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Research report

Pavlovian conditioning to hedonic food cues in overweight and lean individuals ☆

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ABSTRACT

Obese individuals develop heightened reactivity to environmental cues associated with hedonic foods through Pavlovian conditioning. This study examined differences between overweight (n = 16) and lean (n = 17) 18–26 year-olds in their acquisition of a swallowing response to visual cues paired with chocolate milk, tasteless water and no taste stimulus. We hypothesized that, compared to lean participants, overweight participants would demonstrate a heightened conditioned swallowing response to the visual cue paired with chocolate milk as well as a resistance to extinction of this response. Results showed that overweight participants swallowed more in response to the visual cue previously paired with chocolate than the cue previously paired with tasteless water ($t(15) = -3.057$, $p = .008$) while lean participants showed no cue discrimination ($t(16) = -1.027$, $p = .320$). The results evaluating the extinction hypothesis could not be evaluated, as the lean participants did not acquire a conditioned response. In evaluating the conditioned swallow response of overweight participants only, results indicated that there was not a significant decrease in swallowing to cues paired with chocolate milk or water, but overall, overweight participants swallowed more to cues paired with chocolate than cues paired with water. These are the first results to show differential acquisition of Pavlovian conditioned responding in overweight individuals compared to lean individuals, as well as differential conditioning to cues paired with hedonic food stimuli compared to cues paired with neutral stimuli.

Introduction

The modern environment is full of highly palatable foods. A heightened responsiveness to environmental cues that predict food intake has been implicated as one of the mechanisms that promote overeating, and by extension, weight gain in some individuals (Stice, Yokum, Burger, Epstein, & Small, 2011; Volkow, Wang, & Baler, 2011). When palatable food consumption is repeatedly paired with previously neutral cues in the environment, those cues come to elicit the same set of responses that were elicited by the food itself, such as salivation (Pavlov, 1927). Pavlovian conditioning plays an important role in motivating and maintaining food consumption (Woods & Kuskosky, 1976). This study evaluates differences between overweight and lean individuals in their acquisition and extinction of conditioned swallowing responses to visual cues paired with hedonic and neutral taste stimuli.

Through a process of Pavlovian conditioning, cues that typically predict food intake can trigger cue reactivity, a motivational state that may be experienced as an urge to eat and therefore increase the probability of food intake (Jansen, 1998). Several studies have demonstrated that exposure to the sight and smell of food increases subjective craving, desired portion size, and actual food intake (Fedoroff, Polivy, & Herman, 1997; Ferriday & Brunstrom, 2008; Sobik, Hutchison, & Craighead, 2005). Exposure to the sensory qualities of food also elicits physiological forms of cue reactivity (cephalic phase responses), including salivation. Cephalic phase salivary response has been observed in humans (Mattes, 1997; Nederkoorn, Smulders, & Jansen, 2000) and can also be elicited by neutral, non-food stimuli after repeated pairings with food. Early researchers in this field demonstrated a conditioned salivary response in normal weight humans in response to a tone previously paired with citric acid (Brown & Katz, 1967; Feather, Delse, & Bryson, 1967).

More recently, Van Gucht and colleagues (Van Gucht, Baeyens, Vansteenwegen, Hermans, & Beckers, 2010; Van Gucht et al., 2008) developed a paradigm testing Pavlovian acquisition, extinction and renewal in normal weight individuals. In this paradigm, two neutral

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stimuli (serving trays, CSs) are repeatedly presented, one of which (CS+) is consistently paired with chocolate consumption (US) while the other (CS–) is never paired with eating chocolate. In this model, craving and expectancy to eat chocolate, as well as indirect measures of approach and avoiding tendencies are measured. Self-reported craving is reliably acquired using this paradigm; however, to date, the subsequent extinction paradigm consistently fails to reduce the acquired differential craving. It is possible that previous learning histories about trays or other cues may have influenced these responses. Furthermore, this model also includes operant conditioning (picking up the chocolate and eating it) and conditioned cephalic phase responses were not examined.

