & Briley, 1985). Relative to *ad lib* sucrose and chow rats, after a month of the cyclic sugar diet rats spent more time on the closed arms of the EPM following administration of the opiate antagonist naloxone (Colantuoni *et al.*, 2002), or after a 36 h fast (Avena, Bocarsly, Rada, Kim & Hoebel, 2008). Also after three or four weeks of MWF fat access, mice spent more time in the hide-box during a light/dark emergence test (de Araujo-Held, Martin, de Sousa & Luscher, 2002) which is another validated rodent model of anxiety (Crawley & Goodwin, 1980). Collectively, such findings suggests that animals with intermittent access display increased anxiety and the purpose of Experiment 2b was to test anxiety-like behaviour in DisA and ConA rats on two consecutive

anxiety tests; the EPM, and the light/dark emergence test.

Methods

Apparatus

Animals were tested on the EPM for 300 seconds each. The EPM was constructed of 4 arms at right angles 12 cm wide and 52 cm in length. Two opposing arms were walled by opaque Plexiglas ® 40 cm high and the remaining two arms were open without sides. All arms were joined at a central 12 by 12 cm square platform elevated 53 cm from the ground. The maze floor was removed and washed between test trials. The room was illuminated by one 13 W fluorescent red lamp (2 LUX at apparatus floor level). The rat's performance was recorded by an overhead camera and scored with ANY-maze Video Tracking System software (Stoelting Co., Illinois USA). The animal was considered to have entered or left an arm or the central platform when the central point of the tracked animal passed across one of the boundaries. Scored behaviours included percent number of open arm entries, percent time spent in open arms, and number of entries to closed arms.

The light/dark emergence test was conducted in a room illuminated by one 13 W fluorescent lamp (2 LUX at maze floor level) within an apparatus consisting of a 120 x 120 x 45 cm white melamine enclosed arena and a black ABS plastic floor. A 40 x 24 x 17 cm black melamine hide box was located at the midpoint of the edge at one side of the arena. At the start of each trial, animals were placed in the hide box and activity was recorded by an overhead camera and scored with ANY-maze Video Tracking System software. Subjects were followed for 300

s and considered to have entered or left the hide box when their entire tracked area was in either the arena or the hide box. Scored behaviours included latency to emerge from the hide box and, time spent in the open field.

Procedure

On day 56 of Experiment 2a (Phase II), immediately after daily access to saccharin was withdrawn, animals were tested on the two anxiety measures. Because data collection was staggered across two days, 16 animals were tested across two days. Rats were first tested on the EPM. At the beginning of each test, the rat was placed in the centre facing the open arm furthest away from the experimenter and recording was initiated once the experimenter left the room. After 300 s in the EPM, each rat was returned to their home cage. When EPM testing was completed for all animals, rats were subjected to the light/dark emergence test. Each rat was tested in the light/dark emergence test approximately 1.5 h after completing the EPM.

Statistics

Differences between DisA and ConA rats on the EPM measures (percent time spent in open in open arms, percent of open arm entries) and light/dark emergence test (time spent in open field, number of open-field entries) were analyzed using independent samples *t*-tests. Pearson's correlation coefficients were calculated between day 1 saccharin consumption, average Phase II consumption, and the behavioural measures.

Results

There was no difference between DisA and ConA rats on EPM or

light/dark emergence measures which is illustrated in Figure 9. DisA rats spent an average of 56% of their time in the open arms relative to 54% by ConA rats, [$t(30) = 0.66$, $p > 0.05$]. ConA rats made 56% of their entries into the open arms relative to the 54% by DisA rats, [$t(30) = 0.61$, $p > 0.05$]. Day 1 saccharin consumption by all rats was correlated with percent time spent in open arms, [$r = 0.39$, $p < 0.05$] and percent open arm entries, [$r = 0.47$, $p < 0.01$]. Mean Phase II consumption sucrose intake was not significantly correlated with time spent in open arms, [$r = 0.32$, $p < 0.10$] or percent open arms entries, [$r = 0.33$, $p < 0.10$] although both approached significance.

During the light/dark emergence test, the DisA and ConA rats did not differ in latency to exit the hide box. [$t(30) = 1.5$, $p > 0.05$] or in time in open field, [$t(30) = 0.925$, $p > 0.05$]. Emergence test scores were not correlated with either first day and average Phase II saccharin intake or EPM measures.

Discussion

Anxiety was measured on day 56 of Phase II but no differences in behavioural measures were detected between DisA and ConA rats on any measure reported. It is possible that if tests were conducted following a period of food and saccharin restriction, or after naloxone administration, anxiety differences could be detected as reported previously (Avena, Bocarsly, Rada, Kim & Hoebel, 2008; Colantuoni *et al.*, 2002). Our results also contrast with reported increases in anxiety-like behaviour in mice after three to four weeks of MWF fat access (de Araujo-Held, Martin, de Sousa & Luscher, 2002), although in this case MWF access mice were compared to control animals that had lab chow

but no fat access. Therefore, it is not clear if access history or dietary richness had an effect on anxiety.

Although access history did not seem to have an impact on the anxiety measures, EPM measures were inversely correlated with the day 1 saccharin intake, and approached significance for average intake in Phase II. These results agree with the inverse relationship previously reported between EPM anxiety measures and sugar intake (DeSousa, Wunderlich, De Cabo & Vaccarino, 1998). High sucrose intake has also been previously correlated with a faster acquisition of cocaine and amphetamine self-administration (DeSousa, Bush & Vaccarino, 2000; Gosnell, 2000) and an upward shifted amphetamine self-administration dose-response curve (DeSousa, Bush & Vaccarino, 2000). In future studies, it might be useful to obtain EPM and other behavioural measures prior to sugar exposure that may provide information about subsequent consumption.

