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Research report

# How food cues can enhance and inhibit motivation to obtain and consume food ☆

Ben Colagiuri [a,b,*], Peter F. Lovibond [b]

[a] School of Psychology, University of Sydney, Australia
[b] School of Psychology, University of New South Wales, Australia

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ABSTRACT

Learning may play an important role in over-eating. One example is Pavlovian-to-instrumental transfer (PIT), whereby reward cues facilitate responding to obtain that reward. Whilst there is increasing research indicating PIT for food in humans, these studies have exclusively tested PIT under instrumental extinction (i.e. when the food is no longer available), which may reduce their ecological validity. To address this, we conducted two experiments exploring PIT for food in humans when tested under instrumental reinforcement. Participants first underwent Pavlovian discrimination training with an auditory cue paired with a chocolate reward (CS+) and another auditory cue unpaired (CS–). In instrumental training participants learnt to press a button to receive the chocolate reward on a VR10 schedule. In the test phase, each CS was presented whilst participants maintained the opportunity to press the button to receive chocolate. In Experiment 1, the PIT test was implemented after up to 20 min of instrumental training (satiation) whereas in Experiment 2 it was implemented after only 4 min of instrumental training. In both experiments there was evidence for differential PIT, but the pattern differed according to the rate of responding at the time of the PIT test. In low baseline responders the CS+ facilitated both button press responding and consumption, whereas in high baseline responders the CS– suppressed responding. These findings suggest that both excitatory and inhibitory associations may be learnt during PIT training and that the expression of these associations depends on motivation levels at the time the cues are encountered. Particularly concerning is that a food-paired cue can elicit increased motivation to obtain and consume food even when the participant is highly satiated and no longer actively seeking food, as this may be one mechanism by which over-consumption is maintained.



## Introduction

Obesity and over-eating are pervasive problems world-wide, with more than half of the adult population in OECD countries being overweight and 18% being obese (OECD, 2013). The high rates of obesity come at substantial cost to both individuals and communities. Obesity is associated with increased risk of type 2 diabetes, coronary heart disease, and hypertension, among other conditions and in 1998 was estimated to cost $99 billion dollars in the US alone (Wolf & Colditz, 1998) with projected costs of up to $860 billion by 2030 (Wang, Beydoun, Liang, Caballero, & Kumanyika, 2008).

Whilst there are a multitude of factors that contribute to over-eating, there is increasing recognition of the role that learning processes may play. Consumption of food is inextricably paired with numerous cues such as the sight, smell, and taste of the food as well as signals for its availability, including packaging, logos, and advertisements. Over time, these food cues can acquire the ability to influence eating behaviour in and of themselves. Cue-induced eating is one such example. Here, a cue previously paired with food can elicit increased consumption relative to neutral or unpaired cues both in humans (e.g. Cornell, Rodin, & Weingarten, 1989; Halford, Gillespie, Brown, Pontin, & Dovey, 2004) and animals (e.g. Boggiano, Dorsey, Thomas, & Murdaugh, 2009; Petrovich, Ross, Gallagher, & Holland, 2007). However, one of the most interesting learning processes that may contribute to over-eating is Pavlovian-to-instrumental transfer (PIT) – a process whereby a reward-cue can increase actions directed at obtaining that and other rewards. PIT is particularly interesting because it involves the transfer of food-cue learning (Pavlovian associations) onto goal-directed action to obtain food (instrumental responding). Thus, whereas cue-induced eating concerns how cues influence consumption when food is already present, PIT concerns how cues can lead individuals to actively seek out food. As such, a better understanding of PIT may lead to ways of preventing individuals at risk of obesity from engaging

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* Corresponding author.
*E-mail address:* ben.colagiuri@sydney.edu.au (B. Colagiuri).

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in maladaptive food-seeking behaviours and thereby prevent over-consumption before the food is even present.

There have been numerous animal studies conducted on PIT for both food and other rewards (see Holmes, Marchand, & Coutureau, 2010 for a review). The standard PIT procedure involves three phases: Pavlovian training, instrumental training, and a transfer test (e.g. Colwill & Rescorla, 1988; Estes, 1943; Holland, 2004). In Pavlovian training, one cue (e.g. tone) is paired with a food reward (e.g. food pellet) whilst another cue (e.g. light) is paired with no reward. In the separate instrumental training, the animal learns to make a response (e.g. lever press) in order to obtain the food reward. Then, in the transfer test, each cue is presented whilst the animal has the opportunity to make the instrumental response. PIT occurs when the food-paired cue induces greater instrumental responding than the unpaired cue in the test phase. Further, there is evidence that the PIT effect can be both outcome specific and outcome non-specific, such that a food-paired cue can not only induce greater responding to obtain that specific food reward (specific PIT), but can also induce greater responding to obtain other food rewards (general PIT: Colwill & Rescorla, 1988; Corbit & Balleine, 2005; Delamater, 1996), suggesting two distinct motivational effects.

Whilst most research on PIT has been conducted in animals, there are a growing number of studies demonstrating this phenomenon in humans (e.g. Allman, DeLeon, Cataldo, Holland, & Johnson, 2010; Bray, Rangel, Shimojo, Balleine, & O'Doherty, 2008; Hogarth, 2012; Hogarth & Chase, 2011; Nadler, Delgado, & Delamater, 2011; Prévost, Liljeholm, Tyszka, & O'Doherty, 2012; Rosas, Paredes-Olay, García-Gutiérrez, Espinosa, & Abad, 2010; Talmi, Seymour, Dayan, & Dolan, 2008; Watson, Wiers, Hommel, & de Wit, 2014). Whilst most human PIT studies use symbolic rewards (e.g. points, money), some have also used food rewards (Bray et al., 2008; Lovibond & Colagiuri, 2013; Watson et al., 2014). For example, we recently developed a procedure in which participants first learnt associations between different coloured lights and a chocolate reward and then were separately trained to press a button to receive the same chocolate reward (Lovibond & Colagiuri, 2013). In the transfer test, we found a strong PIT effect whereby presentation of the chocolate-paired cue led to a much higher rate of button pressing than the unpaired cue. An important feature of this procedure was that the participants consumed the chocolate rewards throughout the experiment and were free to either respond or not respond during the transfer test. This seems to indicate a genuine motivational effect induced when using a natural-high value food reward, which may explain how food cues contribute to over-consumption.