It is important to evaluate these processes in both overweight and normal weight individuals, to further our understanding about cue reactivity and Pavlovian learning, to begin to develop interventions to directly address putative abnormal or hyperactive cue-reactivity in this population. Jansen's cue reactivity model (Jansen, 1998) proposes that those with a history of overeating, such as binge eaters and overweight individuals, exhibit heightened cue reactivity as a function of their history of eating large amounts of food in the presence of certain cues. Cue reactivity research with these populations supports this hypothesis (Epstein, Paluch, & Coleman, 1996; Jansen et al., 2003; Sobik et al., 2005; Temple, Giacomelli, Roemmich, & Epstein, 2007; Tetley, Brunstrom, & Griffiths, 2009). Overeaters and overweight individuals are thought to develop increased cue reactivity through disparate learning histories related to food intake. Recent studies suggest that obese individuals may anticipate and experience abnormal reward processing of hedonic food (Davis & Fox, 2008; Epstein et al., 2007; Stice, Spoor, Bohon, & Small, 2008; Stice et al., 2011; Wang et al., 2001). Furthermore, some of these abnormalities in reward processing may predate obesity while others may be a consequence of habitual overeating (Bello, Lucas, & Hajnal, 2002; Stice et al., 2011).

To date, no group has studied acquisition and extinction of markers of food cue responsivity to hedonic food cues (such as those in our environment today) in overweight and normal weight individuals. Thus, the purpose of this study is to examine differences in Pavlovian acquisition and extinction of behavioral responses (swallowing) to food cues in overweight and non-overweight college students. We hypothesized that overweight participants would demonstrate a stronger conditioned swallowing response to the visual stimulus repeatedly paired with a hedonic taste stimulus compared to lean participants. Secondly, we hypothesized that the conditioned swallowing responses of overweight participants would be more resistant to extinction than those of lean participants.

Methods

Overview of the study

This study used a mixed quasi-experimental design with one between subjects factor (weight group) and two within subjects factors (cue type and trial block). Overweight and normal weight college students were recruited to complete surveys and participate in a laboratory conditioning paradigm. The conditioning paradigm consisted of the presentation of three visual cues on a computer screen (conditioned stimuli (CS)) repeatedly paired with the delivery of 1 ml of chocolate milk (Hershey's chocolate syrup with equal parts whole milk and half and half), Evian water, or no taste cue (unconditioned stimuli (US)). Both the water and no taste USs were included to distinguish between conditioned swallowing elicited by cues predicting presentation of hedonic food cue (chocolate milk) vs. presentation of liquid (water) in the mouth. The acquisition phase consisted of 27 CS–US pairings and the extinction phase consisted of 27 presentations of the visual cues with no US presentations. Swallowing was measured via electromyograph (EMG) during each visual cue presentation at baseline (before cues were paired with US) and during three blocks (of 9 trials) of extinction (total 27).

Participants

Forty-five college students who report liking sweets were recruited for participation through flyers posted in common high-traffic areas on San Diego college campuses, and through Internet resources, including student web forums, e-mail list serves, and Craigslist. The 45 total participants were divided into two groups based on Body Mass Index (BMI). The overweight group consisted of 25 participants (BMI ≥28) and the lean group consisted of 20 participants (BMI ≤24). Prospective participants contacted the researcher via phone or e-mail and took part in an initial phone screen to determine eligibility.

The inclusion criteria included being between the ages of 18 and 26 years old, BMI ≥28 or ≤24, self-reported liking chocolate, and a willingness to participate in a lab paradigm. Participants were excluded from this study due to self-reported dairy or wheat food allergies, self-reported color blindness, self-reported history of any serious psychiatric condition or eating disorder, a score of 16 or greater on the Center for Epidemiological Studies Depression Scale (CES-D), suggesting clinically significant depression, self-reported medical conditions that could affect weight or eating and self-reported current use of any medications that could affect weight or appetite.