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Tables and Figures

Table 1: Summary of Data Collected in Experiment 1

		Food		Weight		Water		Saccharin	
Concentration	**Block**	**DisA**	**ConA**	**DisA**	**ConA**	**DisA**	**ConA**	**DisA**	**ConA**
1.00%	**Baseline**	28.9	28.6	311	308	43.5	44.5	-	-
	Block 1	29.2	29.5	367	365	38.1	38.9	26.3	14.6
	Block 2	30.2	31.0	426	426	36.5	32.7	43.8	23.5
	Block 3	31.2	31.9	482	477	34.8	28.4	48.1	29.1
0.5%	**Baseline**	29.7	28.6	313	307	42.7	39.6	-	-
	Block 1	29.3	29.4	370	361	31.4	12.0	65.9	49.1
	Block 2	30.4	30.8	425	416	32.9	10.7	88.6	56.8
	Block 3	31.5	31.4	473	465	30.8	8.9	92.2	54.4
0.25%	**Baseline**	29.0	28.8	312	310	40.9	41.9	-	-
	Block 1	28.6	28.8	366	358	29.5	7.7	101.9	61.6
	Block 2	29.1	30.5	418	410	30.2	6.5	116.2	65.0
	Block 3	30.0	30.5	462	456	29.8	6.3	113.6	62.0
0.125%	**Baseline**	29.3	28.7	312	306	41.6	43.3	-	-
	Block 1	29.8	30.4	372	357	30.2	4.3	93.2	73.0
	Block 2	31.3	30.9	434	411	32.8	3.3	119.4	77.3
	Block 3	31.5	30.7	484	459	30.4	2.9	113.4	73.9

Note. Food, water and saccharin data are averaged across the 8 day baseline and blocks 1-3 (days 1–10, days 11-22, and days 23-34). Weight data are reported for last baseline and block days. Saccharin was not provided during the baseline period.

Table 2: Summary of Experiment 2 Design

	Days	Experiment	Second Bottle	Second Bottle Access
Baseline	-7 to 0		None	
Phase I	1 to 45	2a	0.25% Saccharin	45 ConA (24 h/day) or 12 DisA (24h/4 days) exposures
Phase II	46 to 69		0.25% Saccharin	12 alternate day exposures
Anxiety Tests	56		N/A	N/A
DE	70 to 85	2b	0.25% Saccharin	8 abstinence days followed by 4 alternate day exposures
Sucrose	86 to 100	2c	4% Sucrose	8 alternate day exposures

Table 3: Summary of Data Collected in Experiment 2

			Experiment 2a		Experiment 2b	Experiment 2c
		Baseline	**Phase I**	**Phase II**	**DE**	**Sucrose**
Weight (g)	DisA	325	468	574	610	635
	ConA	319	455	557	593	615
Food (g)	DisA	31.2	32.5	33.9	33.1	28.7
	ConA	29.8	32.2	32.7	32.2	29.2
Water (g)	DisA	45.1	36.8	27.1	36.1	25.7
	ConA	44.5	5.7	26.3	35.1	24.1
Solution (g)	DisA	-	147.6	128.7	144.1	252.1
	ConA	-	73.1	80.2	96.3	169.2
Kilocalories	DisA	96.7	101.0	105.3	102.5	110.0
	ConA	92.3	100.0	101.4	99.7	104.9

Note. Food, water, solution, and kilocalorie data is averaged for each section. Weight data are reported for last baseline and section days.

Table 4: Summary of Experiment 3 Design

	Days	Second Bottle Access
Baseline	-7 to 0	
Phase I	1 to 45	45 ConA (24 h/day) or 12 DisA (24h/4 days) 4% sucrose exposures
Phase II	46 to 53	4 alternate day 4% sucrose exposures
Abstinence	54 to 63 (LAb) or 54 to 57 (SAb)	Only NAb group continued to receive alternate day 4% sucrose access
Post-Abstinence	63 to 85 (LAb) or 57 to 85 (SAb)	LAb and SAb 4% sucrose access restored for at least 12 more alternate day exposures

Table 5: Summary of Data Collected in Experiment 3

			Baseline	Block 1	Block 2	Block 3	Phase II	Abstinence	Post-abstinence
Weight (g)	DisA	SAb	252	270	289	303	313	317	329
		LAb	255	269	287	301	311	316	325
		NAb	250	267	286	300	310	315/317	325/330
	ConA	SAb	259	274	294	304	309	316	324
		LAb	253	274	290	303	311	317	331
		NAb	250	267	289	299	305	308/309	314/318
Food (g)	DisA	SAb	21.2	18.7	19.3	19.4	17.4	20.0	18.4
		LAb	21.1	18.7	19.3	18.9	17.7	19.8	17.3
		NAb	20.8	18.5	18.7	19.6	17.7	19.0/18.3	18.0/18.0
	ConA	SAb	20.7	17.3	16.8	17.2	18.0	21.6	19.8
		LAb	22.2	17.8	17.2	17.6	18.2	21.4	20.2
		NAb	20.3	17.1	16.6	17.4	18.3	19.3/19.5	19.5/19.5
Water (g)	DisA	SAb	30.4	22.6	25.0	24.8	18.3	31.9	18.9
		LAb	32.7	24.7	27.3	26.6	19.1	31.9	19.1
		NAb	31.6	23.2	26.7	26.3	19.3	29.2/22.6	18.8/18.4
	ConA	SAb	33.2	5.1	4.2	5.7	23.0	38.3	25.7
		LAb	32.6	6.6	5.3	5.2	21.1	34.4	21.7
		NAb	30.9	4.1	4.3	4.0	21.8	26.3/23.5	20.8/20.9
Sucrose (g)	DisA	SAb	-	211.1	269.2	264.2	248.2	-	229.8
		LAb	-	231.2	269.7	265.0	255.4	-	268.6
		NAb	-	220.4	261.6	272.0	251.3	-	218.8/229.1
	ConA	SAb	-	136.7	132.0	116.7	128.3	-	186.6
		LAb	-	115.8	114.8	115.8	115.5	-	158.4
		NAb	-	153.3	143.5	125.2	125.2	-	145.2/151.2
Kilocalories	DisA	SAb	65.8	68.1	70.5	70.0	71.3	62.1	77.3
		LAb	65.6	69.2	70.4	68.3	72.4	61.5	75.6
		NAb	64.4	68.1	68.4	70.8	72.1	72.3/74.2	75.2/74.5
	ConA	SAb	64.2	73.8	72.8	72.9	66.3	67.0	77.4
		LAb	68.8	74.7	72.7	73.3	67.0	66.4	75.6
		NAb	63.1	75.2	74.4	74.2	67.0	68.2/77.7	73.1/72.9