However, one potential limitation in terms of the applicability of existing PIT research to eating behaviour is that the transfer test is almost always carried out under instrumental extinction, i.e. when the instrumental response no longer leads to food. Whilst this is an intentional design feature of these studies aimed at reducing any ceiling effects that could occur if responding during the test was too high, whereby no facilitation could be observed, it does make it difficult to determine whether PIT can induce food seeking when the food is still available, as is the case outside of the laboratory. To date, only a handful of studies have investigated PIT when tested under instrumental reinforcement and these have been conducted exclusively in animals. The results of these studies have been mixed, with some finding that food-paired cues enhance instrumental responding (Edgar, Hall, & Pearce, 1981; Hamm & Meltzer, 1977; Meltzer & Brahlek, 1970) and others finding that food-paired cues actually inhibit responding (Azrin & Hake, 1939; Lovibond, 1981; Soltysik, Konorski, Holownia, & Rentoul, 1976). Thus, it is currently unclear whether PIT can be observed in humans when tested under more naturalistic conditions in which the response is reinforced and whether any such effect is facilitatory or inhibitory. Testing under reinforcement may be particularly important given that outside of the laboratory, food cues are likely to be most often encountered when the food is still available to obtain, not under extinction. An example would be seeing a pizza advertisement when a viewer knows he or she can order pizza and have it delivered soon after. By contrast, testing under extinction would be more akin to seeing a pizza advertisement after multiple attempts to order pizza without it being delivered.

To address this gap, we conducted two experiments using a standard PIT design with a chocolate reward, but with the transfer test conducted under instrumental reinforcement, such that the participants could still earn chocolate during the test phase. In the first experiment, we allowed a natural reduction in responding due to satiation before implementing the PIT test, whereas in the second experiment we implemented the PIT test fairly soon into instrumental training, when satiation was lower. If there is no PIT effect or an inhibitory one when tested under reinforcement, then it would seem unlikely that PIT could contribute to over-consumption of food. On the other hand, if PIT does produce facilitation under these circumstances, then it seems quite likely that it could be an important mechanism in the maintenance of maladaptive eating behaviours and that these cues could serve as points of intervention. To our knowledge, this is the first study investigating PIT under instrumental reinforcement in humans.

## Experiment 1

The first experiment used a very similar design to our previous work in this area involving a chocolate reward (Lovibond & Colagiuri, 2013). The critical difference was that the transfer test was conducted under instrumental reinforcement. To attempt to avoid potential ceiling effects, in Experiment 1, we implemented the transfer test after a natural reduction in responding (4 min no response) or after 20 min cumulative time irrespective of response rate, which are comparable parameters to those used in animal studies (e.g. Lovibond, 1981).

### *Methods*

#### *Participants*

Eighty-one first year undergraduates from the University of Sydney participated. Fifty-six were first year psychology students who participated in return for partial course credit whilst the remaining 25 were recruited on a university volunteer website and were reimbursed AUD$15 for their participation. In both cases, the advertisement described the study broadly as investigating responses to eating chocolate and associated stimuli, and participants self-selected to enrol in the study. Overall, there were 48 females (64.9%) and participants had a mean age of 19.3 (SD = 1.5). Participants were asked to abstain from eating any food for 3 h prior to the experiment and from eating chocolate for 24 h prior to the experiment. In order to confirm this, two questions were included in the demographic questionnaire asking participants to report the last time they had eaten any food and the last time they had eaten chocolate, without any reminder of the eligibility criteria. Participants were excluded if they were currently dieting. All study procedures were approved by the University of New South Wales Human Research Ethics Committee (HREC) and ratified by the University of Sydney HREC.

#### *Materials*

Participants were seated at a desk in a 2 m × 2 m testing cubicle, facing a 61 cm computer monitor. A keyboard was placed immediately in front of the participant and had every key removed except for the space bar. On the desk to the left of the monitor was a Med Associates M&M's dispenser Model ENV-702 on a pedestal mount, inside a 210 mm × 170 mm × 330 mm sound attenuating plywood box. A clear 20 mm diameter plastic tube delivered individual M&M

**Table 1**
Study design.

| Phase 1: Pavlovian training | Phase 2: Instrumental training | | Phase 3: Transfer test |
|---|---|---|---|
| 6 × A + → Choc<br>6 × B – → No choc<br>(Intermixed, button press inactive) | Button press → Choc<br>(Button press rewarded, VR10) | 4 min no response or 20 min total elapsed time | 2 × A<br>2 × B<br>(Intermixed, button press rewarded, VR10) |

chocolates through a hole in the plywood box into a plastic container within easy reach of the participant's left hand. Stereo speakers (Logitech, Model Labtec S-120) sat on either side of the monitor facing the participant and were used to deliver the instructions and the two auditory cues: 180 Hz (low) tone and 440 Hz (high) tone. In the adjoining control room, a desktop computer with Matlab software was used to present the stimuli and chocolate, and to record button presses.

*Design and procedure*

After providing informed consent, participants provided written demographic data, answered questions about time since eating chocolate and other food, and were asked to rate how much they felt like eating chocolate (cravings) on a 100 mm VAS from 0% (not at all) to 100% (I crave it). They were then seated in the test room with their attention drawn to the monitor, keyboard, speakers, and chocolate dispenser. The experiment followed a three phase PIT design, as shown in Table 1. The design followed Lovibond and Colagiuri (2013) with the critical exception being that the transfer test was conducted under instrumental reinforcement, i.e. with the button press still leading to chocolate rewards.