Procedure

Eligible participants completed a series of surveys, signed consent forms and then took part in a laboratory conditioning paradigm lasting approximately 1½ hours. Participants were instructed to refrain from eating during the four hours prior to their laboratory appointment.

Participants were seated in a Whisperroom™ in a reclining chair with a computer screen in their view. The CSs were presented on the computer screen (see Fig. 1). Participants received both the experimental hedonic unconditioned stimulus (US) (high-fat chocolate milk) and the neutral US (Evian water) through a gustometer. The gustometer was composed of two computer-controlled programmable syringes that dispensed liquid through Tygon beverage tubing, ensuring consistent volume, rate and timing of taste delivery.

Because the US delivery interferes with accurate assessment of swallowing, we limited measurement of swallowing to two minute test trials either immediately before acquisition training (Baseline) or during the extinction (Block 1, 2 and 3) session. During the acquisition phase, the CS+ was paired with the delivery of one mL of chocolate milk and the $CS-_1$ was paired with the delivery of one mL of Evian water. The $CS-_2$ was presented with no US delivery during the acquisition phase. During the extinction phase, all three CSs were presented without any US delivery. Each participant received the same set of visual cues in the same order; however, CS–US pairings were randomized across participants such that half of the participants received chocolate milk with the blue circle and half received it with the red square. The yellow triangle was always presented with no US delivery. CS–US pairings were not made explicit to participants at any point in the instructions. The acquisition phase consisted of nine presentations of each CS–US pairing in a semi-randomized order for a total of 27 acquisition trials. No CS–US pairing was presented more than two consecutive times. Each acquisition trial lasted 10 seconds and began with a 7.5 second CS presentation followed by 2.5 seconds of US delivery. Each acquisition trial was followed by a 20 sec ITI trial in which the computer monitor was black.

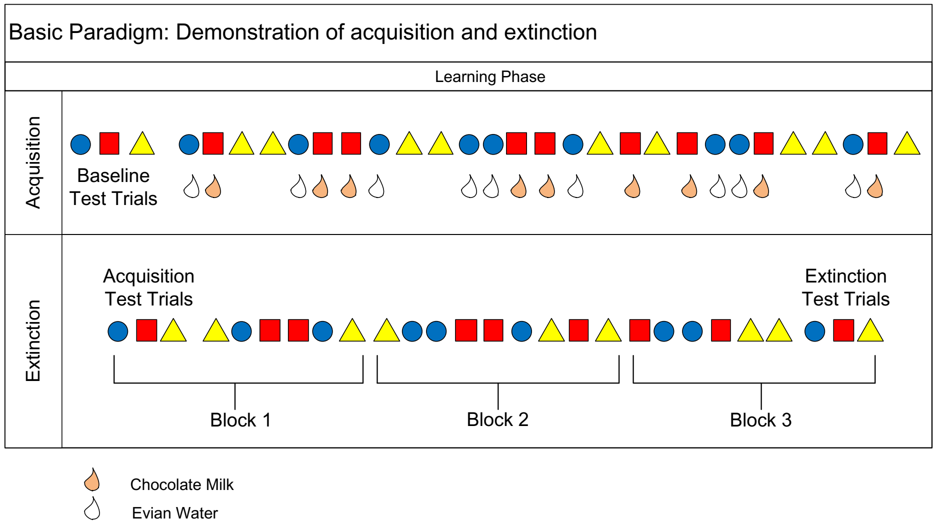

Fig. 1. Basic conditioning paradigm.

During extinction, participants were exposed to nine presentations of each CS type in a semi-randomized order for a total of 27 extinction trials. Each extinction trial lasted 10 seconds and began with a 7.5 second CS presentation followed by 32.5 second ITI in which the computer monitor was black. Participants were compensated $20 for each hour of laboratory participation.