Note. Food, water, solution, and kilocalorie data is averaged for each section. Weight is reported for last baseline and section days. Where necessary, NAb values are reported to correspond with both SAb and LAb values: SAb/LAb.

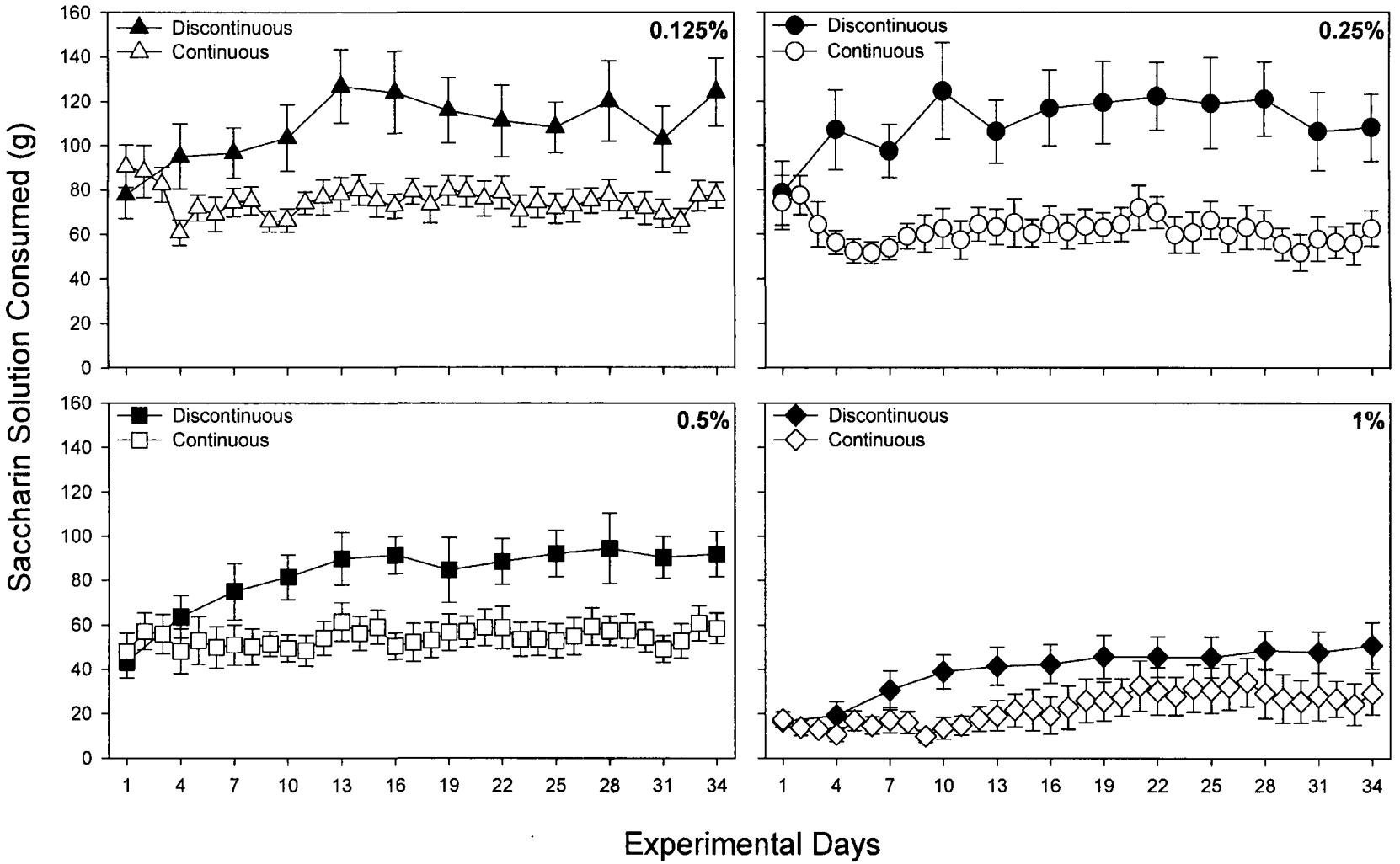

Figure 1: Access-induced saccharin consumption changes by concentration in Experiment 1. Mean (±SEM) daily intake of 0.125, 0.25, 0.5 and 1% saccharin solutions by DisA and ConA rats. Across all concentrations utilized, DisA animals consumed more than ConA animals.