The first phase involved learning the cue–chocolate relationships (Pavlovian training). Pre-recorded voice instructions informed participants that they would hear different sounds and that some of the sounds would be followed by chocolate. They were told that they should eat the chocolate as soon as it was delivered and could not take any with them after the experiment. A differential conditioning procedure was then implemented in which the low and high tones (counterbalanced) served as the two cues: Cue A and Cue B. Each cue was presented for 10 s. Cue A was always followed by delivery of a single M&M chocolate, whereas Cue B was presented with no outcome. On Cue A trials, the chocolate dispenser was activated 8 s after CS onset. Delivery of the chocolate took approximately 1 s, therefore, the chocolate was usually consumed in the last second of cue presentation. There were a total of 12 trials, six of each of the two cues. The order of the trials was randomised with the constraint that no more than two of the same trial occurred in a row. The intertrial interval (offset to onset) varied randomly between 15 and 35 s.

The second phase involved learning the response–chocolate relationship (instrumental training). Participants were instructed via voice recording that they could now press the space bar in order to receive chocolate. They were told that they could press as often or as little as they liked and that they may have to press the space bar multiple times to earn a single chocolate. Button pressing was rewarded on a variable ratio (VR) 10 schedule in which on average, 10 (range 5 to 15) presses were required before a chocolate was delivered. To help shape participants' button pressing, we faded the VR schedule in over the first three trials (fixed ratios 2, 4, 6). Each button press, whether rewarded or not, led to the presentation of a small black square (2.5 mm × 2.5 mm) on the monitor. If the participant had not received three chocolates in the first 10 min, then the experimenter entered the room and informed them that they may need to press the button several times in order to receive chocolate.

The final phase was the transfer test. It began after the participant voluntarily stopped responding for 4 min or after a total of 20 min from the beginning of instrumental training, regardless of responding, whichever occurred first. The transfer test involved four test trials, two of each cue. Each trial lasted 90 s with button pressing recorded in 5 s bins throughout. The first 30 s constituted baseline responding, with the relevant cue being presented at 30 s for 10 s. The test trials were run in two blocks of two trials (one of each cue), with the order of cues randomised within blocks. The intertrial interval randomly varied from 90 to 110 s. Importantly, the transfer test was conducted under instrumental reinforcement such that participants could still earn chocolate by pressing the button on the same VR10 schedule as instrumental training. There were no additional instructions provided for the transfer test. In all stages of the experiment, delivery of a chocolate was accompanied by presentation of the word "Chocolate" in the centre of the computer monitor for 1 s. A live video stream into the room was used to ensure that participants were consuming each chocolate as it was delivered throughout the experiment. After the transfer test, participants rated their cravings and completed the post-experimental questionnaire to assess their knowledge of the cue–chocolate relationships. All participants were debriefed at the end of the experiment.

*Data handling and analysis*

Participants (n = 21) were excluded if they failed to earn at least four chocolates during instrumental training, which was the point at which the VR10 schedule began. Failure to earn four chocolates was always due to satiation. Three participants were excluded for failing to abstain from eating 3 h before the experiment. A further five participants were excluded *ad hoc*; three failed to follow instructions during the experiment and two had computer errors that led to the dispenser failing to deliver chocolate during Pavlovian training. The pattern of results was identical when these five participants were included.

Consistent with our previous work (Lovibond & Colagiuri, 2013), we compared responding in the 30 s before and after cue onset. Thus, for the remaining 52 participants, the total number of responses during the transfer test was calculated for each cue in the 30 s before the relevant cue appeared (baseline) and for the period from CS onset to 30 s after onset (post) summed across the two test presentations. These data were then analysed via a 2 × 2 repeated measures ANCOVA with cue and time as factors and gender as a covariate. The critical test of PIT here was the interaction, which tested whether the two cues elicited differences in responding. These were followed by tests of simple effects comparing responding before and after the cues for each cue separately, controlling for gender. The fact that the transfer test was conducted under instrumental reinforcement meant that we were also able to test whether the cues affected actual consumption. This involved identical analysis to button presses, but with the number of chocolates consumed in the relevant periods as the dependent variable. Finally, a repeated measures ANCOVA compared chocolate cravings at the beginning and end of the experiment, controlling for gender. Covariates were mean centred to avoid multicollinearity. All analyses were conducted in IBM Statistics (v. 20) with $\alpha = .05$.

*Results*

During instrumental training participants made an average of 292 (SD = 271) button presses to receive 29.6 (SD = 25.6)

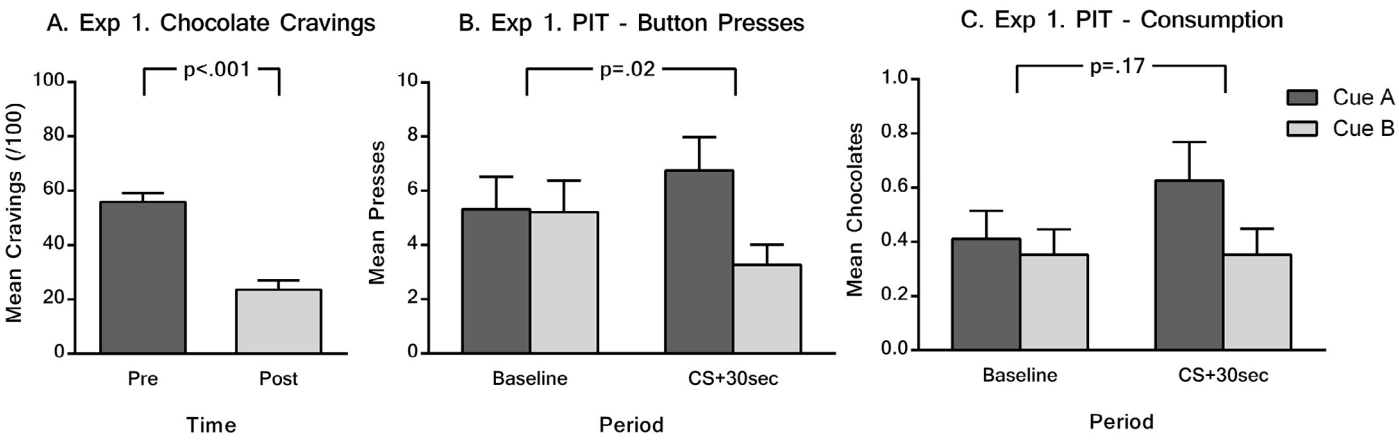


**Fig. 1.** Experiment 1. (A) Covariate adjusted mean (+SE) cravings at the beginning and end of the experiment. (B) Covariate adjusted mean number of responses in the transfer test during baseline and from CS onset to 30 s for the chocolate paired cue (Cue A) and the unpaired cue (Cue B). (C) Covariate adjusted mean number of chocolates consumed in the transfer test during baseline and from CS onset to 30 s for each cue. For B and C, p-values are for the interaction between cue and time.