Measures

Swallowing

Swallowing frequency was measured by EMG recordings as a non-invasive estimate of salivation (Nederkoorn, Smulders, & Jansen, 1999). EMG swallow responses were recorded using two small Ag-AgCl electrodes attached one centimeter apart along the *musculus digastricus* under the left jaw with a reference point at the left mastoid bone. EMG data were sampled at 250 Hz using an SR-Lab EMG amplifier (San Diego Instruments, San Diego, CA) and filtered at collection with a band pass filter of 10 and 300 Hz. Rectified EMG measures were digitized as microvolts (µV) and recorded over the 40 second measurement period during and immediately following the visual CS presentation during the baseline and extinction session only. The recordings were limited to these time points to avoid counting swallowing events of the actual liquid USs. The experimenter recorded artifacts such as coughing and verbalizations and EMG responses accounted for by such artifacts were not analyzed as swallow responses.

EMG swallows were coded by a rater blind to group and CS type (Nederkoorn et al., 1999). Criteria for swallow event detection was adjusted slightly to take into account individual differences in EMG response between subjects. A swallow event showed a gradual increase, peak and decrease of electrical activity and this activity is discernible from baseline electrical activity, which typically measured approximately 0.5 µV. The peak of a swallow event typically met or exceeded two µV and lasted 1–3 sec long. A second rater blind to group coded twenty percent of the data and the inter-rater reliability was high, Pearson's R = .998, Kappa = .959, suggesting reliability of the criteria used for coding swallowing events.

Anthropometrics

Height was measured to the nearest one millimeter (mm) using a portable Schorr height board (Schorr Inc., Olney, MD). Weight was measured to the nearest 0.1 kilogram (kg) using a Tanita Digital Scale (model WB-110A). Each measure was taken in duplicate and the average value will be used in the analyses. BMI was calculated as the weight (kg) divided by the height (m) squared.

Center for Epidemiological Studies Depression Scale (CES-D)

Symptoms of depression were assessed with the CES-D, a 20-item self-report Likert scale depression inventory focused on depressive symptoms within the past week, yielding a total score ranging from 0 to 60, with higher scores indicating the presence of greater depressive symptomatology (Radloff, 1977). Participants with a score of 16 or higher were excluded from participation due to the potential of clinically significant depression (Radloff, 1977).

Three Factor Eating Questionnaire (TFEQ)

The TFEQ is a 51-item self-report measure with three scales designed to assess cognitive restraint, disinhibition, and hunger as dimensions of human eating behavior (Stunkard & Messick, 1985). The cognitive restraint scale is designed to assess restraint of food intake with the intent to control body weight or shape. The disinhibition scale measures loss of control over eating and the hunger scale is designed to assess feelings of hunger. Both the cognitive restraint and the disinhibition scale significantly discriminate between dieters and non-dieters (Stunkard & Messick, 1985).

Demographics questionnaire

Participants were given a demographics questionnaire on which they were asked to self-report their age, gender, race, ethnicity, level of education, income, height, and weight in an online demographics survey. In addition, questions regarding the participant's marital status, parental status, eyesight, food allergies, medical history, psychiatric history, current medications, and liking of chocolate were asked during a five-minute phone screen to determine initial eligibility for participation in the study.

Analyses

Prior to analyses, the data were visually inspected to assess for participants with no measurable swallow response across the entire recording period either before or immediately after acquisition (baseline and block 1 respectively), which may be an indication of technical difficulties and low signal to noise ratios for a given individual. EMG swallowing data from twelve participants were not used in analyses due to failure to demonstrate any measurable EMG swallow response to the CS+ during baseline or during Block 1 due

Table 1
Participant characteristics.