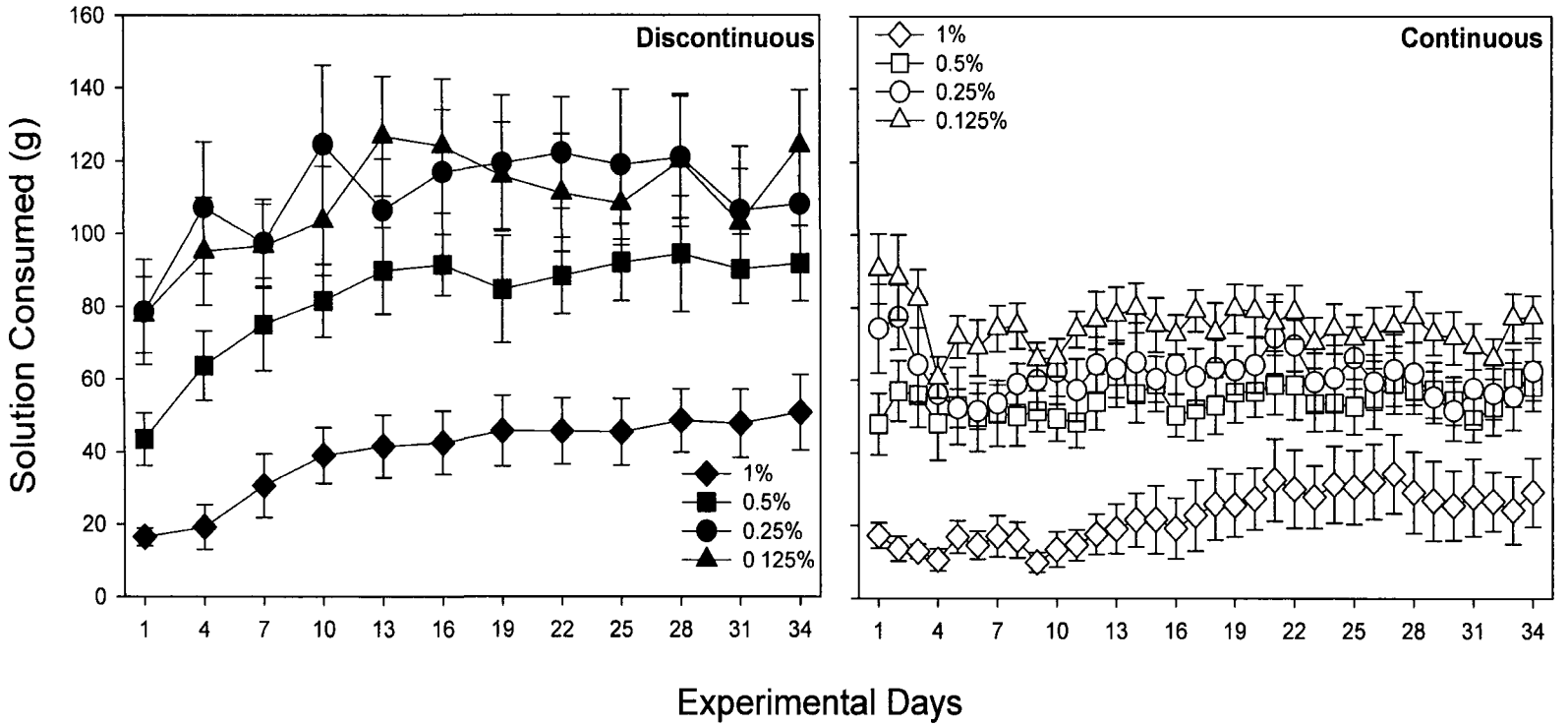

Figure 2: Access-induced saccharin consumption changes by access schedules in Experiment 1. Mean (±SEM) daily intake of 0.125, 0.25, 0.5 and 1% saccharin solutions by DisA and ConA rats. For DisA and ConA schedules saccharin intake differed with saccharin concentration.

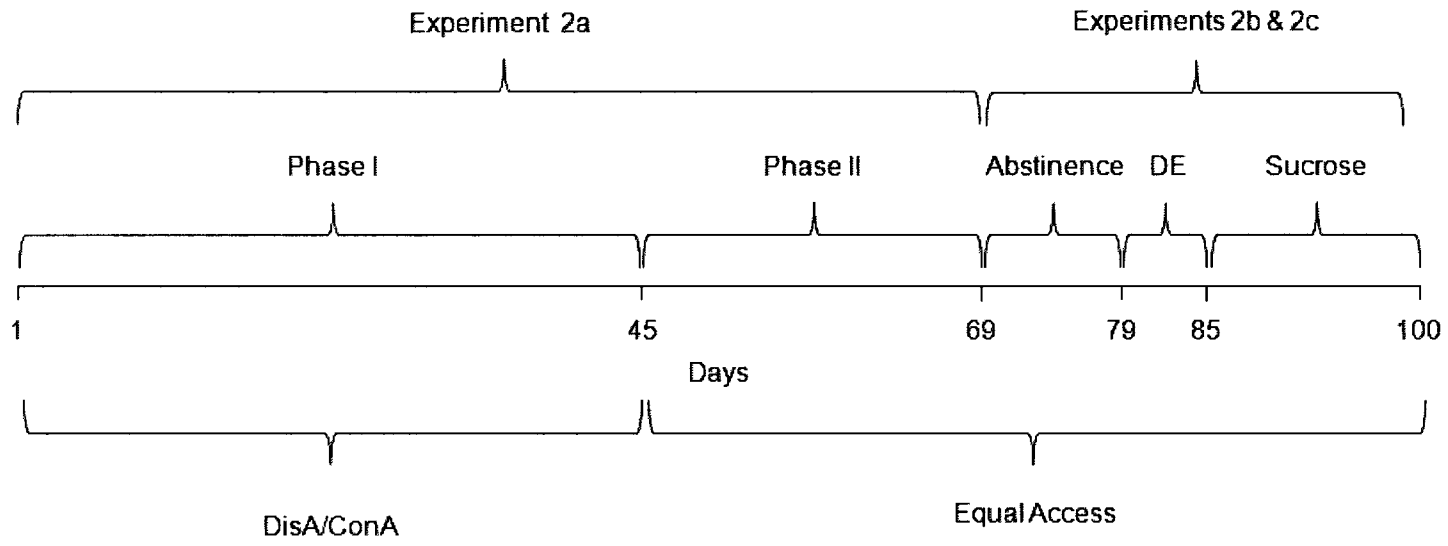

Figure 3: Timeline for Experiment 2. In Phase I of Experiment 2a, DisA and ConA groups were maintained on different access schedules (every fourth day vs. continuous saccharin exposure) for 45 days. During Phase II of Experiment 2a, Experiments 2b, and 2c, both groups received identical treatments.