chocolate rewards. During the test phase they made a total of 43.7 (SD = 46.1) presses to earn a total of 4.0 (SD = 4.3) chocolates. Including Pavlovian training, this meant that the average total number of chocolates consumed in the experiment was 39.6 (SD = 27.4). As shown in Fig. 1A, there was a statistically significant reduction in chocolate cravings from 55.9 out of 100 at the beginning of the experiment to 23.5 by the end of the experiment, $F_{1,50} = 52.0$, $p < .001$.

Mean response rates during the transfer test are presented in Fig. 1B. A repeated measures ANCOVA revealed no main effect of either cue or time, $F_{1,50} = 2.98$, $p = .09$ and $F < 1$, respectively. There was, however, a statistically significant interaction between cue and time, indicating that response rates differed over time according to which cue was presented, $F_{1,50} = 5.43$, $p = .02$. Analysis of simple effects indicated that whilst responding to Cue A increased by 1.42 (SD = 10.7) presses from baseline to 30 s after CS onset, this difference was not statistically significant $F < 1$. Responding to Cue B decreased by 1.94 (SD = 7.94) presses from baseline to 30 s after CS onset, which approached but did not reach statistical significance, $F_{1,50} = 3.11$, $p = .08$.

Mean number of chocolates consumed during the transfer test is shown in Fig. 1C. The pattern of consumption was fairly similar to button presses, with neither a main effect of cue nor time, $F_{1,49} = 2.85$, $p = .10$ and $F < 1$. However, in this case there was no interaction between cue and time nor any significant simple effects in terms of cue elicited consumption, highest $F_{1,49} = 2.05$, $p = .16$, suggesting that the differential effect of the cues of button pressing did not translate into differences in consumption.

*Discussion*

Experiment 1 found evidence of a PIT effect to food cues in terms of button presses with a transfer test conducted under instrumental reinforcement. Somewhat surprisingly, the PIT effect appeared to be driven more by suppression to the unpaired cue, rather than facilitation to the food-paired cue. That is, there was a near significant trend for a reduction in responding following the CS– with little evidence of an increase to the CS+. The lack of statistical significance of the suppression of button presses to the unpaired cue may have resulted from a floor effect. At the time of test, baseline response rates were fairly low – approximately five presses/min – presumably due to high levels of satiation at the time the transfer test was implemented. This may have meant that no further suppression of responding could be detected. Further, whilst the consumption data suggested a similar pattern to the button press data, there was no statistically significant evidence that the cues differentially affected actual consumption. But, again, this could have been attributable to floor effects created by low baseline responding during the transfer test.

## Experiment 2

In order to further explore the possibility of inhibitory learning to the unpaired cue, Experiment 2 used an identical design to Experiment 1, with the exception that the transfer test was implemented after 4 min instrumental training, irrespective of response rate. Given that instrumental training and the transfer test were conducted under instrumental reinforcement with no intervening instrumental extinction, the length of training is likely to affect baseline responding during test. Specifically, shorter instrumental training provides less opportunity to satiate and is, therefore, likely to lead to higher baseline motivation to consume chocolate during test, relative to longer instrumental training. Thus, the intention of implementing the transfer test after only 4 min of instrumental training was to reduce satiation during test and thereby increase baseline response rate and consumption such that any inhibitory effects to the unpaired cue could be observed.

*Methods*

*Participants*

Sixty first year psychology students from the University of Sydney participated in return for partial course credit. Thirty-nine (65%) were female and participants had a mean age of 20.0 (SD = 3.12). Selection criteria were the same as in Experiment 1.

*Materials*

The materials were identical to those used in Experiment 1.

*Design and procedure*

The design and procedure was the same PIT design used in Experiment 1, with Pavlovian acquisition, instrumental acquisition, and transfer test under instrumental reinforcement. The only difference was that the transfer test was implemented after 4 min of instrumental training irrespective of responding, compared with the up to 20 min instrumental training allowed in Experiment 1.

*Data handling and analysis*

Participants (n = 17) were excluded for failing to earn at least four chocolates during instrumental training, again this was always the

