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# Single-response appetitive Pavlovian to instrumental transfer is supressed by aversive counter-conditioning

Short title: Counter-conditioning suppresses PIT

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**Abstract**

Background – Environmental stimuli, when paired with reward, can influence behaviour in maladaptive ways, e.g. by encouraging overeating or addiction. Such behaviour can be sensitive to reward value manipulations, under circumscribed conditions, but whether reward-seeking is also sensitive to stimulus value manipulations remains unclear. Thus, the current experiment investigated whether reducing the hedonic value of a reward-paired stimulus would reduce reward-seeking behaviour.

Methods – 36 participants successfully completed a single-response Pavlovian-instrumental transfer (PIT) task with a counter-conditioning procedure. The Pavlovian phase associated three CSs with money at 100%, 50%, or 0% contingency. Counter-conditioning then followed for the experimental group, who saw the 100% CS paired with unpleasant pictures, while the control group saw only neutral images. Instrumental training required participants to learn a button-pressing response to win money. The transfer phase contrasted instrumental responding during baseline and CS presentation.

Results – Both experimental and control groups liked the 100% CS more than the other CSs after Pavlovian training, but counter-conditioning reduced this 100% CS liking. In transfer, the experimental group showed an abolition of appetitive PIT, while the control group showed maintenance of appetitive PIT. However, this group difference was only evident in response vigour, not response initiation.

Conclusions – CS hedonic value influences cue-potentiated instrumental responding. More specifically, hedonic value of a reward-paired cue influences the vigour of instrumental responses, but not the decision to initiate a response. These data may have relevance to smoking cessation policies, where the introduction of health warnings may be viewed as a real-world example of counter-conditioning.

Keywords: Pavlovian-instrumental; transfer; emotion; motivation; counter-conditioning.



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## Introduction

Conditioned stimuli associated with rewarding outcomes, such as food or drugs, can usurp behaviour such that animals (including humans) will work to procure a reward despite detrimental net effects, such as obesity (Watson, Wiers, Hommel, Gerdes, & de Wit, 2017) or addiction (Garbusow et al., 2016).

Such interactions between a conditioned stimulus (CS) and an instrumental response (R) can be modelled experimentally via the Pavlovian-instrumental transfer (PIT) paradigm (Corbit & Balleine, 2005; Estes, 1943; Holland, 2004). While multiple methodological permutations of the PIT paradigm exist (Cartoni, Balleine, & Baldassarre, 2016; Holmes, Marchand, & Coutureau, 2010), all include separate Pavlovian and instrumental phases, where predictive and causal associations are trained, respectively. These training phases are followed by a test phase, where the ability of the CS to 'transfer' control onto the response is measured, often by comparing instrumental response rate in the presence versus absence of the CS.

A recent resurgence in PIT investigations has shown PIT to be composed of two complementary processes (Cartoni et al., 2016). *Specific*-PIT refers to the ability of a CS to influence instrumental responses that procure the same outcome as that predicted by the CS. For example, Hogarth & Duka (2007) trained participants to associate one visual stimulus with cigarettes, and another stimulus with chocolate. In a separate session, participants learned that they could win cigarettes or chocolate pressing the 'D' or 'H' key, respectively. Finally, in the test phase, specific-PIT was demonstrated by participants pressing 'D' more often in the presence of the cigarette CS, and 'H' more often in the presence of the chocolate CS.

In parallel, *general*-PIT refers to the ability of a CS to influence instrumental responses that procure the outcomes beyond those predicted by the CS. For example, Watson et al (2014) trained participants to associate three CSs with three distinct food outcomes, e.g. popcorn, smarties, and nuts, before training two responses with two of these same food outcomes, e.g. popcorn and smarties. In the test phase, general-PIT was demonstrated by the nuts CS augmenting instrumental responding indiscriminately across response options.