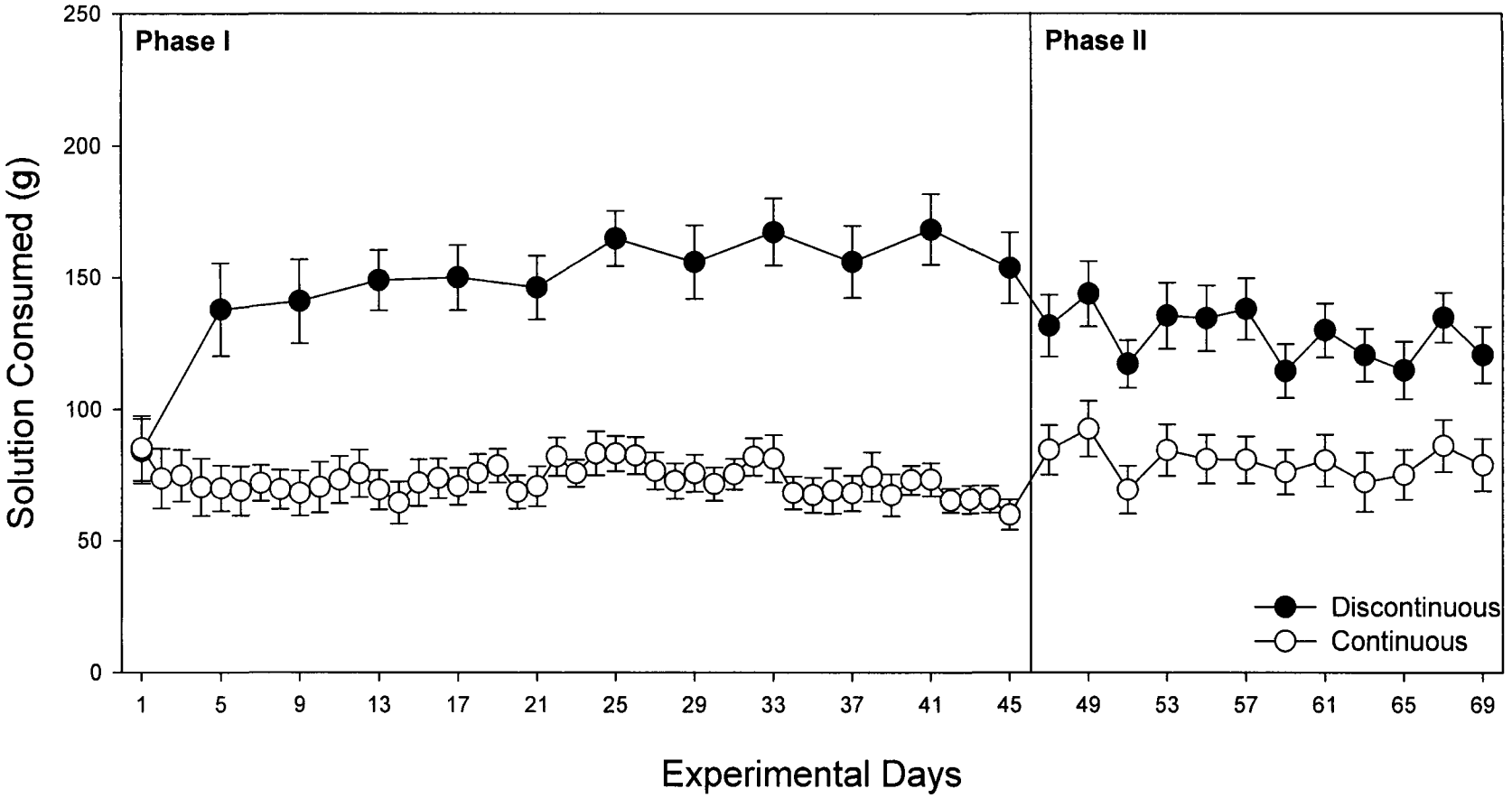

Figure 4: Access-induced saccharin consumption changes in Experiment 2a. Mean (±SEM) daily 0.25% saccharin solution intake by DisA and ConA groups during discontinuous/continuous saccharin access (Phases I) and alternate day saccharin access (Phase II).