		Lean (n = 17) % (n)		Overweight (n = 16) % (n)	
Gender	Male	29 (5)		37 (6)	
	Female	71 (12)		63 (10)	
Race	Caucasian	59 (10)		19 (3)	
	Asian	18 (3)		19 (3)	
	Hispanic	12 (2)		43 (7)	
	African American	6 (1)		19 (3)	
	Native American	6 (1)		0 (0)	
Education	Some high school	0 (0)		6 (1)	
	High school diploma	12 (2)		6 (1)	
	Some college	41 (7)		63 (10)	
	Bachelor's degree	35 (6)		25 (4)	
	Graduate degree	12 (2)		0 (0)	
Income	<$20K	76 (13)		63 (10)	
	$20K–$40K	12 (2)		19 (3)	
	$40K–$60K	12 (2)		6 (1)	
	$60K–$80K	0 (0)		12 (2)	
		M	*SD*	*M*	*SD*
BMI		21.66*	1.84	33.29*	6.41
Age		23.12	2.26	22.06	2.02
CES-D		5.00	3.74	4.13	3.61
TFEQ		17.06	7.05	20.88	8.57
	TFEQ Cog. restraint	8.18	4.59	7.75	4.16
	TFEQ Disinhibition	4.00*	1.94	6.31*	3.22
	TFEQ Hunger	4.88	3.53	6.81	4.22

BMI, Body Mass Index; CES-D, Center for Epidemiological Studies Depression Scale; TFEQ, Three Factor Eating Questionnaire.
* $p < .05$.

to low signal to noise ratios. The final dataset for analyses contained a total of 33 participants (Overweight = 16, Lean = 17; see Table 1 for demographics and Fig. 1a and b for swallowing at each time point).

Data reduction: To determine the best CS– control for the responses to the CS+ paired with chocolate milkshake we compared the swallowing responses to the CS_{-1} and CS_{-2}, to assess whether there were swallowing differences when water or no CS was presented. The number of swallows at Baseline and at Block 1 were compared using a 2 (condition: water and no CS) × 2 (time; Baseline and Block 1) repeated-measures ANOVA (see Fig. 2). The Condition × Time interaction effect was not significant, $F(1, 32) = .388$, *NS*. The tests associated with the Condition ($F(1, 32) = .168$, *NS*) and Time ($F(1, 32) = .214$, *NS*) main effects were also nonsignificant. Because there were no differences, we chose water as the CS_{-} comparison.

To measure the amount of conditioned swallowing to the hedonic food cue specifically, we then derived a difference score by calculating the average number of swallows induced by the CS+ trials in each test block and subtracting the average number of swallows in the CS– trials in each respective block.

Statistical Models: Using this difference score as the outcome measures we tested two hypotheses. First, we tested the hypothesis that acquisition of a response among overweight participants would be demonstrated by a significantly greater number of swallows among overweight participants to the cues paired with chocolate compared to cues paired with water than lean participants immediately following acquisition training (i.e. Block 1). Second, we hypothesized that the swallowing response for the cues paired with chocolate compared to cues paired with water would demonstrate a resistance to extinction in overweight compared to lean participants.

Demographic characteristics of the sample (age, gender, race, ethnicity, income, and level of education) as well as socio-cognitive variables (CES-D score, dietary restraint, disinhibition, and hunger) were examined to determine baseline group equivalence and the presence of any moderators.

Results

Differential swallowing in response to ($CS+ - CS_{-1}$) in overweight and lean individuals

Differences in swallowing to cues paired with chocolate milkshake and cues paired with water in the overweight group compared to the lean group was evaluated with a 2 × 2 repeated-measures ANOVA (see Fig. 3). Group (overweight and lean) was the between subjects factor and Block (Baseline and Block 1) was the within subjects factor, with the difference between swallowing for the CS+ and CS_{-1} as the dependent variable. The Group × Block interaction effect was significant, $F(1, 31) = 5.235$, $p = .029$. The test associated with the Block main effect was significant ($F(1, 31) = 10.526$, $p = .003$) while the Group main effect was not significant ($F(1, 31) = 2.045$, *NS*).