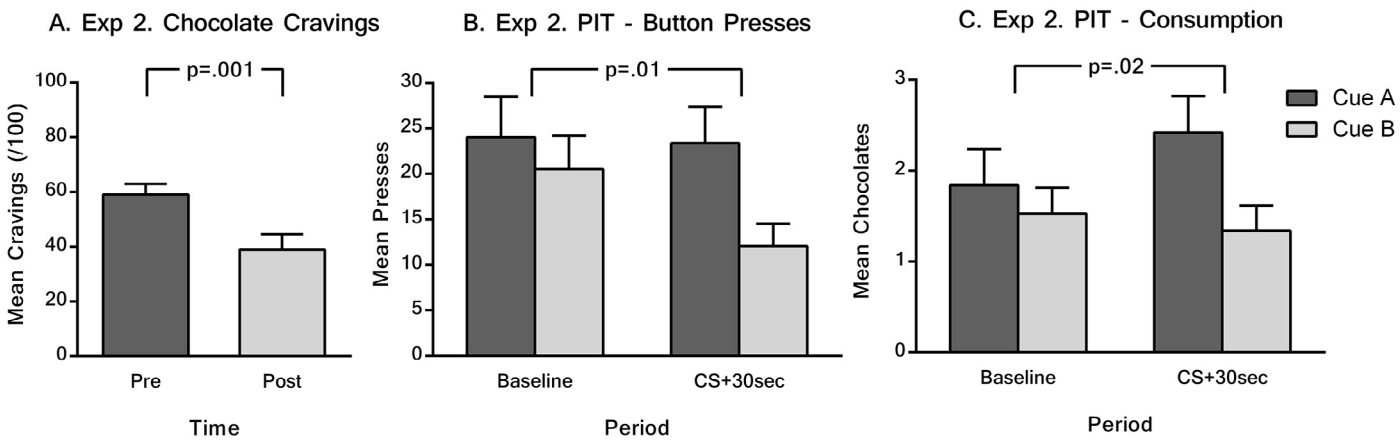


**Fig. 2.** Experiment 2. (A) Covariate adjusted mean (+SE) cravings at the beginning and end of the experiment. (B) Covariate adjusted mean number of responses in the transfer test during baseline and from CS onset to 30 s for the chocolate paired cue (Cue A) and the unpaired cue (Cue B). (C) Covariate adjusted mean number of chocolates consumed in the transfer test during baseline and from CS onset to 30 s for each cue. For B and C, p-values are for the interaction between cue and time.

result of satiation. Three were excluded for failing to abstain from food 3 h prior to the experiment. A further two participants were excluded *ad hoc*: one who failed to follow instructions and one for whom there was technical error such that chocolate was not delivered during Pavlovian training. The pattern of results was identical when these two participants were included. Data handling and analysis of the remaining 38 participants were identical to Experiment 1.

*Results*

In Experiment 2, during instrumental training participants made an average of 134 (SD = 129) button presses to receive 14.8 (SD = 12.3) chocolates. During the test phase, they made an average of 173 (SD = 206) presses to receive 16.2 (SD = 19.3) chocolates. Including Pavlovian training, the average total number of chocolates consumed in the experiment was 37.1 (SD = 29.8). Cravings for chocolate reduced significantly from 59.0 out of 100 at the beginning of the experiment to 39.0 at the end of experiment, $F_{1,36} = 12.7$, $p = .001$, as shown in Fig. 2A.

Mean response rates during the transfer test are presented in Fig. 2B. Participants averaged a total of 22.3 responses in the baseline periods preceding each cue during the transfer test. This level of baseline responding was statistically significantly higher than in Experiment 1, $t_{115} = 3.95$, $p < .001$. A repeated measures ANCOVA revealed a statistically significant main effect of cue, with less overall responding for Cue B than Cue A, $F_{1,36} = 14.0$, $p = .001$. There was also a significant main effect of time, with less overall responding following cue presentation, $F_{1,36} = 4.28$, $p = .046$. These main effects were qualified by a statistically significant interaction between cue and time, such that the lower responding to Cue B was significantly more marked in the 30 s period following cue presentation than before presentation, $F_{1,36} = 6.89$, $p = .01$. As in Experiment 1, simple effects analysis revealed no statistically significant change in responding to Cue A from baseline to 30 s after CS onset, $F < 1$, but in this case there was a statistically significant reduction of 8.47 (SD = 17.8) button presses following presentation of Cue B, $F_{1,36} = 8.65$, $p = .006$, suggesting an inhibitory PIT effect to this cue for button presses.

Mean number of chocolates consumed are presented in Fig. 2C. The pattern of consumption was somewhat different to the pattern of button pressing. Here, there was also a significant main effect of cue with overall less consumption on Cue B trials than Cue A trials overall, $F_{1,36} = 14.4$, $p = .001$, and a significant interaction with time suggesting that the cues differentially influenced consumption, $F_{1,36} = 5.53$, $p = .02$. However, in this case, the simple effects revealed no significant reduction in consumption to Cue B from baseline to 30 s after CS onset, $F < 1$, but there was a statistically significant increase in the consumption of .58 chocolates following presentation of Cue A, $F_{1,36} = 6.12$, $p = .02$.

*Discussion*

In Experiment 2, we implemented the transfer test sooner than in Experiment 1, which led to higher baseline response rates during the transfer test. Under these circumstances, as in Experiment 1 there appeared to be no significant effect of the chocolate-paired cue on response rates. However, the unpaired cue produced a marked decrease in responding, suggesting an inhibitory PIT effect to this cue. The analysis of actual consumption, however, revealed a different pattern. Here, consumption appeared unaffected by the unpaired cue, but increased to the chocolate paired cue, suggesting a facilitatory PIT effect on consumption.

## High versus low baseline responders

The pattern of results from Experiments 1 and 2 suggested a PIT effect for button presses driven by inhibition to the unpaired cue. The consumption data suggested a different pattern, however, whereby there was no statistically significant effect of the cues on consumption in Experiment 1, but the food paired cue increased consumption in Experiment 2, with no effect of the unpaired cue. A dissociation between button presses and consumption may seem counterintuitive at first. However, it is quite possible that differences in button presses and consumption could result from differences in whether cues influenced motivation to press in general versus motivation to press in order to obtain a chocolate. In the former case, the participant's pressing might increase but not reach the current criterion for reinforcement. In the latter case, a participant's responding would increase until they reached the criterion and obtained a chocolate. Thus even with fairly similar rates of button presses, different patterns of consumption can be obtained.