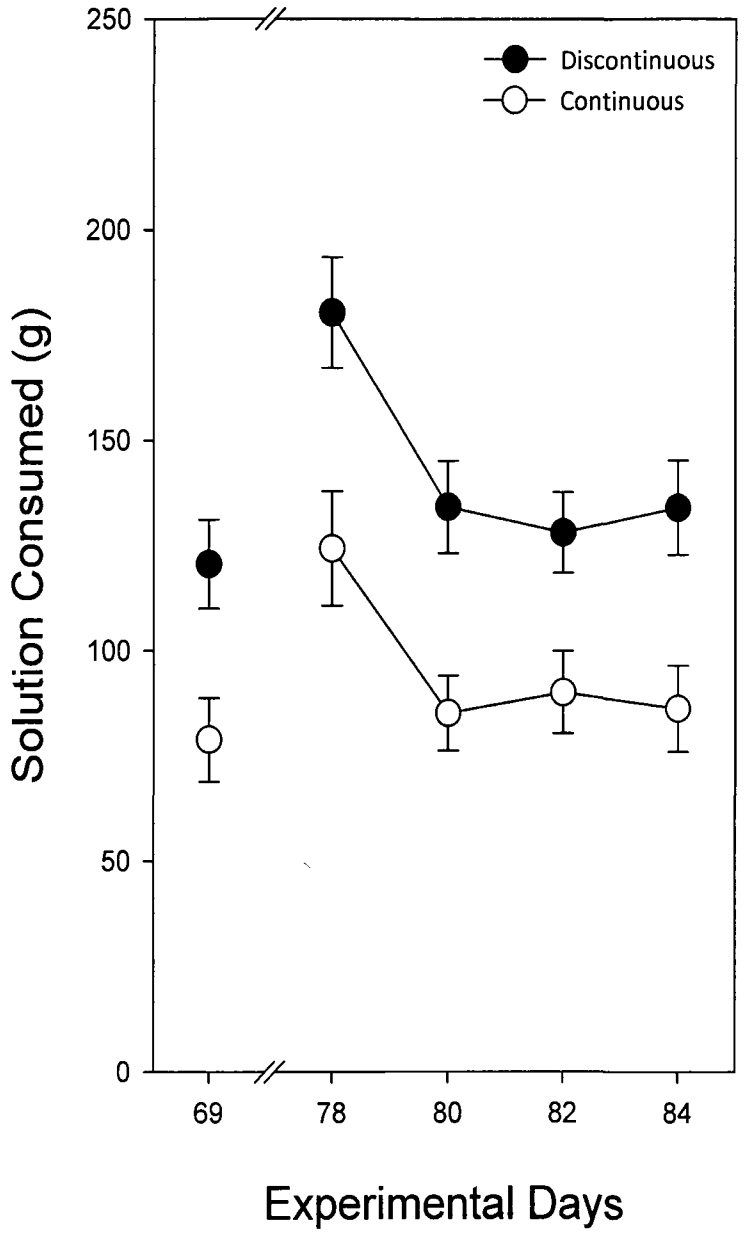

Figure 5: Saccharin DE after DisA/ConA experience in Experiment 2b. Mean (±SEM) daily 0.25% saccharin solution intake before and after 8 days of abstinence.

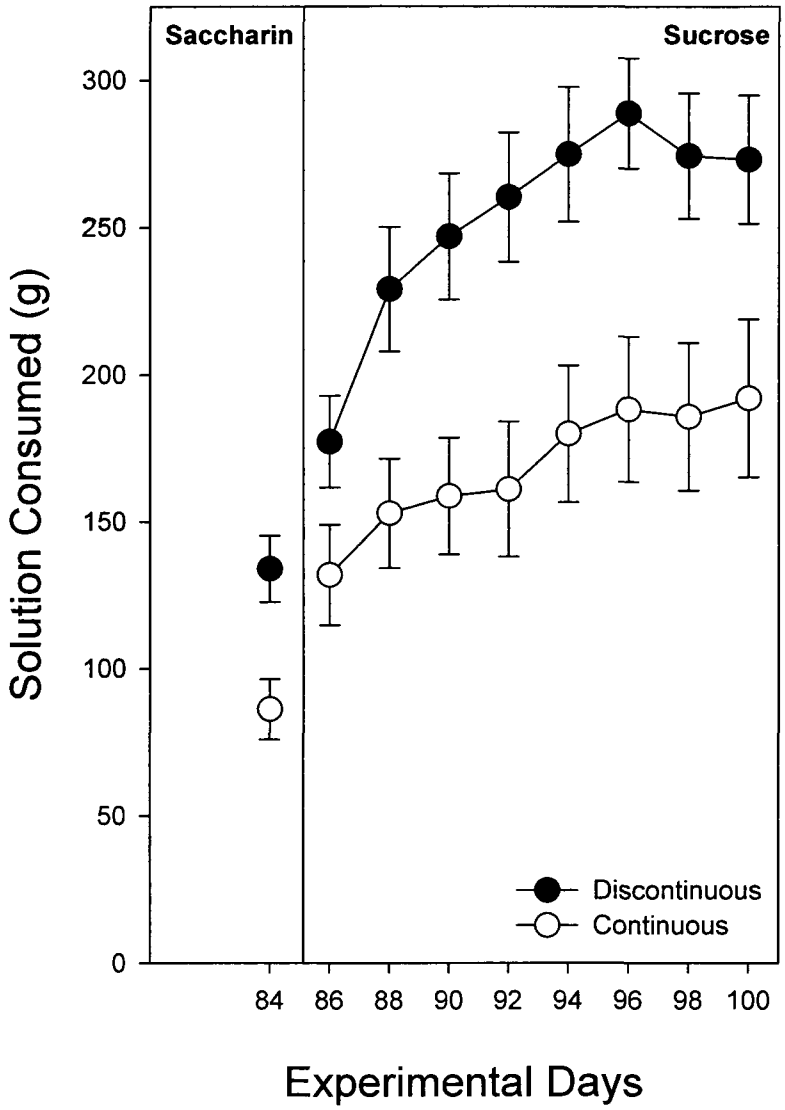

Figure 6: Transfer of access-induced saccharin consumption differences to sucrose in Experiment 2c. Mean (±SEM) daily 0.25% saccharin solution intake on the last saccharin day and 4% sucrose solution intake on sucrose days.

