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UNCERTAIN ACCESS TO SHORTENING ALTERS HOME CAGE INTAKE BUT NOT OPERANT PERFORMANCE IN RATS

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ABSTRACT

Current practices in the treatment of binge-related eating disorders in humans include the introduction of structured meal plans as well as re-introduction of “forbidden foods” [Murphy et al., 2010]. This approach eliminates the uncertainty associated with consuming highly palatable foods rich in fat and sugar. In rats, certainty has previously been shown to reduce appetitive behavior associated with bingeing. Whether or not uncertainty promotes bingeing has not been systematically evaluated. The current study aimed to study the effects of uncertainty on binge-type behavior. Rats in the present study were separated into a daily group (D) which received 30 minutes of access to Crisco® every day at the same time, an intermittent group (INT) which received 30 minutes of access to Crisco® every other day and a daily uncertainty group (DU) which received 30-minute access to Crisco® on an uncertain schedule. Uncertainty was introduced to the schedule of the DU group by the presentation of three jars in the home cage for 30-minute periods, with only one of the three presentations containing Crisco® and no cues as to which presentation it would be. After five weeks under these schedules of access in the home cage, operant performance under various schedules was assessed. In the home cage, the intermittent group consumed more than the daily group, but the daily uncertain group did not significantly differ from either the daily or intermittent group. The INT group earned significantly more reinforcers than the D and DU groups under various operant schedules, i.e uncertainty provoked a slight stimulation of intake. These results suggest that uncertainty may modestly stimulate consummatory behavior (home cage intake) without stimulating appetitive (operant) behavior.

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Chapter 1

Introduction

According to the American Psychiatric Association, binge eating is defined as eating, in a discrete period of time, an amount of food larger than most people would eat in a similar period of time under similar circumstances accompanied by a reported loss of control over eating during the episode [Diagnostic and Statistical Manual of Mental Disorders, 2000]. Eating disorders such as Bulimia Nervosa and Binge Eating Disorder are characterized, in part, by recurrent episodes of binge eating. Bulimia Nervosa is defined by recurrent episodes of binge eating followed by inappropriate compensatory behavior to prevent weight gain, such as vomiting or use of laxatives or diuretics [Diagnostic and Statistical Manual of Mental Disorders, 2000]. Binge Eating Disorder is defined by recurrent episodes of binge eating without compensatory behaviors as seen in Bulimia Nervosa [Diagnostic and Statistical Manual of Mental Disorders, 2000].

Current research suggests a strong correlation between eating disorders and several psychosocial conditions in adolescents [Swanson et al., 2011] and adults [Hudson et al., 2007]. Specifically, 76% of adults and 85% of adolescents who reported bingeing expressed psychosocial co-morbidities such as anxiety, altered mood and substance use [Swanson et al., Hudson et al., 2007]. Among adolescents and adults alike, 78% of those with Bulimia Nervosa and 62.6% with Binge Eating Disorder reported impairment of everyday functions. 66.2% of adolescents with Bulimia Nervosa and 65.2% of those with

Binge Eating Disorder reported an anxiety disorder of some sort [Swanson et al., 2011.], while 80.6% of adults with Bulimia Nervosa and 65.1% of adults with Binge Eating Disorder reported anxiety disorders of some sort [Hudson et al., 2007]. Most significantly, among *adolescents,* 61% of those with Bulimia Nervosa and 34.4% of those with Binge Eating Disorder reported suicidal ideations [Swanson et al, 2011]. These statistics are clearly significant in a clinical setting and highlight the need for further investigation of the physiological underpinnings and causes of binge eating and its associated disorders.

As reported in numerous studies, intermittent presentation of a palatable substance can elicit binge-like behavior in rats [Corwin, 2011]. A recent review suggests, however, that while intermittency alone can stimulate ingestive behavior, elicitation of binge- type behavior may be particularly powerful when combined with uncertainty [Corwin, 2011]. Current clinical literature regarding uncertainty focuses predominantly on the trait of uncertainty intolerance, which is defined as the tendency to respond with negative emotional, cognitive, and behavioral reactions to uncertain situations and events [Dugas et al. 2004]. Intolerance of Uncertainty (IU) has been linked to anxiety disorders such as General Anxiety Disorder and Social Anxiety [Dugas et al. 2004], as well as an impairment of function when exposed to uncertainty. In parallel to the psychosocial implications of eating disorders mentioned above, individuals with Anorexia and Bulimia Nervosa have been reported to be highly intolerant of uncertainty [Frank et al., 2012]. In a recent study focusing on IU, participants received mild electrical shocks under various conditions; individuals who were identified as highly intolerant of uncertainty displayed

smaller startle responses when receiving shocks at uncertain intervals as a result of a reported loss of control [Nelson & Shankman, 2011]. The reported loss of control by individuals intolerant of uncertainty in this study was thought to be due to a loss of control over their circumstances or the uncertainty of the circumstances to which they were subjected. Individuals who were not intolerant of uncertainty were able to retain some sense of control over the uncertain circumstances they were subjected to, which resulted in greater startle responses when compared to IU individuals. The intolerance of uncertainty, described above in IU individuals, paired with the stimulatory effects of intermittency on ingestive behavior, may contribute to binge behavior.