A paired-samples *t* test was conducted for the lean and obese groups separately to follow up on the significant interaction. Using the Bonferroni method to control for family-wise error rate at the .05 level, the alpha was corrected for significance $p < .025$. In the lean group, there was no significant difference between chocolate and water swallowing at Block 1 ($M = .412$, $SD = 2.399$) compared to Baseline ($M = -.118$, $SD = 1.576$) $t(16) = -1.027$, *NS*. Conversely, in the overweight group, there was a significantly greater difference between chocolate and water swallowing at Block 1 ($M = 2.688$, $SD = 3.962$) compared to Baseline ($M = -.375$, $SD = 1.708$), $t(15) = -3.057$, $p = .008$. These findings show that for the overweight group, swallowing in response to chocolate versus water significantly increased from Baseline to Block 1.

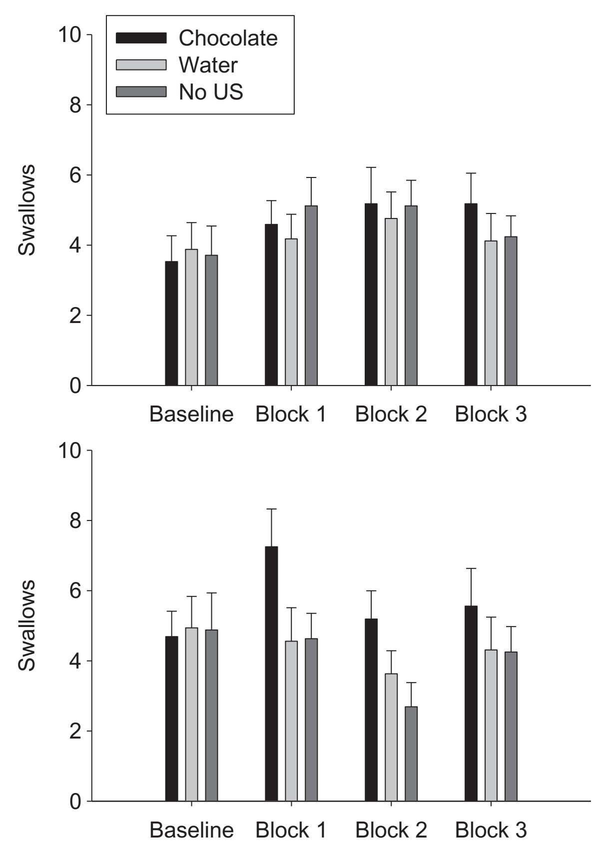

Fig. 2. Swallows by condition in lean (above) and overweight (below) participants over time.

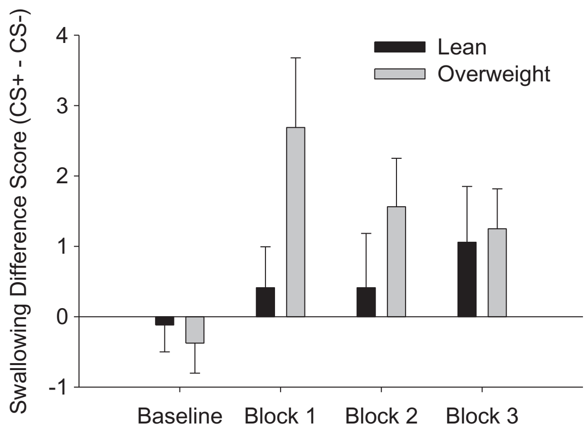

Fig. 3. Swallowing difference score (CS+ – CS₋) for lean and overweight group over time.

Extinction of swallowing in response to (CS+ – CS₋₁) in overweight individuals

Because the lean group did not acquire a response, we were unable to look at extinction in that group. So, we conducted a 2 × 3 repeated-measures ANOVA to test the hypothesis that overweight participants would demonstrate a resistance to extinction by a lack of significant changes in swallowing to the cues paired with chocolate compared to cues paired with water from Block 1 to Block 3 (see Fig. 2). The dependent variable was the number of swallows. The within-subjects factors were Condition (water and no CS) and Block (Block 1, Block 2, and Block 3). The Condition × Block interaction effect was not significant, $F(2, 14) = .763$, *NS*. The tests associated with the Condition main effect was significant, $F(2, 15) = 15.647$, $p = .001$. Specifically, there were significantly more swallows paired with the chocolate cue ($M = 6.000$, $SE = .841$) than the water cue ($M = 4.167$, $SE = .718$). There was no significant main effect of Block, $F(2, 14) = 1.712$, *NS*. These data show that there was not a significant decrease in chocolate and water swallowing for overweight participants during the extinction phase, but that overall, participants swallowed more to chocolate cues than to water cues.