Nonetheless, the discrepancy between inhibitory PIT for button presses versus facilitatory PIT for consumption is difficult to reconcile in terms of which processes are responsible for producing these effects. One possibility is that there were different subsets of individuals responsible for producing each pattern. This could be the case if differences in motivational state at test affect

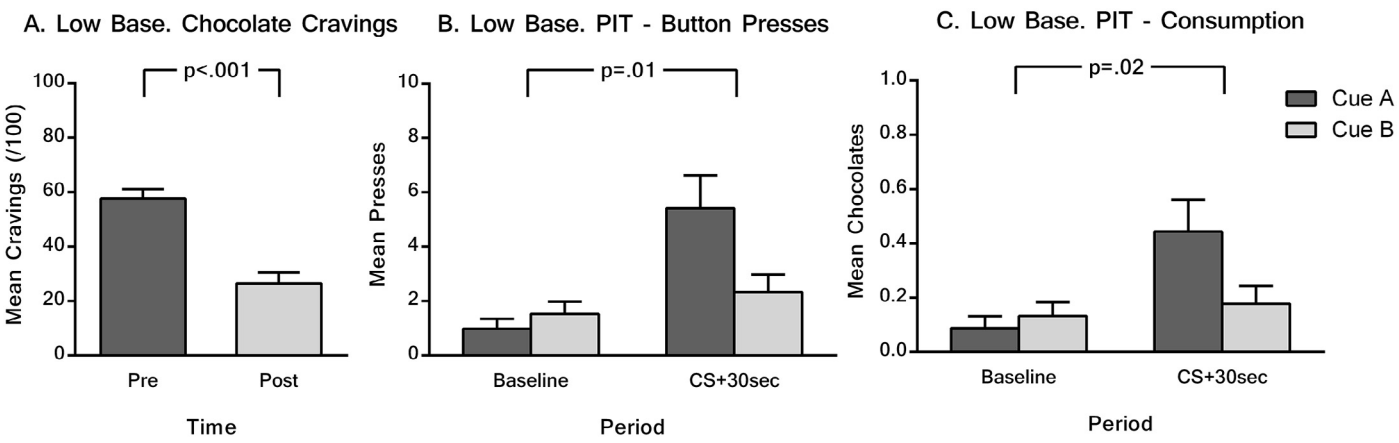


**Fig. 3.** Low baseline responders. (A) Covariate adjusted mean (+SE) cravings at the beginning and end of the experiment. (B) Covariate adjusted mean number of responses in the transfer test during baseline and from CS onset to 30 s for the chocolate paired cue (Cue A) and the unpaired cue (Cue B). (C) Covariate adjusted mean number of chocolates consumed in the transfer test during baseline and from CS onset to 30 s for each cue. For B and C, p-values are for the interaction between cue and time.

what type of PIT effect is observed, particularly if high levels of responding produce ceiling effects whereby facilitation is more difficult to detect and low levels of responding produce floor effects whereby inhibition is difficult to detect. Supporting this possibility, although manipulating the timing of the transfer test across experiments produced differences in baseline responding during the transfer test, over half (n = 28, 53%) of the participants in Experiment 1 did not reach the 4 min no instrumental response criterion. This meant that for those participants, the transfer test was implemented whilst they were still actively seeking and consuming chocolate. Similarly, in Experiment 2, a quarter (n = 10, 26%) of the participants had very low response rates during the transfer test, making a total of less than five button presses before each cue. Thus, despite the manipulation of baseline responding across experiments, different patterns of baseline responding and motivation among participants were evident within each experiment. To explore whether motivational state determines the type of PIT effect, we therefore combined the data from both experiments and analysed participants with low baseline responding during the transfer test separately from those with high baseline responding. The expectation was that a facilitatory PIT effect to the chocolate paired cue would be most likely in low baseline responders, whereas an inhibitory PIT effect to the unpaired cue would be most likely in high baseline responders. To a large extent, this provides a more direct test of the effect of motivational state on PIT than the cross experiment comparison, because it considers the extent to which each individual was actively seeking chocolate at the time of the transfer test.

The 90 participants analysed in Experiments 1 and 2 were classified as either low or high baseline responders. The cut-off was based on the average number of button presses an individual made during the baseline period before presentation of each cue in the transfer test. The median number of button presses was 5.25. Accordingly, we used a cut-off of five or fewer responses for low baseline responders (n = 45) and greater than five button presses for high baseline responders (n = 45). The statistical analysis applied to high and low responders was identical to that employed in Experiments 1 and 2.

*Low baseline responders*

Low baseline responders averaged a total of 204 (SD = 225) button presses to obtain 21.2 (SD = 21.0) chocolates during instrumental training, but averaged only 22.0 (SD = 23.4) presses to obtain 2.1 (SD = 2.2) chocolates during the transfer test. Overall, they consumed 29.3 (SD = 21.2) chocolates over the course of the experiment. Their cravings significantly reduced from 57.6 points at the beginning of the experiment to 26.4 points at the end of the experiment, $F_{1,43} = 46.9$, $p < .001$, as shown in Fig. 3A.

Button pressing data for low baseline responders are presented in Fig. 3B. Here, there was a clear facilitation effect observed to the food paired cue. The repeated measures ANCOVA revealed no main effect of cue, $F_{1,43} = 3.06$, $p = .09$, but a statistically significant main effect of time, with greater responding following cue presentation, $F_{1,43} = 14.5$, $p < .001$. The main effect of time was qualified by a significant interaction in which the increase in responding following cue presentation was significantly more marked for Cue A compared with Cue B, $F_{1,43} = 7.00$, $p = .01$. This was supported by a simple effects analysis, which revealed a statistically significant increase in responding of 4.4 button presses to Cue A from baseline to 30 s after CS onset, $F_{1,43} = 13.2$, $p = .001$, but no significant change in responding following presentation of Cue B, $F_{1,43} = 1.61$, $p = .21$.

Consumption data for low baseline responders are presented in Fig. 3C. As with button presses, there was a clear facilitation effect to the food paired cue for consumption in low baseline responders. Again the main effect of cue was not statistically significant, $F_{1,43} = 2.83$, $p = .10$, but the main effect of time indicated significantly greater consumption following cue presentation, $F_{1,43} = 6.99$, $p = .01$. A significant interaction indicated that the increased consumption following cue presentation was greater for Cue A than Cue B, $F_{1,43} = 4.74$, $p = .04$. The simple effects analysis confirmed the facilitation effect, with Cue A eliciting a statistically significant increase in consumption of .36 chocolates from baseline to 30 s after CS onset, $F_{1,43} = 8.65$, $p = .005$, and no change in consumption for Cue B, $F < 1$. This meant that in the low baseline responders there was a facilitatory PIT effect to the food paired cue both in terms of button presses and consumption, with no effect of the unpaired cue on either.