In addition to the personality *trait* of intolerance to uncertainty described above, environmental conditions may create a *state* of uncertainty that stimulates food intake. Even in a developed country such as America, uncertainty has the potential to affect food intake. Recent studies have shown that time spent in the average American home preparing food is decreasing [Jabs & Devine, 2006] and consumption of food outside the home is increasing. [Guthrie et al., 2002] Furthermore, eating meals together as a family is becoming less common [Sisson et al., 2011]. All of this suggests that eating patterns are becoming increasingly uncertain, which may contribute to binge vulnerability. Current therapeutic methods for treating binge eating disorders focus on cognitive behavioral therapy, which includes the elimination of uncertainty in meal times. [Murphy et al., 2010] In addition, a recent study of nutritional information provided on food products showed that young women had difficulty understanding nutritional information [Wahlich et al., 2012] and displayed lower restraint and over-consumption of foods due

to health value claims [Wansink & Chandon, 2006]. Specifically, “low-fat” labels on food products increased intake due to an over estimation of serving size and decreased guilt associated with excessive consumption. A general lack of understanding of nutritional information and health value claims has the potential to create an indefinitely uncertain environment in regards to food choice. Together, these reports indicate that the effects of uncertainty on ingestive behavior warrant further study

Ingestive behavior can be studied in a laboratory setting using numerous models. Data collected from three particular rodent models has proven invaluable in understanding neuro-circuitry of human binge-type behavior. The sugar addiction model involves twelve-hour food deprivation followed by twelve hours of access to a 25% glucose or 10% sucrose solution. Rats in this model displayed escalating intake of sugar to the point of bingeing and displayed characteristics of addiction [Hoebel, 1989]. The neuro-circuitry of binge-like behavior has been examined using this model. Specifically, this model has shown that sugar displays similarities to drugs of abuse in the alteration of DA receptors and mesolimbic regions of the brain [Bello & Hajnal, 2010, Berridge et al., 2010].

The second model involves the history of dieting and stress in relation to binge-like behavior. In this model, four groups of rats are compared: no history of dieting (HD) and no stress (control group), no HD+stress, HD and no stress and HD+stress. When given an optional palatable food (cookies), rats exposed to HD+stress ate significantly more than rats from the other three groups. This intake was driven by factors other than homeostatic regulation. Following the intake of the palatable substance, HD+stress rats

also displayed an increased intake of chow. This suggests that stress can drive overeating and binge type behavior when palatable foods are available [Corwin et al., 2011]. The presence of optional palatable foods and stress are representative of conditions commonly experienced in cases of human binge eating, making this model appropriate for the study of human binge type behavior.

The final model involves limited access to a palatable substance at various time intervals and was used in this study. In this model, non-food deprived rats are presented with a palatable food (Crisco® vegetable shortening) at various intervals along with regular access to chow and water. Rats are separated into groups that receive the palatable food according to various access schedules. One group is given access to Crisco intermittently every Monday, Wednesday and Friday (INT group) for a brief period of time (typically 1 hour) while a second group is given Crisco access every day for the same brief period. After a few weeks, rats belonging to the INT group eat significantly more Crisco® in the home cage and earn significantly more reinforcers under a variety of operant schedules than do the rats in the daily group [Wojnicki et al., 2013]. As no food deprivation is involved in this model, hunger does not serve as a confounding factor in analysis of intake, making this model relevant to human binge eating disorders [Corwin, et al., 2011]. Further, the “forbidden food” hypothesis of human binge type behavior suggests that restricted access to a palatable substance promotes bingeing on that substance, as is seen in this model [Corwin, et al., 2011]

While the rats in the MWF group received Crisco intermittently (every other day), the entry of researchers into the room and the opening and closing of the cages which

housed rats from the D group on “off days” (Tues, Thurs, Sat.) provided uncertain signals as to when Crisco would be available. Thus, in addition to intermittency, the MWF schedule inherently carries a certain degree of uncertainty. The increased intake in the MWF group suggests that intermittency in the opportunities to consume palatable substances combined with uncertainty in regards to when those opportunities will occur may contribute to binge-like behavior.

This concept is supported through a study by Fiorillo in which differential firing of dopamine (DA) neurons in the ventral tegmental area of the brains of monkeys in response to uncertain delivery of a juice reward was reported [Fiorillo, 2011]. The firing of the DA neurons is thought relevant in reward signaling, particularly to constructs such as intense craving [Berridge et al., 2010], and to learning. Such results suggest that dopaminergic signaling may vary among rats with predictable (certain) and unpredictable (uncertain) access to palatable foods and thus may drive binge-type behavior.

Predictability was examined in a recent study from this lab that is related to the current study [Wojnicki et al., 2013]. In that study, predictable intermittent and unpredictable intermittent 1-hr access to Crisco was compared to rats given daily 1-hr access. While home-cage intake of both intermittent groups was significantly higher than that of the daily group, the predictable intermittent group earned the same number of reinforcers as the daily group and less than the unpredictable intermittent group under various schedules in an operant chamber [Wojnicki et al., 2013]. These results suggest that intermittency will drive binge type behavior regardless of uncertainty, but that uncertainty may be important to craving or other appetitive-related constructs. The

current study seeks to further understand the role of uncertainty in binge-type behavior, which could expand the understanding of binge eating and related behaviors as a whole and provide new therapeutic options for individuals suffering from eating disorders. Specifically, in the present study, *uncertainty* in the Daily control group was increased, whereas in the previous study *certainty* in the Intermittent (binge) group was increased.

Briefly, a group of rats with daily access to Crisco® was subjected to uncertainty through manipulation of the timing of access in the present study. These rats were labeled as the DU group and received Crisco® only once over the course of three jar presentations. During the two remaining presentations, empty jars were placed in the cages. The presentation which contained Crisco changed each day and was maintained on a random schedule over six weeks. Following this protocol, home cage intake was measured and rats were then placed in operant chambers for behavioral analyses. Rats were returned to the uncertain home cage protocol for one week following the operant chamber studies to compare home cage intake trends to the previous six week period.

Chapter 2

Methods

Animals

Thirty six male Sprague Dawley rats (Harlan, Indianapolis, IN), 60 days of age and weighing 262-306 g (273.6 ± 1.3 g) at the start of the study were individually housed in hanging stainless steel wire cages in a temperature- and humidity-controlled environment placed on a 12:12 light:dark cycle. All rats were maintained on a nutritionally complete commercial laboratory rodent chow (Laboratory Rodent Diet 5001, PMI Feeds, Richmond IN; percent of energy as protein: 28.05%, fat: 12.14%, carbohydrate: 59.81%; 3.3 kcal/g), with chow and tap water available ad libitum throughout all parts of the study. The Pennsylvania State University Institutional Animal Care and Use Committee approved all procedures.