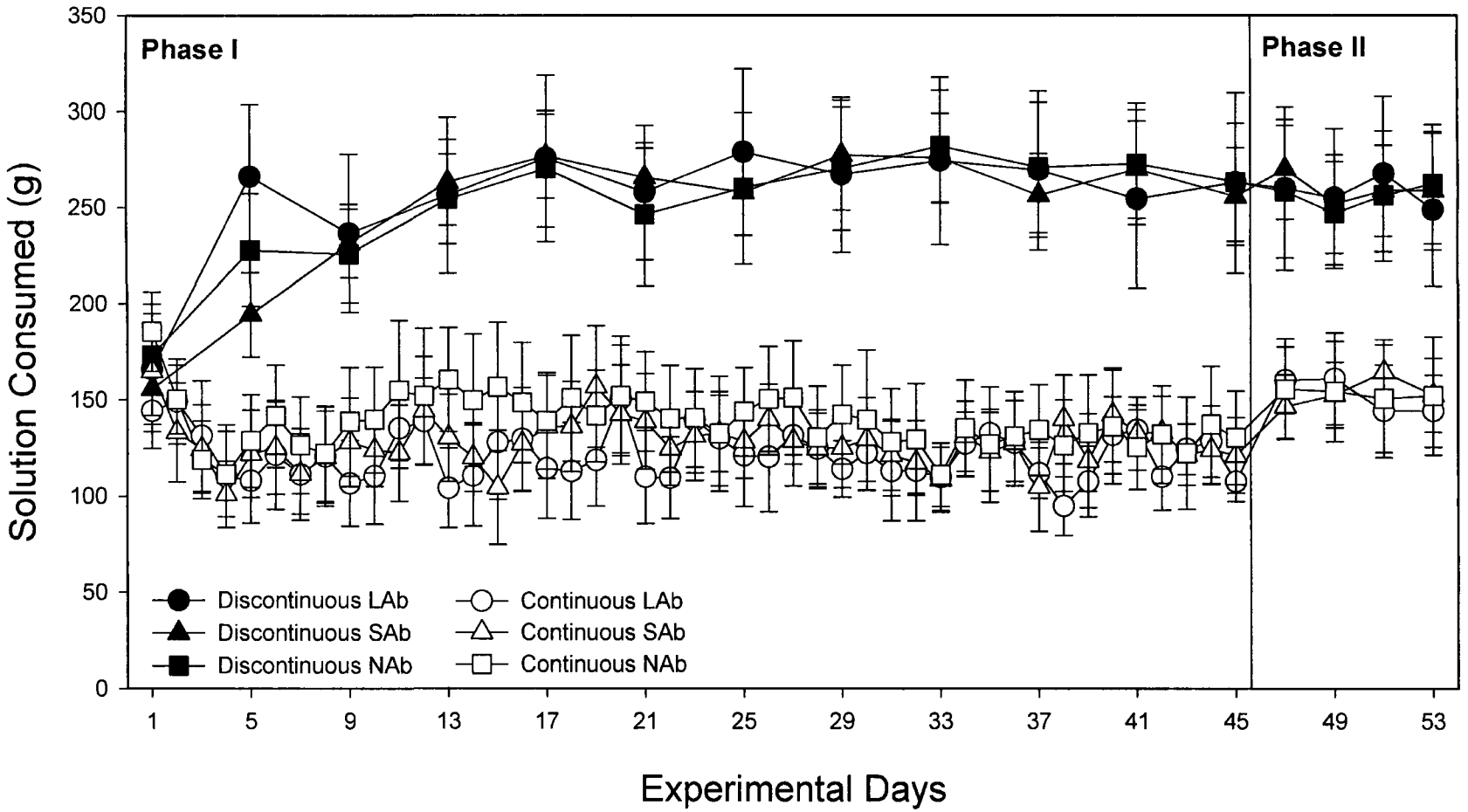

Figure 7: Access-induced sucrose consumption changes in Experiment 3. Daily mean (±SEM) 4% sucrose intake by DisA and ConA and by different abstinence conditions, LAb, SAb and NAb, during discontinuous/continuous sucrose access (Phases I) and alternate day sucrose access (Phase II).