Both forms of PIT appear sensitive to [human] participant outcome (O) expectancy (Hogarth et al., 2014; Jeffs & Duka, 2017; Prevost, Liljeholm, Tyszka, & O'Doherty, 2012; Talmi, Seymour, Dayan, & Dolan, 2008). That is, only participants who have some degree of propositional knowledge of the relationship between CS→O, and R→O, will show PIT; participants considered 'unaware' of these contingencies have so far failed to show PIT.

However, general-PIT appears more sensitive to outcome value (Cartoni et al., 2016; Corbit, Janak, & Balleine, 2007; Dickinson & Dawson, 1987; Holland, 2004). For example, Corbit and colleagues (2007) trained rats on a 'full' PIT procedure, where both specific- and general-PIT could be assessed in parallel. Corbit et al used two stimuli (S1 & S2) that predicted two specific outcomes (O1 & O2) shared with two instrumental responses (R1 & R2), whereas a third stimulus (S3) was paired with a third outcome (O3) that had no corresponding response. Thus S1 & S2 would allow specific-PIT, whereas S3 would allow only general-PIT. Following devaluation of all food outcomes through satiety, instrumental



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responding was reduced in the presence of the S3 only, thus only general-PIT was sensitive to outcome value.

Moreover, indirect evidence suggests that CS value itself, aside from outcome value, may also influence PIT (Manglani, Lewis, Wilson, & Delgado, 2017; Trick, Hogarth, & Duka, 2011). These studies have shown associations between CS liking following Pavlovian conditioning, and strength of PIT. Indeed, CS hedonic value can track outcome value through the process of alliesthesia (Cabanac, 1979), as demonstrated by appetitive reactions to food or drug CSs being attenuated by outcome devaluation (Field, Mogg, & Bradley, 2004; Toates, 1986).

However, despite this circumstantial evidence that CS hedonic value may influence PIT, no study has directly tested the effect of CS hedonic value manipulation on PIT. Such a hypothesis may be tested by directly altering the hedonic value of a reward-paired CS through the process of counter-conditioning (CC; Baeyens, Eelen, Van den Bergh, & Crombez, 1989; Dickinson & Pearce, 1977). After pairing an initially neutral CS with an appetitive US, the CS is then paired with a US of opposite valence to the original. Although the initial appetitive pairing successfully increases the liking attributed to the CS, the subsequent aversive pairing is able to abolish or even reverse this emotional conditioned response (Hollands, Prestwich, & Marteau, 2011; Kerkhof, Vansteenwegen, Baeyens, & Hermans, 2011; Van Gucht, Baeyens, Vansteenwegen, Hermans, & Beckers, 2010). CC has a lasting effect on CS evaluative ratings (Kerkhof et al., 2011), and has been shown to reduce consumption of appetitive food (Hollands et al., 2011; Van Gucht et al., 2010).

However, the effects of counter-conditioning on behaviour may be confounded by changes in outcome expectancy, in that counter-conditioning studies so far either confirm that outcome expectancy tracks CS emotional value (Van Gucht et al., 2010), or are unable to falsify such a claim (Baeyens et al., 1989; Kerkhof et al., 2011). Thus a technique to deliver counter-conditioning while dissociating its effects on expectancy and emotion is required. Such a technique may be informed by the finding that changes in context have dissociable effects on cue-elicited expectancy and emotion (Van Gucht, Baeyens, Hermans, & Beckers, 2013). Using an ABA context shift design, where appetitive conditioning occurred in context A (a well lit room), followed by counter-conditioning in context B (a dimly lit room), it was demonstrated that a return to context A renewed outcome expectancy but did not renew CS liking. Thus expectancy appears sensitive to contextual changes, whereas evaluative responses are less sensitive to such shifts. Conducting any counter-conditioning in a session distinct from that of the rest of an experiment may therefore allow for changes in CS liking that are dissociated from changes in outcome expectancy.