After six days of adaptation to the vivarium, 24 hr chow intake was recorded for 3 days. On the following day body weights were recorded and all rats were provided overnight access to vegetable shortening (Crisco^{TM}, J.M. Smucker Co., Orrville, OH) emulsion (3.67 kcal/gm). Three groups of 12 rats each were then matched by 3 day average chow intake, body weight, and overnight shortening intake [$F(2,33) = 0.06$ chow, $= 0.06$ body weight, $= 0.01$ shortening, NS all].

Procedures

HOME CAGE ACCESS

One group of rats (INT) was provided 30 min of access to a jar of shortening clipped to the front of the home cage 1 hr prior to the start of dark cycle on an intermittent (Mondays, Wednesdays, and Fridays) basis. A second group of rats (D) was provided 30 min of access to a jar of shortening clipped to the front of the home cage 1 hr prior to the start of the dark cycle on a daily (7 days/week) basis. Both of these groups were housed in the same colony room along with other groups of rats in other studies. A third group of rats (DU) was housed in a separate colony room adjacent to the hallway with no other rats in the room. This group of rats was also provided 30 min access to a jar of shortening clipped to the front of the home cage on a daily basis (7 days/week), but with an altered presentation schedule. Starting 3 hours prior to the start of the dark period a jar was placed in the home cage on the hour and removed on the ½ hour for a total of 3 daily jar presentations. On two of these presentations an empty jar was provided and on one of these jar presentations the jar contained shortening. The shortening jar was provided on the first presentation on Sundays and Tuesdays, the second presentation on Mondays, Wednesdays, and Fridays, and the third presentation on Thursdays and Saturdays. The second presentation was the same time prior to start of the dark cycle that the D and INT groups received their shortening jars. These procedures were in effect for the entire study. Operant sessions were conducted during the second presentation time. During week 5 of the study total energy intake (shortening plus 24 hr chow) was recorded.

OPERANT PROCEDURES

The rats were tested in twelve identical operant chambers (Model H10-11R-TC; Coulbourn Instruments, Allentown, PA) located in a dedicated room in the vivariums. The back wall of each chamber contained a house light (Model H11-01R) located at the top of the middle panel of the chamber. The front wall of each chamber contained a response lever (Model H21-03R) located in the middle panel and a triple cue lamp (H11-02R) located above it. Located in the right panel was a lip to collect shortening delivery and a triple cue light above the tray to indicate shortening delivery. Whipped vegetable shortening was used as the reinforcer for the experimenter-defined operant: lever release under some schedules and lever pressing under others. Whipped shortening was delivered in 0.1 g units from a 20 ml glass syringe (Popper & Sons, New Hyde Park, NY) driven by an infusion pump (Model E73-01-3.3 rpm) onto the collection tip located below the triple cue lamp adjacent to the response lever. Care was taken to minimize any air pockets in the 20 ml syringe that would affect the amount delivered. This was accomplished by placing whipped shortening into a self-lock plastic bag, squeezing the shortening into a 60 ml syringe, and then squeezing this into the entire 20 ml syringe. The plunger of the 20 ml syringe was then used to compact the shortening up to the 20 ml marker and thus removing any air pockets. When a reinforcer was scheduled to be delivered all three cue lamps flashed for 2 s prior to the start of the reinforcer delivery, during the 2 s while the whipped shortening was being delivered, and for 1 s after the delivery. All experimental contingencies were programmed with Graphic State 2™ state notation (Coulbourn Instruments, Allentown, PA).

Following the first five weeks of home cage access, all rats were overnight food-deprived and trained to lever press under a fixed ratio 1 schedule of reinforcement. Rats that failed to lever press during the first session were given 5 g of food and trained on the second day. After all rats learned to lever press they were placed on ad libitum food access for at least 4 days. On the fifth day, all rats were again overnight food deprived and placed on a signaled Differential Reinforcement of Low Rate 3 sec schedule of reinforcement (DRL 3) in which lever release was defined as the operant response to prevent lever holding. While the DRL timer was timing out, the triple cue lights above the lever were off. All lever presses during this time stopped the timer and lever release reset the timer. After 3 sec had lapsed without a lever press occurring, the triple light above the lever was illuminated signaling that a reinforcer was available for a lever release. Two days later, all rats were again placed under the signaled DRL3 schedule, but they were not food deprived. For the next 6 sessions a signaled DRL 6 sec schedule of reinforcement was in effect. Stable operant performance was determined a priori as three consecutive sessions with no significant differences among them with respect to the number of earned reinforcers. Following the last operant session, all three groups were placed on the home cage protocol for 1 week to assess if the relationship among the groups with respect to home cage intake was not altered by the operant sessions.

The experimenter-defined operant response of lever release during the signaled DRL 6 schedule was changed to lever press for the remaining sessions. In order to facilitate the functional change in response topography all rats were placed on a Fixed Ratio 3 schedule of reinforcement for one session when overnight food-deprived and for two sessions non-food deprived. For the next 5 sessions a Random Ratio 5 schedule of

reinforcement was in effect with the same stability criterion as the DRL 6 reinforcement schedule. Rats were then placed under a Progressive Ratio 1 (PR1 for 4 sessions) and a PR 3 (PR3) for one session. Following the last operant session, all three groups were placed on the home cage protocol for 1 week to assess if the relationship among the groups with respect to home cage intake was not altered by the operant sessions.