Evaluation of moderators associated with responding

The demographics and socio-cognitive characteristics of the overweight and lean groups were found to be equivalent at baseline with the exception of the Disinhibition scale of the TFEQ such that overweight participants ($M = 6.31$, $SD = 3.22$) endorsed greater food disinhibition than lean participants ($M = 4.00$, $SD = 1.94$) at baseline, $F(1, 31) = 6.34$, $p = .017$. We evaluated TFEQ Disinhibition as a moderator; however, the non-significant Group × Disinhibition interaction, $F(1, 29) = .08$, *NS*, suggests that Disinhibition does not moderate the relationship between group and EMG swallowing response.

Discussion

This is the first study of which we are aware that rigorously tested Pavlovian conditioning to hedonic food cues compared to water in overweight and lean subjects. The results indicated that overweight participants, compared to lean participants, acquired a conditioned swallowing response to the chocolate compared to water. By comparing the swallowing to chocolate to swallowing to water, we are able to distinguish the differential swallowing for a hedonic chocolate milkshake instead of swallowing because something is placed in the mouth. These results are the first to demonstrate that overweight participants acquire a stronger response to hedonic cues compared to lean individuals.

Significant acquisition of a conditioned response among overweight but not lean participants supports previous literature that overweight individuals demonstrate greater reactivity to hedonic food cues than lean individuals as measured by self-reported desire to eat, craving, attention to the cues, affect change, as well as desired and actual portion size (Jansen et al., 2003; Sobik et al., 2005; Tetley et al., 2009). Overweight participants may have acquired the conditioned swallowing response to the chocolate-predicting cue more easily than lean participants due to the heightened food reward or reward specific learning (Davis, Strachan, & Berkson, 2004; Stice et al., 2008; Volkow et al., 2011). Research suggests that obese individuals experience increased reactivity to the hedonic properties of food as a result of biologically determined differences in dopamine reward circuitry (Davis et al., 2009; Epstein et al., 2007; Stice & Dagher, 2010). Our study adds to this body of literature and indicates that overweight participants in our study swallowed more to innocuous cues after only 9 pairings between the chocolate milk and the innocuous cue compared to the same amount of pairings with water.

We were only able to examine extinction among overweight participants as the lean participants did not acquire a response. Because the extinction paradigm could not be examined among lean participants, no group comparisons could be made so we are unable to determine whether the extinction paradigm failed to extinguish the conditioned swallow response because the hedonic food learning of overweight participants was resistant to extinction or because the paradigm itself was inadequate. If the overweight participants' failure to extinguish the swallowing response is related to challenges in extinction, then these results would support previous literature in which obese individuals have been shown to habituate slower to food cues (Epstein et al., 1996). This result would also support previous findings in which an extinction paradigm did not produce significant reductions in subjective ratings of cue-induced craving, while counterconditioning was effective in reducing cravings (Van Gucht et al., 2010). Previous literature supports the efficacy of extinction in reducing subjective craving, salivation and cue-induced overeating (Boutelle et al., 2011; Van Gucht et al., 2008). Further research is needed to evaluate whether a more effective extinction procedure (e.g. increased unpaired presentations) will enable obese subjects to extinguish conditioned cephalic responses to appetitive foods.

Groups were found to be equivalent at baseline on all demographic and socio-cognitive variables except the Disinhibition subscale of the Three Factor Eating Questionnaire (TFEQ). Overweight participants scored significantly higher at baseline on the disinhibition scale, which measures loss of control over eating and significantly discriminates between dieters and non-dieters (Stunkard & Messick, 1985). Disinhibition was evaluated as a moderator but no Group × Disinhibition interaction was found, suggesting that this group difference at baseline does not moderate the relationship between BMI and conditioned swallowing.