Additionally, greater effect sizes have been shown when reversing an originally appetitive CS compared to an originally aversive CS (Baeyens et al., 1989; Dickinson & Pearce, 1977; Stevenson, Boakes, & Wilson, 2000). Thus attempting to reduce the pleasantness of a reward-predictive CS may be more effective than reducing the anxiety attributed to a reward-absence predictive CS.

Moreover, indirect evidence suggests that any behavioural effect of manipulating CS pleasantness may manifest itself in response vigour more than response initiation (Garofalo & Robbins, 2017). For example, Garofalo & Robbins (2017) showed that general-PIT was more apparent in response vigour, measured through grip strength, than response initiation, measured through joystick movement. Therefore, to the extent that general-PIT is sensitive



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to value manipulations, any change in general-PIT may be more pronounced in response vigour than response initiation.

In light of these converging data, the present experiment was devised to test whether reducing the positive emotional value of a reward-predictive CS would bring about concomitant reductions in appetitive PIT. A previous experiment (Jeffs & Duka, 2017) showed that a specific-PIT procedure was not sensitive to differential CS hedonic value. Thus the present experiment used a single-response PIT procedure, which is sensitive to outcome value manipulations (e.g. Holland, 2004), and likely easier than a 'full' PIT paradigm for participants to learn (e.g. Watson et al., 2014, whose full PIT paradigm was not learned by 8% of participants). Money was used as the outcome, rather than a primary reward such as food, to ensure the outcome's general appeal to participants regardless of satiety. Money showed equivalence to food in a previous PIT experiment (Lehner, Balsters, Herger, Hare, & Wenderoth, 2017).

We predicted that reduced PIT would be shown only by participants aware of the Pavlovian CS→O contingencies, as no study has shown PIT in unaware participants (e.g. Jeffs & Duka, 2017; Talmi et al., 2008). We also predicted that reduced PIT would be most evident in response vigour, rather than response initiation, due to response vigour being more sensitive to outcome value (Garofalo & Robbins, 2017).

## Method

*Participants*

67 University of Sussex students (22 males and 45 females), with a mean age of 19.3 years (range 18-27), undertook the procedure. Recruitment stopped after 18 aware participants had completed the task per counter-conditioning group. Sample size was determined a priori using G*Power software (Faul, Erdfelder, Lang, & Buchner, 2007), inputting $\alpha = .05$, $\beta = .20$, $d = 0.6$ (based on PIT pilot data). Participants were recruited via an online participant database and were compensated for their time financially or with course credit. Participants gave written consent before beginning the study, with ethical approval granted by the University of Sussex Life Sciences ethics committee, approval code [DDSJ10].

*Materials*

The experiment was run using E Prime v1.2 software. An LCD monitor presented CSs (see Fig. 1, left) at size 10.2 $cm^2$ and resolution 1280 x 1024 pixels. During Pavlovian training, responses were recorded using a QWERTY keyboard with the horizontal number keys 1-9 coloured green. During counter-conditioning (CC), responses were recorded using a different QWERTY keyboard with the left and right arrow keys coloured yellow. During instrumental training and transfer, the keyboard was replaced by a five button box, oriented such that the buttons were aligned sagittally. Only the button nearest the participant was active, and was coloured blue, whereas the other buttons were grey. Throughout the experiment, to the left and right of the response manipulandi, was a small metal box with its lid open. Inside the left box were 64 fifty pence coins. The right box was initially empty, but was labelled with "Your 50p box".

[insert Fig. 1]



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*Design & Procedure*

Pavlovian training

The entire procedure comprised four phases – Pavlovian, CC, Instrumental, Transfer. See Fig. 2 for a diagram of the procedure.