*High baseline responders*

High baseline responders averaged a total of 246 (SD = 245) button presses to obtain 25.5 (SD = 23.4) chocolates during instrumental training and 175 (SD = 186) presses to obtain 16.2 (SD = 17.4) chocolates during the transfer. This meant that they consumed a total of 47.8 (SD = 31.5) chocolates including the Pavlovian phase. As shown in Fig. 4A, their cravings reduced significantly from 56.7 points at the beginning of the experiment to 33.7 points at the end of the experiment, $F_{1,43} = 17.9$, $p < .001$.

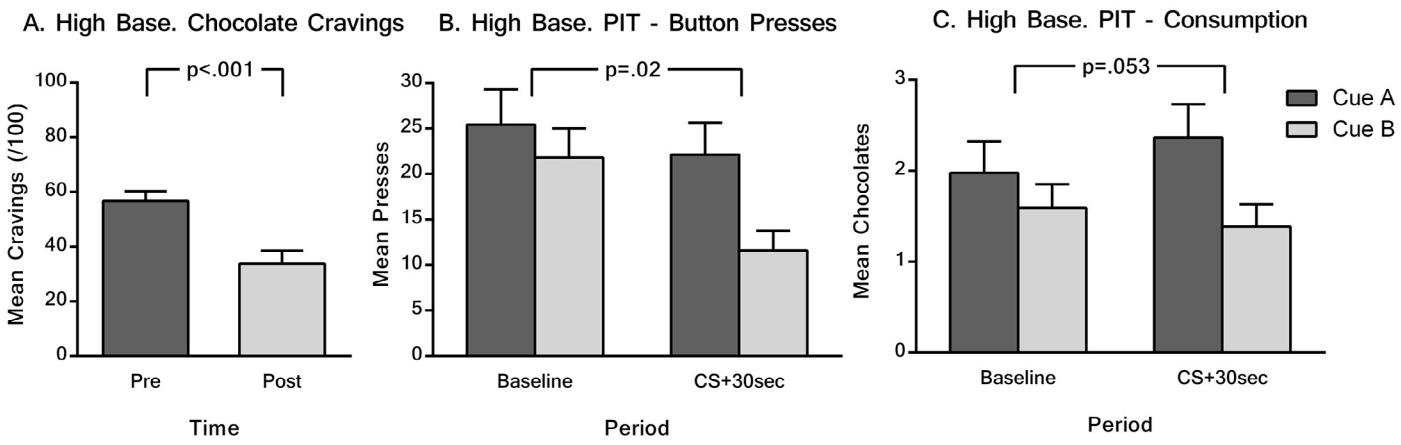


**Fig. 4.** High baseline responders. (A) Covariate adjusted mean (+SE) cravings at the beginning and end of the experiment. (B) Covariate adjusted mean number of responses in the transfer test during baseline and from CS onset to 30 s for the chocolate paired cue (Cue A) and the unpaired cue (Cue B). (C) Covariate adjusted mean number of chocolates consumed in the transfer test during baseline and from CS onset to 30 s for each cue. For B and C, p-values are for the interaction between cue and time.

Button press data for high responders are shown in Fig. 4B. A significant main effect of cue indicated less responding on Cue B trials overall, $F_{1,43} = 13.0$, $p = .001$ and a significant main effect of time indicated less responding overall following cue presentation, $F_{1,43} = 11.5$, $p = .002$. A significant cue by time interaction indicated that the reduction in responding following cue presentation was significantly more marked for Cue B than Cue A, $F_{1,43} = 6.33$, $p = .02$. Simple effects analysis revealed no statistically significant change in responding to Cue A over time, $F_{1,43} = 2.17$, $p = .15$. However, there was a statistically significant reduction in responding of 10.5 presses following presentation of Cue B, $F_{1,43} = 17.5$, $p < .001$. Thus, for button pressing in high baseline responders, there was an inhibitory PIT effect to the unpaired cue and no effect of the food paired cue.

In terms of consumption in high baseline responders shown in Fig. 4C, there was a statistically significant main effect of cue, with more chocolates consumed on Cue A trials than Cue B trials, $F_{1,42} = 8.65$, $p = .005$. A near-significant interaction between cue and time suggested that this difference was larger following cue presentation than during baseline, $F_{1,43} = 3.95$, $p = .053$. The main effect of time was not statistically significant, $F < 1$. Simple effects analysis revealed no statistically significant change in consumption to Cue A, $F_{1,42} = 2.60$, $p = .11$, nor to Cue B, $F < 1$. Overall, these results suggest that the suppression of responding elicited by Cue B did not translate into significant differences in consumption in high baseline responders.

*Cravings and consumption in high versus low baseline responders*

A between-subjects ANCOVA revealed no statistically significant difference in chocolate cravings at the end of the experiment between high and low baseline responders, $F_{1,87} = 1.26$, $p = .27$. However, given that cravings were recorded after the transfer test and that high responders consumed almost seven times more chocolates during the test, post-experimental cravings may not be a valid indicator of cravings at the time of test. Instead, button pressing and consumption during the test may be a better indicator of motivational state and cravings at the time of test. They indicate the extent to which participants were actively seeking chocolate when they encountered the cues. Here, high responders pressed an average of 23.2 times before each cue compared with an average of only 1.7 presses in low responders, $F_{1,87} = 39.4$, $p < .001$, and over the entire test period high baseline responders consumed an average of 16.0 chocolates compared with an average of only 2.4 chocolates in the low baseline responders, $F_{1,87} = 26.8$, $p < .001$. Thus, it seems clear that the low baseline responders were much more highly satiated than the high baseline responders when the cues were encountered during the transfer test.

## General discussion

In the current study, we examined whether PIT for a food reward, i.e. chocolate, could be observed in humans when tested under reinforcement. The most interesting finding was that the direction of the PIT effect observed depended on the participants' motivational state when the food cues were encountered. In participants who were no longer actively seeking food, the food cue elicited a facilitatory PIT effect leading to increased responding and, importantly, consumption. In participants who were still actively seeking food, the food cue had no effect on responding or consumption, but the unpaired cue produced an inhibitory PIT effect whereby responding following it was markedly decreased. These findings have a number of important implications.