It is also interesting that there were no significant differences between the acquisition of swallowing responses in any of the participants for the cues associated with water or the lack of a taste stimuli. We included three conditions in this study to be able to distinguish conditioned responding to the hedonic features of chocolate milk vs. responding to liquid (water) being placed in the mouth or no liquid. Based on these results, we can propose that the responses to the cues predicting chocolate milk could be related to the hedonic properties of the taste stimulus, not to swallowing a liquid.

However, we also need to consider that the conditioning paradigm at this stage is limited. In particular, the lean participants had a nonsignificant increase in swallows to chocolate compared to water at the acquisition point. The most parsimonious explanation is that the paradigm needs to be adjusted to include more pairing trials during conditioning to allow the lean group to acquire a conditioned response. Alternatively, the hedonic properties of the US may have been at subthreshold levels for conditioning in lean subjects. More than one ml of the taste stimuli may be needed to develop a true swallowing response to a neutral cue, or the US that we chose (chocolate milk) was not potent enough to drive a detectable change in swallowing in the lean group. Finally, we need to consider that measuring EMG swallows may not be sensitive enough to detect small changes in responding.

In contrast, a conditioned swallowing response was acquired in the overweight group to the cues paired with chocolate milk compared to the cues paired with water, and this response did not significantly decrease during the extinction trials. These findings emphasize the importance of evaluating an extinction paradigm that allows adequate time for the response to extinguish. The possibility that these acquired responses are resistant to extinction and other methods, such as counterconditioning (Van Gucht et al., 2010), still need to be evaluated in future research.

This study addressed the basic science of Pavlovian conditioning and extinction of hedonic cues in a highly controlled laboratory setting, producing high internal validity. Two CS–'s were included in this study to address the internal validity of the hedonic taste condition and demonstrate whether conditioned swallow responses could be attributed specifically to the hedonic properties of the chocolate milk rather than simply anticipation of liquid being introduced into the mouth. The results showed that EMG swallow responses to the visual cue previously paired with water were not significantly different than those previously paired with no US, supporting the attribution of conditioned swallowing to the hedonic properties of the chocolate milk and the internal validity of the CS manipulation. Limitations include the lack of acquisition of responding in the lean group, which suggests that the paradigm may need to be adjusted. It also could suggest that responding in the lean group was an anomaly, since the sample was relatively small. The measurement of swallowing by EMG may include inherent variability, and is not a direct measurement of salivation, although it is highly correlated with salivation (Nederkoorn et al., 1999). While all participants self-reported liking chocolate in order to qualify for this study, there were no direct measurements of self-reported liking of the specific chocolate stimulus used or subjective hunger at the time of the experiment. Group differences in hunger and subjective ratings of the pleasantness of the chocolate milk stimulus could influence the effectiveness of the conditioning paradigm in producing an acquired swallow response. Finally, it is possible that this study was underpowered, as data were lost due to mechanical issues. Future studies should include larger samples, and should consider exploring any differences in acquired responses as related to the perceived magnitude of the US or to differences in learning between groups.

The results of this study pose a number of questions for future research in appetitive Pavlovian conditioning and extinction in humans. This study could be replicated with adjustments made to the conditioning paradigm to examine the effects of changing the taste stimulus, increasing the number of acquisition or extinction trials, extinguishing in different contexts, and adding additional conditioning or extinction sessions on different days. Future studies may also incorporate additional conditioned response variables including subjective ratings of craving and physiological measures such as insulin response, cardiac activity and brain activity. Overall, this study suggests that people who are overweight may have differential learning to food cues, which could drive motivation to overeat. It would be important in future studies to evaluate whether there are differences in Pavlovian learning before weight gain and if any differences in learning can be used to predict risk for overweight and obesity.

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