[insert Fig. 2]

First, the Pavlovian conditioning phase used a trace conditioning procedure to associate visual stimuli with monetary reward at 100%, 50%, or 0% contingencies. In short, participants viewed a pair of CSs, then estimated their likelihood of winning (i.e. gave online expectancy awareness ratings), before being told whether they had won or not. Winning was contingent solely upon the CS displayed (and so not contingent on responses to the likelihood question). See Supplementary Method, Section 1.2.1, for further detail of Pavlovian training.

There were 128 trials in total, 64 winning and non-winning trials, divided into 8 blocks of 16. After each block, participants saw a screen that detailed their winnings for that block (always £4.00), and moved the amount from the left hand box into their 'winnings' box. The act of moving money was designed to give participants tangible experience of reward.

The four CSs were assigned to one of four roles, counterbalanced across participants. One CS was 100% contingent with winning (CS+), while another CS was 0% contingent with winning (CS-). Two further CSs occurred equally alongside both CS+ and CS-, and so were equally (50%) contingent with winning and not winning (CS±a & CS±b). As such, both of these CS± were non-predictive (Rescorla, 1987), and so acted as a 'mere exposure' baseline (Murphy, Monahan, & Zajonc, 1995). The CS- served as a contrast to the CS+, to emphasise the appetitive nature of the CS+. Each trial presented CS+ or CS-, combined with CS±a or CS±b. Therefore no trial contained both CS+ and CS- together, or CS±a and CS±b together. Horizontal position (left or right) of CS was counterbalanced within participants, order of presentation was random.

Immediately after the final block of Pavlovian conditioning, participants rated the subjective pleasantness and anxiety evoked by each of the four CSs, using the horizontal number keys 1-9. Order of CS and emotion was randomised, thus questions pertaining to the same CS were not necessarily consecutive.

Counter-conditioning

Participants then took a 1min break before they experienced the counter-conditioning phase. During the 1min break, the experimenter switched keyboards (from green numbers to yellow arrows), and then described CC to participants as "a different task". The combination of these changes (time, colour, and description) from Pavlovian to CC phases was designed to emphasise the change in context, as per similar manipulations that have produced successful ABA context shifts previously (Leer & Engelhard, 2015; Orinstein, Urcelay, & Miller, 2010).

Briefly, Participants viewed a stimulus pair, comprising one Pavlovian CS, and one IAPS image (International Affective Picture System; Lang, Bradley, & Cuthbert, 2008). They then pressed an arrow key (left or right) corresponding to the image they recognised. This



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response requirement was designed to encourage participants to view both images, rather than avoiding the aversive pictures. Stimulus pairs remained on screen for 7.5s, regardless of how quickly participants responded. Participants were randomly allocated to either neutral or aversive conditions. Participants assigned to the neutral condition saw all CSs paired with neutral photographs; participants in the aversive condition saw the CS+ paired with aversive images, while the other CSs were paired with neutral images.

The CC phase used 12 aversive and 36 neutral images from the IAPS database. Fig. 1 (middle and right) displays exemplar images; details of full set can be found in Supplementary Method, Section 1.1.1. CC images were displayed at the same size and resolution as Pavlovian CSs. There were 36 trials in total, thus 12 trials each of CS+, CS±, and CS- (the two CS± were presented 6 times each). A different IAPS picture was used in each trial. Order of presentation was random, horizontal position of CS was counterbalanced within participants. See Supplementary Method, Section 1.2.2, for further detail of CC.

Upon completion of CC, participants took a further 1min break, before completing a second evaluative rating session. This second session began with the instruction:

“In a moment you will perform a different task where you can win money. But first please answer some questions about the pictures you have seen. Press the spacebar to start.”

The purpose of making reference to winning money was to change the context again to better match the transfer phase, to increase the probability that participants' second evaluative rating of each CS would generalise into the transfer phase. The rest of the rating session continued as had that following Pavlovian training.

Instrumental training

Participants next took a five minute break, while the experimenter replaced the keyboard with the response box. In short, participants were reinforced with 50p at the end of each trial for sustaining a > 1Hz response rate throughout each trial response window. There were 40 trials in total, divided into 4 blocks of 10. Each block ended with a screen displaying participants’ winnings for that block (in this phase the amount was response-contingent), and asked participants to move the specified amount into their winnings box.