STATISTICS

Data were analyzed using analysis of variance (ANOVA) followed by post-hoc tests as appropriate. Specifically, a 1-way ANOVA was used to analyze differences among groups in home cage shortening intake, average total energy intake for week 5, and average number of reinforcers earned under the last 3 sessions for each schedule of reinforcement. For within group analysis of the number of reinforcers earned across schedules, a 1-way repeated measures ANOVA was used with time as the repeated measure. To analyze the total daily energy intake among groups that occurred across days in week 5, a 2-way ANOVA was used, with day as a repeated measure. Tukey's Studentized Range (HSD) was used for all post-hoc testing, except for the number of reinforcers earned under the RR5 schedule. In that case, there was a significant main effect of group but Tukey's did not reveal significant differences among group means. Therefore, Duncan's Multiple Range Test was used for that analysis. Finally, independent t-tests were used to determine if intakes of the D and DU groups differed at the different times that Crisco® was presented to the DU group.

Chapter 3

Results

HOME CAGE INTAKE

Throughout the study, there were significant differences among the groups with respect to home cage shortening intake when the rats were maintained on the home cage protocol and no operant sessions were conducted: week 6 [$F(2,33) = 4.33$, $p < 0.0213$]; post DRL 6 [$F(2,33) = 8.71$, $p < 0.0009$] and post PR 3 [$F(2,33) = 6.97$, $p < 0.0030$]. As shown in Figure 1 (top panel), the INT group consistently consumed significantly more shortening than the D group. However, the DU group was consistently no different than either the INT or D group. While there were differences among the groups with respect to shortening intake, there were no differences among the groups with respect to the average total energy (shortening plus chow) intake for week 5 ([$F(2,33) = 0.03$, $p < 0.9732$] despite the fact that there were significant daily differences ([$F(6,198) = 13.35$, $p < 0.0001$] and a daily x group interaction ([$F(6,198) = 18.41$, $p < 0.0001$] (Figure 1, bottom panel). The differences in daily energy intake were due to the high caloric intake of shortening by the INT group on Mondays, Wednesdays, and Fridays, and the under consumption of chow on the other days. The amount of shortening consumed by the DU group did not differ among the three different presentation times.

OPERANT PERFORMANCE

The INT group earned significantly more reinforcers than either the D or DU groups under the DRL 6 [$F(2,33) = 7.84$, $p < 0.0016$], RR5 [$F(2,33) = 3.65$, $p < 0.0371$], and PR 1[$F(2,33) = 5.82$, $p < 0.0069$] schedules of reinforcement, but not the PR 3 [$F(2,33) = 2.67$, $p < 0.0840$] (Figure 2, top panel). The number of reinforcers earned under the DRL 6, RR 5, and PR 1 schedules by the DU and D groups did not statistically differ within each group regardless of whether the data used in the analysis was restricted to the number of reinforcers per se or the number of reinforcers expressed as energy normalized to body weight [Heusner, 1985]. For the INT group, however, there were significantly fewer reinforcers earned under the PR 1 schedule when compared to the DRL 6 regardless of the type of analysis. The shortening energy under the RR 5 schedule was no different than either the DRL 6 and PR 1, while the number of reinforcers earned was no different than the DRL 6, but significantly greater than the PR 1. All groups earned significantly less shortening regardless of the type of analysis under the PR 3 schedule than the other three schedules.

Under the DRL 6 sec schedule of reinforcement there were no differences among the groups with respect to the false alarm (inter-response times less than 6 sec) to hit ratios (inter-response times equal to or greater than 6 sec) [$F(2,33) = 0.36$ $p < 0.7021$].

The INT consumed significantly more total (reinforcers + home cage) shortening than the D group under the signaled DRL 6 [$F(2,33) = 8.56$ $p < 0.001$] schedule of reinforcement, significantly more than the DU group under the RR 5 [$F(2,33) = 4.06$ $p <$

0.0265], and significantly more than both groups under the PR 1 [$F(2,33) = 11.67$ $p < 0.0001$] and PR 3 [$F(2,33) = 5.85$ $p < 0.0067$] schedules (Figure 2, bottom panel).

Chapter 4

Discussion

The present results suggest that uncertainty as an isolated factor may contribute modestly to consummatory (home cage intake), but does not appear to contribute to appetitive (operant) behavior in rats (see table 1). This is illustrated by the fact that home cage Crisco intake in the DU group was somewhat higher than the D comparison group, although significant differences between these two groups was not achieved. In addition, the home cage Crisco intake in the DU group was not significantly different from the INT (binge) group, although it is clear that the DU intakes were somewhat lower than that of INT. While the DU group was subjected to uncertain access to a palatable reinforcer through the presentation of three jars, daily presentation of Crisco still carries a certain degree of certainty when compared to intermittent access, which may have limited the elevation of intake. Conversely, the INT group received inadvertent uncertain cues regarding palatable substance presentation such as the opening and closing of cage doors and the presence of researchers in the room in addition to the uncertainty inherent in the intermittent (Monday, Wednesday, Friday) access schedule. Subjecting the INT group to further uncertainty through an access schedule similar to that of the DU group could have theoretically elevated intake to an even greater extent. Elevation of intake in the INT group to statistical significance beyond the DU group could not be assured, however, as the intake of the INT group in the present study was already approaching gastric capacity [Bull et al. 1970].

In contrast to results obtained in the home cage, reinforcers earned in the operant chamber by the DU group were significantly less than the INT group and did not differ from the D group, for all schedules except PR3. There were no differences in reinforcers earned among any of the groups on the PR3 schedule, suggesting that the slope of increase was too steep. Rats from all groups responded maximally and then stopped responding, i.e. they were no longer willing to work for a Crisco® reinforcer. The present findings suggest that home cage consumption and operant performance are not directly related, which is consistent with another study recently published from this lab. In that study, predictable intermittent and unpredictable intermittent 1-hr access to Crisco were compared to daily 1-hr access. While home-cage intake of both intermittent groups was significantly higher than that of the daily group, the predictable intermittent group earned the same number of reinforcers as the daily group and less than the unpredictable intermittent group under various schedules in an operant chamber [Wojnicki et al, 2013]. Thus, the appetitive (operant) behavior of the two groups in that study with highly predictable food-cue associations (daily/predictable; intermittent/predictable) was lower than that of the intermittent group with unpredictable food-cue associations. In the present study, on the other hand, the appetitive behavior of both daily groups, regardless of food-cue predictability, was lower than that of the intermittent group. While the results of the previous study suggest that the pairing of intermittency and uncertainty can stimulate appetitive behavior (operant responding), the present study shows that uncertainty as an isolated factor may modestly stimulate consummatory behavior (home cage intake). It is important to note that uncertainty in the present study was manipulated in a group with daily access to shortening. As mentioned above, the daily access schedule

itself inherently carries a certain degree of certainty, and therefore the uncertainty imposed by rotating the time of presentation may not have been sufficient to promote appetitive behavior.