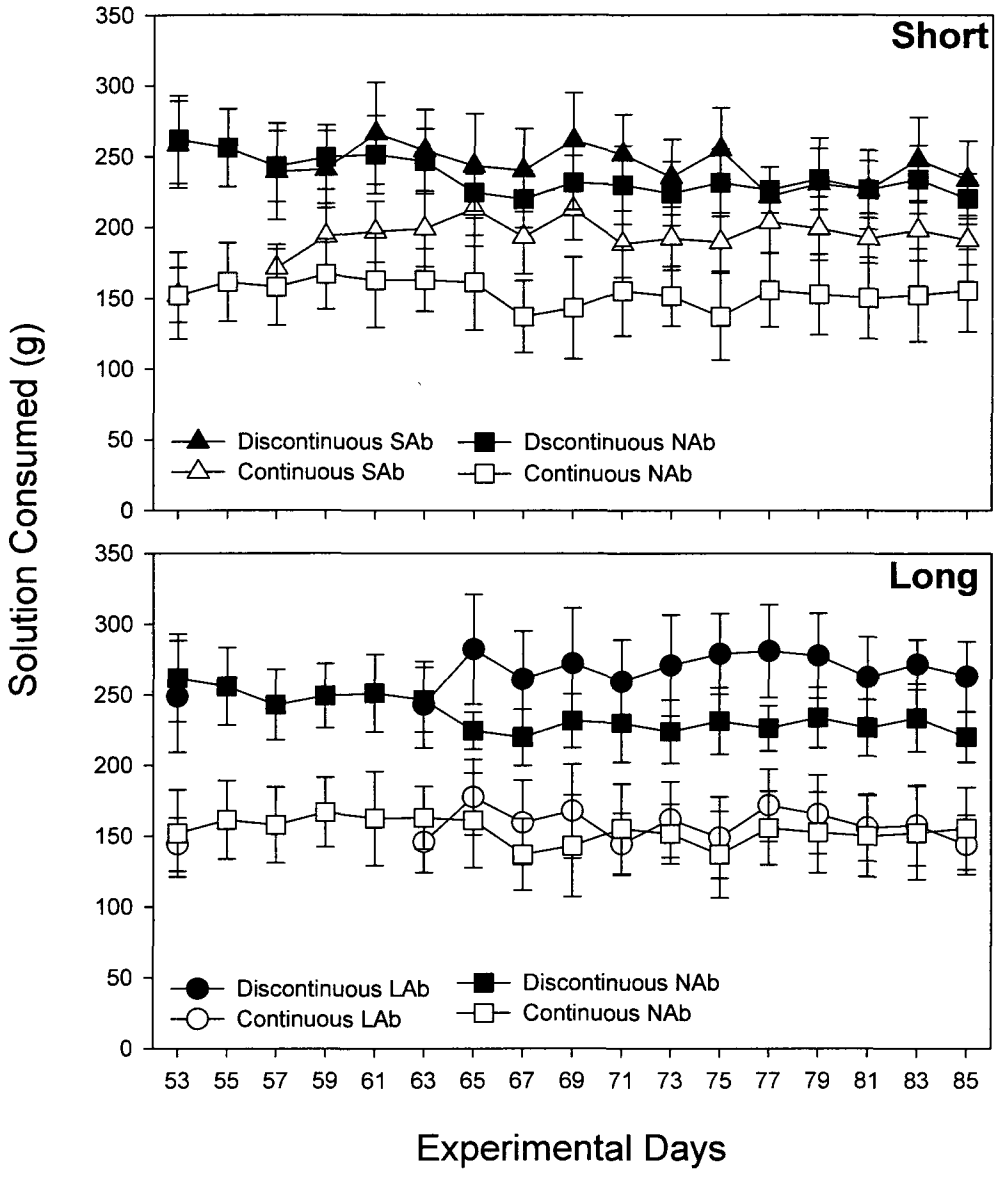

Figure 8: Sucrose intake after ConA or DisA experience by abstinence group in Experiment 3. Mean (±SEM) daily 4% sucrose solution intake by rats with DisA and ConA histories on the last pre-abstinence day (day 53) and after three day SAb (top panel) or nine day LAb periods (bottom panel) relative to NAb intake (identical in both panels).

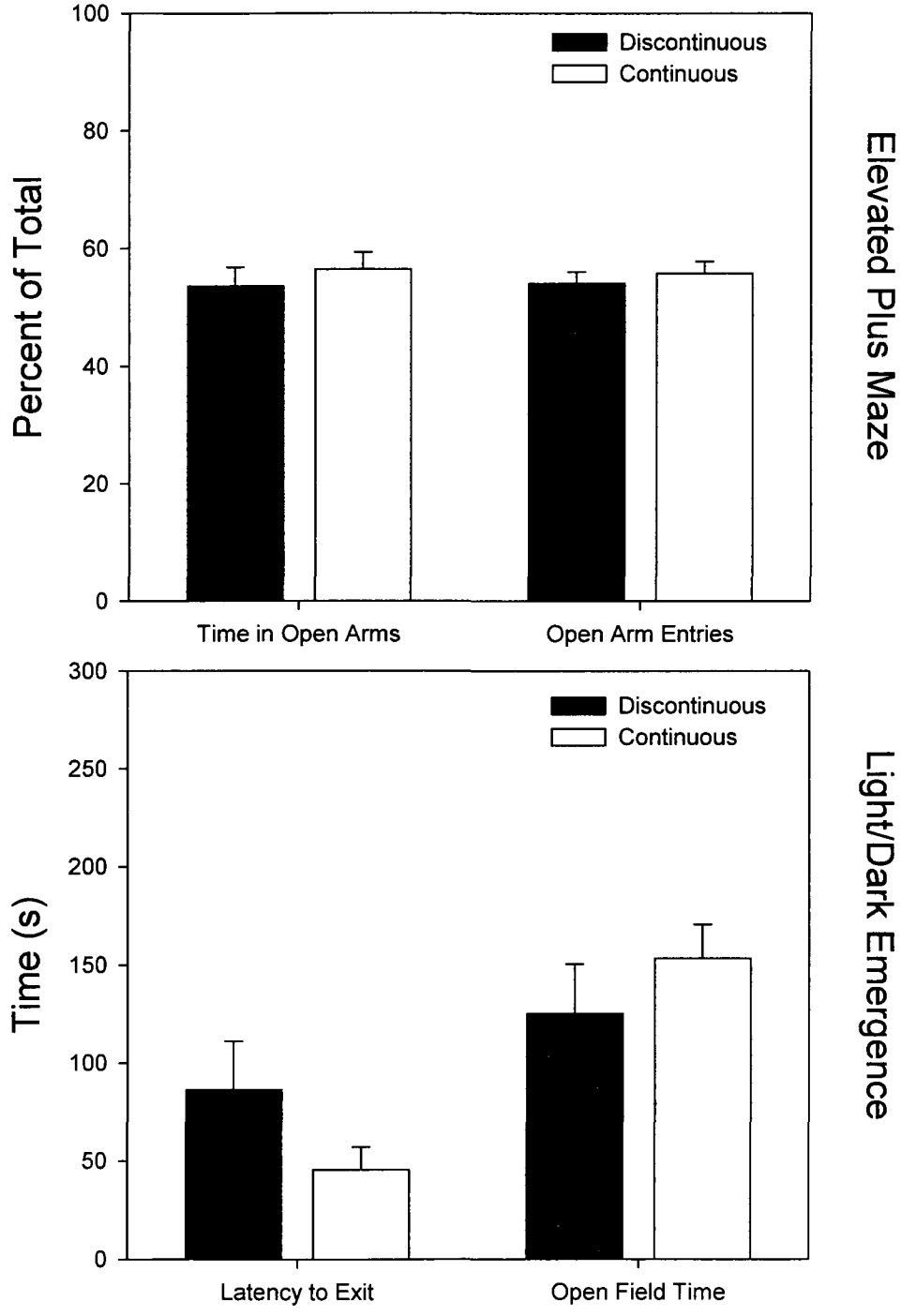

Figure 9: Anxiety tests in Experiment 2 (Appendix A). DisA and ConA rats were subjected to two consecutive anxiety tests on day 56: the EPM followed by the light/dark emergence test.