Each trial was signalled by a fixation cross followed by two dark grey squares which occupied the same position as had Pavlovian CSs. Participants then saw the question *“Press the button?”*. If participants pressed, the screen went blank for 18.5s, during which time they were required to maintain a response rate of > 1Hz to win. This schedule was adapted from the single-response PIT task of Talmi et al (2008). Participants were not made explicitly aware of these response requirements, and so the experimenter supervised participants during the first block. After 3 trials, the experimenter explained that participants would “have a better chance of winning by pressing multiple times during the blank screen”. This multiple-press requirement encouraged participants to express response vigour, while still allowing variation in press-rate due to the difficulty participants would have in comprehending the reinforcement contingency. If participants pressed at the required rate, they were reinforced at 50% contingency. This contingency ensured that the dark grey squares (hereafter S±) were non-discriminative of reinforcement, and so would create a baseline condition for the ensuing transfer phase. Trials where participants pressed at least once, but did not win, were explicitly indicated with the text *“You win nothing”*. Trials where participants did not press at all contained no information as to winning.



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Fig. 3 presents a timeline of this instrumental trial sequence; Supplementary Method, Section 1.2.3, contains further detail of instrumental training.

[insert Fig. 3]

Transfer

Instrumental training transitioned into the transfer phase so as to appear as a continuation of the same task. It therefore began immediately after the final trial of instrumental training with the following instructions:

“Now you will continue to earn money as before, but you will only be told how much at the end of the session. Sometimes the pictures you saw earlier will be presented. Press the blue button to continue.”

Transfer proceeded in much the same way as instrumental training, except that no reinforcement was earned. Thus conditions of nominal extinction were evoked, in that participants believed they were still winning money, but no reinforcement was provided. This ensured that no new learning occurred. Furthermore, in place of the S±, in 1/3 of trials the CS+ was presented alongside CS±a or CS±b, in another 1/3 the CS- was presented alongside CS±a or CS±b, with the remaining 1/3 retaining the S±. As with Pavlovian training, position of CS (left/right) was counterbalanced, with presentation order random. There were 96 trials in total, thus each CS occurred 32 times.

Post-transfer Awareness

Participants provided their propositional knowledge of CS contingencies in two ways immediately after the transfer phase. One measured hierarchical awareness, i.e. expectancy that a CS would alter the efficacy of the instrumental response; the other tested Pavlovian contingency awareness, i.e. knowledge of CS-US pairing in the Pavlovian phase.

First, to test hierarchical knowledge, participants retrospectively rated their estimated probability of winning 50p during the different transfer trial-types (CS+, S±, CS-). The experimenter gave participants a sheet of paper with five visual-analogue scales (VASs), anchored with "Low chance" at the left extreme and "High chance" at the right extreme. Participants viewed on screen each of the four CSs and the S± individually, in random order, and marked each VAS consecutively with their estimated chance of winning during the transfer phase. This new form of response collection, i.e. VAS versus numeric scale, was designed to emphasise the different question now being asked compared to the earlier Pavlovian phase. Supplementary Method, Section 1.2.4, contains verbatim instructions used with the VASs.

After presenting the final CS, the end of the task was signalled, and participants called the experimenter. At this point, to test Pavlovian contingency awareness, the experimenter asked the participant: *“in the first part of the experiment, when you saw the shapes and then won 50p, which of these shapes was most likely to win?”* while presenting each CS in random order.

The entire PIT procedure took approximately 90mins to complete - 30mins for Pavlovian training, 60mins for the instrumental and transfer phases combined.



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*Statistical analyses*

Given the specificity of the experimental hypotheses – to investigate the effect of CC on *appetitive* PIT – analyses focussed on responses to the CS+ versus appropriate baselines within each experimental phase. CS- data is presented in Supplementary Results, Section 2.1.