The differential effects of uncertainty on consummatory and appetitive behavior may be due to various factors. For instance, rats in the DU group of the present study were given access to Crisco between the hours of 9am-12pm (2-4 hours before lights out) during one of three jar presentations, creating differential intervals between feeding and lights out each day. Four hours prior to lights out is a period of time during which rats normally do not eat very much. However, as the dark period approaches, the rats become more active and begin to eat. Thus, the differential times of presentation may have resulted in differential intakes, which would not have shown up statistically, since mean intakes across all three possible presentation times were used in the data analysis.

The idea that the different times may have resulted in different intakes is supported by prior research in which feeding patterns of rats were assessed. A seminal study concerning food intake by rats during a normal dark-light period by Siegel (‘61) showed that rats display differential intakes across a 24-hour period. Specifically, with the dark cycle beginning at 7PM, intake was slightly elevated at 5PM, i.e. intake was somewhat elevated two hours before lights out. In the present study, rats in the DU group were given access to Crisco between the hours of 9AM-11:30AM with lights going out at 1PM. To study the effects of these different presentation times, shortening intake on early feeding days (Crisco® access during first jar presentation from 9AM-9:30AM, 4 hours prior to lights out) was compared with intake that occurred on intermediate feeding days (Crisco access during second presentation from 10am-10:30am, 3 hours prior to lights

out) and on late feeding days (Crisco access during final jar presentation from 11am-11:30am, 2 hours prior to lights out). It was determined that the timing of Crisco presentation had no significant effect on shortening intake. Thus, the lack of effect in the DU group was not due to differential intakes during the different presentation periods.

Although timing of presentation did not significantly affect shortening intake, the resultant 24-hour intake patterns may have contributed. Previous studies have reported a "sawtooth pattern" for total 24-hr energy intake in rats with intermittent access to palatable substances [Corwin et al.,1998, Corwin 2004, Dimitriou et al., 2000, Thomas et al., 2002, Wojnicki et al., 2006, Wojnicki et al., 2008]. Rats with intermittent access to a palatable substance respond to the high energy density through compensatory behavior. Specifically, chow intake is decreased on days when the palatable energy dense substance is unavailable such that overall energy intake across the study is equivalent to that of a control group that only has access to chow [Corwin et al., 1998]. It is important to note, however, that rats will generally defend protein status if possible even when faced with dietary challenges [Leathwood et al., 1983]. For maximal growth, protein must comprise 15% of total intake (by weight) in the diet of young rats [National Research Council, 1995]. The only source of protein for rats in the present study was chow, and following access to Crisco®, rats from all groups decreased chow consumption. Therefore, the intake of protein in all of the groups was calculated to determine if protein status was maintained. Indeed, all groups maintained protein status. Therefore, it is possible that the defense of protein nutriture limited shortening intake in the DU group.

To determine if this was the case, chow consumption associated with the maintenance of protein status was analyzed for limitation of home cage Crisco intake in

the DU group. Maximum possible consumption of Crisco® for the DU group was estimated by calculating the minimum amount of chow necessary to maintain protein status and the corresponding amount of Crisco® that would make total energy intake consistent with recorded values. It was determined that maintenance of protein status did not restrict Crisco® intake to the point that the DU group could not have consumed as much shortening as the INT group.

In addition to maintaining protein status, the rats in the present study never became obese, which is consistent with all prior studies from this lab. It has been reported that timed high fat, restricted feeding patterns are associated with increased insulin sensitivity, fat oxidation and decreased body weight in rats [Sherman et al., 2012]. An average of approximately 45% of the energy intake of rats in the DU group of the current study was comprised of Crisco, therefore the findings of the Sherman et al. study are relevant. While the entire diet was not time restricted, the high fat portion of the diet was limited by 1-hr access to Crisco. The absence of obesity among rats in the present study is consistent with findings in rats given a timed high fat, restricted eating diet.

The results of the present study indicate that uncertainty as an isolated factor may modestly contribute to consummatory, but not appetitive behavior in rats. In terms of food consumption, the appetitive phase is defined as the actions of approach to a goal object and is loosely represented by intense craving in humans. In contrast, the interaction with the goal object is defined as the consummatory phase and is represented by the actual amount of food consumed [Adler, 1985] In relation to the current study, the appetitive phase is operationalized by the “motivation” to work for a palatable substance in an operant chamber, i.e. the number of reinforcers earned. Uncertainty had no effect

on operant performance, as D and DU did not differ, but both earned fewer reinforcers than I. Consummatory behavior in the current study relates to the amount of Crisco® consumed, and in this case subtle differences did emerge. In particular, the DU group consumed somewhat more Crisco® than the D group, and somewhat less than the INT group, i.e. the DU group did not differ statistically from INT. Overall, these results indicate that uncertainty may contribute mildly to consummatory behavior, but not appetitive behavior.