To our knowledge this is the first demonstration of PIT effects in humans when the transfer test is conducted under reinforcement. As such, the enhancement of responding to the chocolate-paired cue suggests that PIT may well be one mechanism by which over-consumption of food is maintained, with food cues inducing motivation to obtain food even when that food is known to be already available. What is particularly interesting about the facilitation observed is that it occurred when the participants were not currently motivated to obtain and consume chocolate, i.e. in low baseline responders. This suggests that the enhancement of responding to the chocolate-paired cue was independent of the desirability of the chocolate at that given time. This lack of sensitivity to satiation is consistent with numerous studies showing no effect of devaluation manipulations on PIT for various rewards in both humans (Hogarth, 2012; Hogarth & Chase, 2011; Watson et al., 2014) and animals (Corbit, Janak, & Balleine, 2007; Holland, 2004; Rescorla, 1994). For example, Watson et al. (2014) recently found that food-paired cues enhanced instrumental responding to obtain the food even after it had been devalued by allowing participants to satiate themselves by providing free access prior to the transfer test. This apparent insensitivity of PIT to satiation is concerning, because it indicates a way in which biological mechanisms that should inhibit eating at appropriate times, such as satiety, can be overridden by food-cues in the environment. In the current case, it suggests that when motivation to obtain food has naturally decreased due to satiation, encountering a cue paired with that food can instigate actions directed towards obtaining and consuming the food despite the satiation.

The inhibition of responding to the unpaired cue observed in high baseline responders is also noteworthy. Typically, manipulations aimed at weakening the relationship between a cue and food have little effect on PIT. For example, facilitatory PIT effects in rats are still observed to food-paired cues even after the cue has undergone a period of extinction in which the cue is presented, but no food is delivered (Delamater, 1996). Whilst the current inhibition is distinct in the sense that the unpaired cue had never previously been paired with chocolate and so never had excitatory associations that needed to be overcome, it does suggest that at least in principle, establishing inhibitory links between cues and food may be one method of dampening motivation to work for and obtain food in humans. Only one published study has tested the effects of extinction on PIT in humans. It used a computer game PIT task and it found no effect of extinction (Rosas et al., 2010). However, we have recently conducted a study in which we extinguished the Pavlovian association of a cue paired with a real food reward and found that extinction did dampen, but not eradicate PIT (Lovibond, Satkunarajah, & Colagiuri, under review). Thus, extinction may be effective for reducing PIT for food in humans and it would be interesting to test this under instrumental reinforcement.

The fact that the occurrence of facilitation to the chocolate-paired cue or inhibition to the unpaired cue depended on the level of responding at the time of the test has broader implications for PIT research. The results suggest that the level of baseline responding is an important factor in the expression of PIT. Thus, PIT studies involving transfer tests under instrumental extinction that typically find evidence of facilitation to the food-paired cue, with no effect of the unpaired cue (Bray et al., 2008; Lovibond & Colagiuri, 2013; Watson et al., 2014), should not be taken as evidence that no learning has occurred to the unpaired cue. It may simply be that the instrumental extinction produces floor effects whereby no inhibition can be observed. Similarly, the failure of the food-paired cue to enhance responding in high baseline responders is likely attributable to the fact that participants were still motivated and actively seeking chocolate at a relatively high rate (approx. 23 button presses per minute). As such, these results suggest that food-paired and unpaired cues may acquire excitatory and inhibitory associations, respectively, but that the effect of this learning on responding is dependent on the level of responding when those cues are encountered. One of the most likely factors to affect baseline level of responding is satiation. This means that when satiation is low and current motivation to obtain food is high, inhibitory cues can dampen responding with little effect of excitatory cues. On the other hand, when satiation is high and baseline responding is low, inhibitory cues have little effect, but excitatory cues can elicit new motivation to obtain and consume food.

There are some limitations to the current findings. First, we employed a single type of reward, chocolate, and thus could not determine whether the effects observed reflected specific PIT, general PIT, or a combination of the two. It would, therefore, be interesting to extend the current findings and test for PIT under reinforcement using multiple food rewards. One potential difficulty with such a design when using real food rewards in humans, however, is that a substantially greater amount of food needs to be consumed during Pavlovian training – up to three times the amount – and this could interfere with participants' willingness to complete instrumental training. In the current study involving only a single reward, one quarter of the participants failed to complete the minimum instrumental training due to satiation. This would likely inflate greatly with multiple rewards. Second, we did not include formal tests of conditioned inhibition. As such, the suppression of responding induced by the unpaired cue may be attributable to more general inhibitory processes than conditioned inhibition. Future studies could, therefore, incorporate summation and retardation tests to explore this. Third, we only assessed craving before and after the experiment, not during it, which meant that we had to rely on button pressing and consumption as indicators of satiation at the time of test. Future studies could incorporate cravings ratings throughout the experiment. This would also allow for determination of whether responding is mediated by temporarily increased or decreased desire induced by the cues in the transfer test. Any inclusion of online ratings of craving would, of course, need to be implemented in such a way that they did not interfere with the ongoing instrumental responding and the PIT effect itself. Finally, we did not screen participants for eating disorders prior to study entry, apart from dieting in general, and it is plausible that PIT may differ in such populations.

In summary, we found novel evidence of PIT for food-related cues in humans under more ecologically valid test conditions in which participants could still earn the reward. The results suggest that learning occurs to both food-paired and unpaired cues, such that food-paired cues can enhance motivation to obtain and consume food, whereas unpaired cues can inhibit motivation, with the expression of either depending on the level of motivation when the cue is encountered. The enhancement to the food-paired cue is particularly concerning because it occurred despite high levels of satiation, suggesting that food-cues can override natural inhibitory mechanisms aimed at regulating eating and weight. As such, food cues may be an important point of intervention for attempting to reduce maladaptive patterns of over-eating.

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