Participant awareness was classified using online expectancy ratings from the final block of Pavlovian training, and correct CS+ identification from the post-experiment interview. 'Aware' participants were those whose mean CS+ expectancy rating was significantly greater than 5 (where 5 = 'don't know') at the end of Pavlovian training, and who could also correctly identify the CS+ in the post-experiment interview. All other participants were classified as 'unaware'.

The non-predictive CS±a & CS±b were rated as non-significantly different throughout all analyses, and so a single CS± score was calculated as their mean.

Instrumental responses were divided into two aspects – initiation, and rate. Response initiation (RI) was defined as the percentage of available trials where at least one response was made. Response rate (RR) was calculated by taking the total number of presses, then dividing by the available response window (18.5s per trial) to give a per-second measure of vigour. (Response latency was also analysed, but the pattern of results was the same as for RI, and so latency data are not reported here to remain concise.)

Investigation of the full cohort found that 6 participants failed to receive positive reinforcement during instrumental training, and so were excluded from all analyses. Of the remaining 61 participants, 36 were aware (18 per CC group), 25 were unaware (14 aversive CC, 11 neutral). The main analysis included only aware participants; data for unaware groups is presented in Supplementary Results, Section 2.2 and 2.3.

Primary outcome measures, i.e. evaluative ratings and transfer motor behaviour, were analysed initially using mixed ANOVA, and were further scrutinised using planned contrasts. These contrasts were performed using repeated-measures t-tests, comparing CS+ to either CS± (for evaluative ratings), or S± (for motor responses) within each CC group. Greenhouse-Geisser method was used to correct for non-sphericity in within-subjects ANOVA. Partial eta squared was calculated by SPSS 23; Cohen's dz was calculated as mean difference divided by standard deviation of that difference.

## Results

*Pavlovian expectancy awareness*

Both groups showed increasing CS+ expectancy awareness as Pavlovian training progressed, regardless of subsequent CC allocation. Mean CS+ Expectancy for each Group at each Block of the Pavlovian phase are presented in Fig. 4. A mixed ANOVA of CS+ Expectancy rating, with Block (1 – 8) and CC Group (Neutral, Aversive) as factors, confirmed this description: there was a significant effect of Block [F(3.70, 126) = 39.2, p < .001, $\eta_p^2$ = .535], but non-significant effects of CC Group or Block*CC Group [Fs < 2.41, ps > .130, $\eta_p^2 s$ < .066]. Polynomial contrasts showed that the Block main effect followed a linear trend [F(1,34) = 147, p < .001, $\eta_p^2$s = .812].



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[insert Fig. 4]

*Evaluative conditioning*

Immediately following Pavlovian training, prior to CC, both groups rated the CS+ as more pleasant that the CS±. However, following CC, the Aversive group lost this CS+ vs CS± difference, and showed an increased anxiety rating for the CS+ from Pavlovian to CC rating phases. However, these effects were of small size, and were only detected through simple comparisons.

A mixed ANOVA of Pleasantness rating, with CS (CS+, CS±), Phase (Pavlovian, CC), and CC group as factors, reported a main effect of CS (CS+ > CS±), and a CS*Phase interaction [Fs(1, 34) > 9.72, ps < .004, $\eta_p^2 s$ > .222]. All other effects were non-significant [Fs(1, 34) < 0.77, ps > .378, $\eta_p^2 s$ < .023].

Planned contrasts, comparing CS+ to CS± pleasantness rating following the Pavlovian phase, confirmed this initial difference to be significant in both groups [ts(17) > 2.28, ps < .036, dzs > 0.54].

However, while this CS pleasantness difference remained significant following CC in the Neutral group [t(17) = 2.75, p = .014, dz = 0.65], the difference became non-significant for the Aversive CC group [t(17) = 0.92, p = .370, dz = 0.22]. Mean Pleasantness rating for each CS by each Group after each rating phase is presented in Fig. 5.