In a recent study conducted by Arce et al. 2013, rhesus monkeys were subjected to chronic stress using social subordination. The subordinate monkeys expressed higher levels of serum cortisol and subsequently consumed greater amounts of a high calorie diet than the dominant monkeys. Further, a decrease in anxiety-like behavior in the subordinate monkeys coincided with the consumption of the high calorie diet. An early prediction by Hoebel supports the role of stress in binge eating, postulating that stress induces the release of DA, which may stimulate areas such as the nucleus accumbens (NAc) which processes feeding stimuli [Hoebel et al., 1989]. Recent studies have shown that stress induced corticosterone can indeed increase DA release in the NAc [Rouge-Ponte et al, 1998, Marinelli, 2002]. Boggiano has also used elevated plasma corticosterone levels to identify bingeing rats, including those with no history of dieting, in the HD+ stress model [Corwin et. al, 2011]. These studies suggest that stress may stimulate consummatory behavior, which may in turn decrease stress, thus creating a cycle of increased intake. While the uncertainty imposed in the current study was likely only a mild stressor, it is possible that prolonged exposure to an uncertain access

schedule may elicit a small stress response, slightly increasing the intake of Cricso® (consummatory behavior).

The results of the current study suggest that uncertainty may mildly stimulate consummatory behavior. In conjunction with a previous study [Wojnicki et al., 2013] that displayed the effects of intermittency on appetitive and consummatory behavior, the results of this study suggest the efficacy of eliminating both uncertainty and intermittency for clinical treatment of binge-related eating disorders. This is consistent with current cognitive behavioral therapeutic methods of introducing structured meal plans and introducing forbidden foods back into the diets of individuals suffering from eating disorders [Murphy et al., 2010].

References

Adler, *Handbook of Behavioral Neurobiology*, 2nd. Ed. 1985:16-17.

Arce M, Michopoulos V, Shepard K, Ha Q, and Wilson M. Diet choice, cortisol reactivity and emotional feeding in socially housed rhesus monkeys. Physiol Behav. 2013.

Bello NT, Hajnal A. *Dopamine and binge eating behaviors*. Pharmacol Biochem Behav 2010;97:25–33.

Berridge K., Ho C., Richard J., DiFeliceantonio A. *The tempted brain eats: Pleasure and desire circuits in obesity and eating disorders.* Brain Research; 2010; 1350, 43-64.

Bull L, Pitts G. *Gastric Capacity and Energy Absorption in the Force Fed Rat.* J. Nutrition, 1970, 101: 593-596.

Corwin RL. Binge-type eating induced by limited access in rats does not require energy restriction on the previous day. Appetite. 2004; 42:139-42.

Corwin RL. The face of uncertainty eats. Curr Drug Abuse Rev. 2011; 4:174-81.

Corwin RL, Avena NM, Boggiano MM. Feeding and reward: perspectives from three rat models of binge eating. Physiol Behav. 2011; 104:87-97.

Corwin RL, Wojnicki FE, Fisher JO, Dimitriou SG, Rice HB, Young MA. Limited access to a dietary fat option affects ingestive behavior but not body composition in male rats. Physiol Behav. 1998; 65:545-53.

Dimitriou SG, Rice HB, Corwin RL. Effects of limited access to a fat option on food intake and body composition in female rats. Int J Eat Disord. 2000; 28:436-45.

DSM-IV™-TR. Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision. Washington, DC: American Psychiatric Association, 2000, pp. 583-95, 785-7.

Dugas MJ, Buhr K, Ladouceur R. The role of intolerance of uncertainty in etiology and maintenance. In: Heimberg RG, Turk CL, Mennin DS, editors. Generalized Anxiety Disorder: Advances in Research and Practice. New York, NY: Guilford Press; 2004. pp. 143–163.

Fiorillo, C. *Transient activation of midbrain dopamine neurons by reward risk.* Neuroscience 2011, December 1; 197: 162-171.

Fisher JO, Birch LL. Restricting access to foods and children's eating**.** Appetite 1999, 32**:**405-419.

Frank GK, Roblek T, Shott ME, Jappe LM, Rollin MD, Hagman JO, Pryor T. *Heightened fear of uncertainty in anorexia and bulimia nervosa.* Int J Eat Disord. 2012 Mar; 45(2): 227-32.

Foltin RW, Evans SM. The effects of D-amphetamint on responding for candy and fruit drink using a fixed ratio and a progressive ratio schedule of reinforcer delivery. Pharm Biochem Behav 2001 69; 125-31.

Guthrie, JF; Lin, BH; Frazao, E. *Role of food prepared away from home in American Diet. 1977-78 vs. 1994-96 changes and consequences* 2002 May-Jun;34(3):140-50.

Heusner AA. Body Size and Energy Metabolism. Ann Rev Nutr. 1985; 5:267-93.

Hoebel BG, Hernandez L, Schwartz DH, Mark GP, Hunter GA. *Microdialysis studies of brain norepinephrine, serotonin, and dopamine release during ingestive behavior*. Theoretical and clinical *implications*. Ann NY Acad Sci 1989;575:171–91.

Hudson JI, Hiripi E, Pope Jr HG, Kessler RC. The prevalence and correlates of eating disorders in the National Comorbidity Survey Replication. Biol Psychiatry 2007;61:348–58.

Kales EF. Macronutrient analysis of binge eating in bulimia. Physiol Behav 1990; 837-40.

Jabs J, Devine, C.M *time scarcity and food choices; an overview. Appetite, 2006.*

Leathwood, P. D.; Ashley, D. V. M.: Strategies of protein selection by weanling and adult rats. Appetite 4:97–112; 1983.

Marinelli M, Piazza PV. *Interaction between glucocorticoid hormones, stress and psychostimulant drugs*. Eur J Neurosci 2002;16:387–94.

McGee HM, Amare B, Bennett AL, Duncan-Vaidya EA. Behavioral effects of withdrawal from sweetened vegetable shortening in rats. Brain Res 2010;1350:103-11.

Murphy R, Straebler S, Cooper Z, Fairburn CG. Cognitive behavioral therapy for eating disorders. Psychiatr Clin North Am. 2010; 33:611-27.

Naleid AM, Grimm JW, Kessler DA, Sipols AJ, Aliakbari S, Bennett JL, Wells J, Figlewicz DP. Deconstructing the vanilla milkshake: The dominant effect of sucrose on self-administration of nutrient–flavor mixtures Appetite 2008;50:128–138.

National Research Council. *Nutrient Requirements of Laboratory Animals: Fourth revised Edition. 1995; 22-26.*

Nelson B, Shankman S. Does intolerance of uncertainty predict anticipatory startle responses to uncertain threat? International Journal of Psychophysiology , 81(2), August 2011, 107-115.

Overduin J., Figlewicz, DP.,Bennett-Jay J., Kittleson S, Cummings DE. Ghrelin increases the motivation to eat, but does not alter food palatability. Am J Physiol Regul Integr Comp Physiol 2012 303:R259-R269.

Rao, R. E., Wojnicki, F. H. E., Coupland, J., Ghosh, S., & Corwin, R. L. W. (2008). Baclofen, raclopride, and naltrexone differentially reduce solid fat emulsion intake under limited access conditions. Pharmacology Biochemistry & Behavior, 89(4), 581–590.

Richardson, N.R., Roberts, D.C.S. Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. J. Neurosci. Methods 1996, 66:1-11.

Rougé-Pont F, Deroche V, Le Moal M, Piazza PV. *Individual differences in stressinduced dopamine release in the nucleus accumbens are influenced by corticosterone*. Eur J Neurosci 1998;10:3903–7

Sherman H, Genzer Y, Cohen R, Chapnik N, Madar Z, Froy O. *Timed high fat diet restricts circadian metabolism and prevents obesity.* FASEB Journal, August 2012. (26) 3493-3501

Siegel P. *Food Intake in the Rat in Relation to the Dark-Light Cycle. Journal of Comparative and Physiological Psychology* 1961, 54 (3), 294-301

Swanson SA, Crow SJ, Le Grange D, Swendsen J, Merikangas KR. Prevalence and correlates of eating disorders in adolescents: results from the National Comorbidity Survey Replication.

Thomas MA, Rice HB, Weinstock D, Corwin RL. Effects of aging on food intake and body composition in rats. Physiol Behav. 2002; 76:487-500.

Ventura AK, Birch LL. Does parenting affect children's eating and weight status? Int J Behav Nutr Phys Act. 2008; 5-15.

Wahlic C, Gardner B, McGowan L. *How, when and why do young women use nutrition information on food labels? A qualitative analysis.* Psychol. Health, 2013; 28(2): 202-16

Wansink B, Chandon P. *Can "Low-Fat" Nutrition Labels Lead to Obesity?* Journal of Marketing Research, 2006. 43; 605-617

Wojnicki FHE, Roberts DCS, Corwin RLW. Effects of baclofen on operant performance for food pellets and vegetable shortening after a history of binge-type behavior in non-food deprived rats. Pharm Biochem Behav 2006;84:197–206.

Wojnicki FHE, Johnson DS, Corwin RLW. Access conditions affect binge-type shortening consumption in rats Physiol Behav 2008(a);95:649–57.

Wojnicki FHE, Charny G, Corwin RLW. Binge-type behavior in rats consuming trans-fat-free shortening. Physiol Behav 2008(b);94:627–29.

Wojnicki FHE, Babbs RK, Corwin RLW. Reinforcing efficacy of fat, as assessed by progressive ratio responding, depends upon availability not amount consumed. Physiol & Behav 2010;100(40):316-21.

Wojnicki FHE, Babbs RK, Corwin RLW. Environments predicting intermittent shortening access reduce operant performance but not home cage binge size in rats. Physiol Behav 2013;116-117:35-43.

Wong, K. J., Wojnicki, F. H. E., & Corwin, R. L. W. (2009). Baclofen, raclopride, and naltrexone differentially affect intake of fat/sucrose mixtures under limited access conditions. Pharmacology Biochemistry & Behavior, 92(3), 528–536.

Figure Legends

Fig. 1. Top panel. Mean (± sem) amount of shortening consumed in the home cage at various points throughout the study. Different letters indicate significant differences among groups within each time period. Bottom panel. Total energy intake (shortening + chow) during each day of week 5 and average for the week. Asterisks indicate significant difference between INT and the other two groups.

Fig. 2. Top panel. Mean (± sem) reinforcers earned under each schedule of reinforcement. Different letters indicate significant differences among groups within each schedule. Different numbers indicate significant differences within each group across the different reinforcement schedules. Bottom panel. Total shortening intake. Mean (± sem) shortening intake (grams earned in operant sessions + supplemental home cage) for each schedule of reinforcement. Different letters indicate significant differences among groups within each schedule.

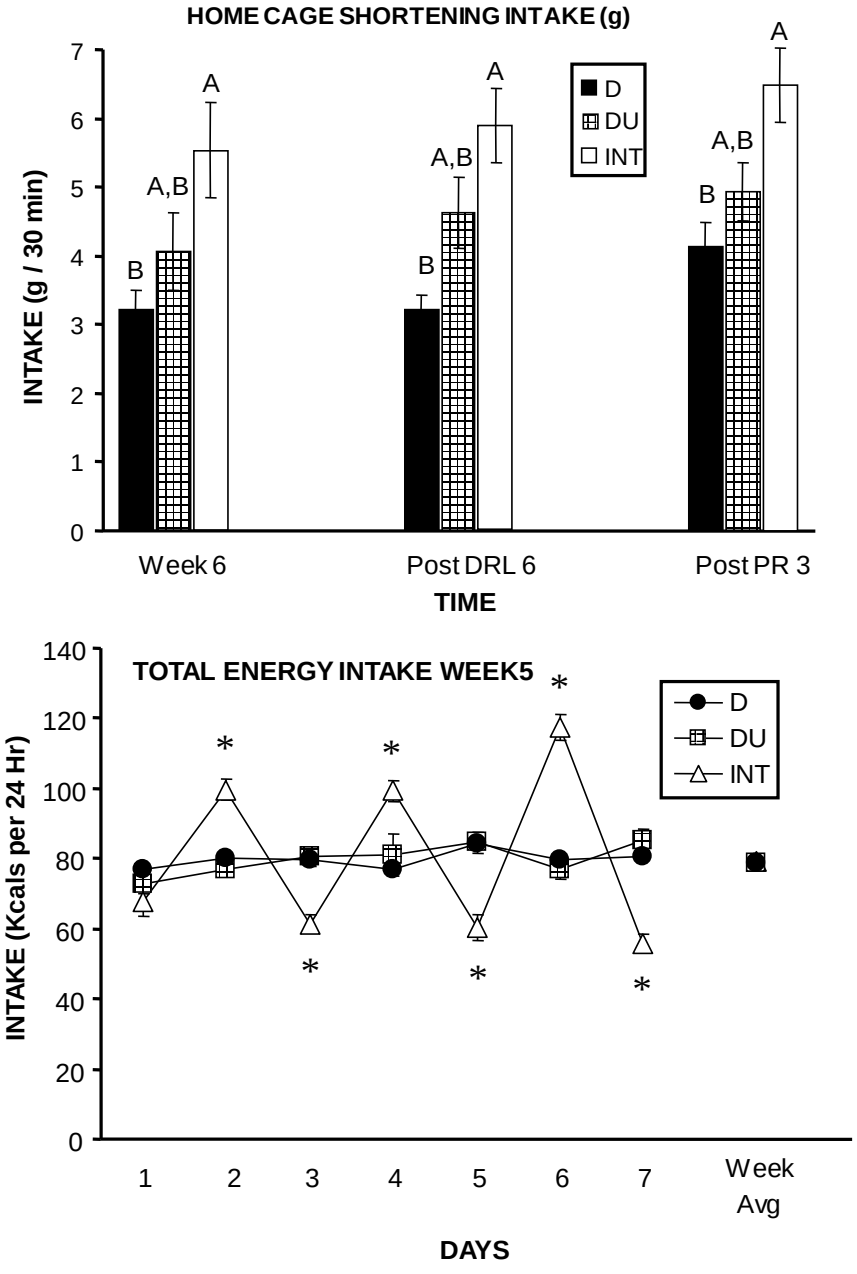

Figure 1. Vlassis et al.

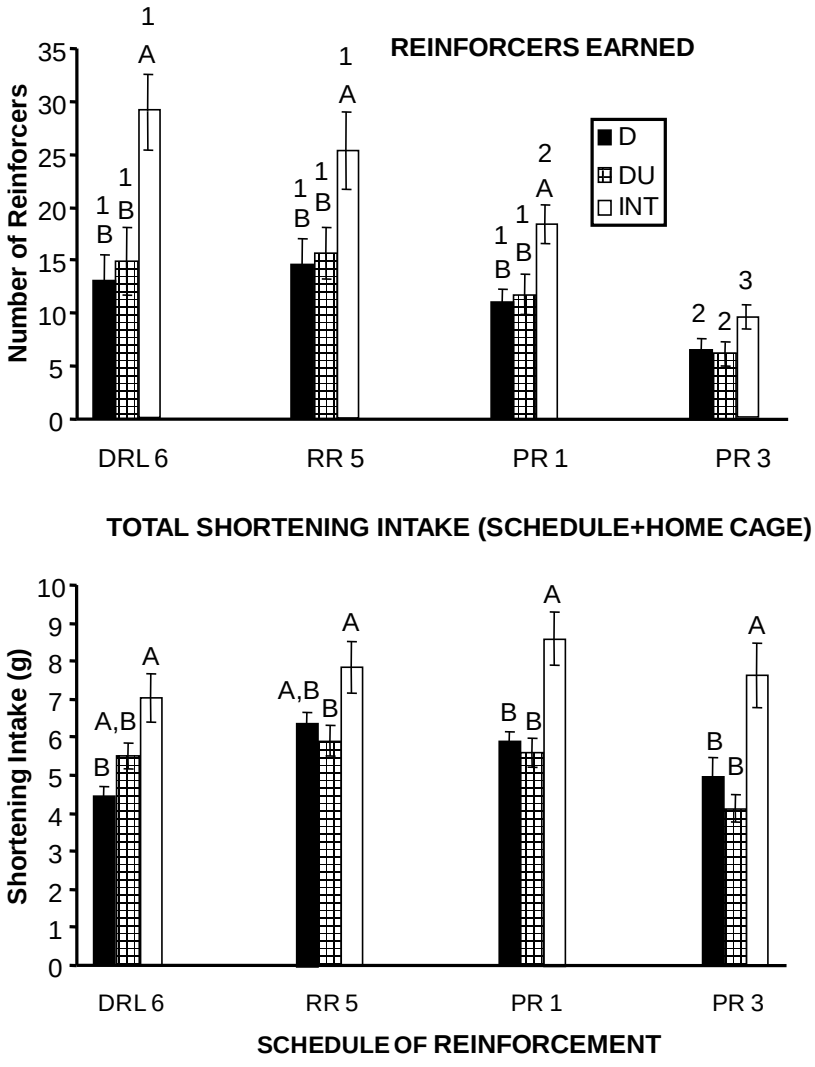

Figure 2. Vlassis et al.

Table 1: Effects of Intermittency and Uncertainty on Intake and Appetitive Behavior[1]

Home Cage Intake		
	Intermittent	Daily
Certain	↑↑[2]	↓
Uncertain	↑↑	↑
Appetitive Behavior (Operant)		
	Intermittent	Daily
Certain	↓	↓
Uncertain	↑↑	↓

1: Summary of results from the present study and Wojnicki et al. 2013

2: ↑↑ Indicates large intakes or large numbers of reinforcers earned; ↑ Represents moderate intakes; ↓ Represents low intakes or low number of reinforcers earned

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The Pennsylvania State University, Schreyer Honors College — December 2013
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ACTIVITIES

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