[insert Fig. 5]

Further RM t-tests investigated the change in CS pleasantness rating from Pavlovian to CC rating phases, separately for each CS, and each CC group. All four contrasts were non-significant [ts(17) < 1.73, ps > .102, dzs < 0.41].

Due to the aversive nature of the CC manipulation, CS anxiety ratings were scrutinised in the same manner as pleasantness ratings. The mixed ANOVA of anxiety ratings reported only a main effect of Phase (CC > Pavlovian) [F(1, 34) = 5.67, p = .023, $\eta_p^2$ = .143]. All other effects were non-significant [Fs(1, 34) < 3.37, ps > .075, $\eta_p^2 s$ < .090].

The planned contrasts, comparing CS+ to CS± anxiety rating, were non-significant [ts < 1.24, ps > .233, dzs < 0.29]. However, the RM t-tests investigating the change in CS anxiety rating revealed a significant increase for CS+ within the Aversive group only [t(17) = 2.29, p = .035, dz = 0.54]. The other three comparisons were non-significant [ts(17) < 1.37, ps > .187, dzs < 0.32]. See Table 1 for mean anxiety ratings.

Thus, overall, the CC manipulation was successful in its aim to abolish the pleasantness difference between CS+ and CS±, but appears to have done so by *increasing* CS+ anxiety more selectively than *reducing* CS+ pleasantness.

[insert Table 1]



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*Instrumental training*

All participants (except the 6 already excluded) learned the instrumental response, as evidenced by them pressing at a rate sufficient to receive monetary reinforcement, and response patterns were similar between CC groups. Fig. 6 shows mean Response Initiation and Response Rate for each CC group across blocks of instrumental training.

For Response Initiation, responding was stable across blocks, and similar between groups. A mixed ANOVA, with Block (1 – 4) and CC Group as factors, showed non-significant effects [Fs < 1, $\eta_p^2 s$ < .018].

For Response Rate, responding was initially < 1Hz during Block 1, but plateaued from Block 2 onwards in both groups as participants learned the response requirements. A mixed ANOVA, with Block and CC Group as factors, showed a main effect of Block [F(2.30, 78.0) = 53.4, p < .001, $\eta_p^2$ = .611], but non-significant effects of CC Group and Block*CC Group [Fs < 2.66, ps > .109, $\eta_p^2 s$ < .073].

[insert Fig. 6]

*Transfer*

For the Neutral CC group, appetitive transfer was shown in both Response Initiation and Response Rate. In contrast, for the Aversive CC group, appetitive transfer was shown only in Response Initiation; transfer was suppressed in Response Rate. Fig. 7 displays mean Response Initiation and Response Rate during the transfer phase for each CS, and each CC group.

[insert Fig. 7]

A mixed ANOVA of Response Initiation, with Stimulus (CS+, S±) and CC group as factors, reported only a main effect of Stimulus (CS+ > S±) [F(1, 34) = 13.0, p = .001, $\eta_p^2$ = .276]. The main effect of CC Group, and the Stimulus*CC Group interaction, was non-significant [Fs(1, 34) < 0.82, ps > .372, $\eta_p^2 s$ < .024].

The planned contrasts of CS+ vs S± trials, within each CC group, confirmed the Stimulus effect [ts(17) > 2.53, ps < .021, dzs > 0.60], indicating appetitive transfer in both groups. Additional independent groups t-tests compared each CC group's Response Initiation within each Stimulus; both CS+ and S± comparisons were non-significant [ts(34) < .844, ps > .404, ds < 0.28].

For Response Rate, the mixed ANOVA reported a main effect of Stimulus (CS+ > S±) [F(1, 34) = 8.14, p = .007, $\eta_p^2$ = .193]. The main effect of CC Group, and the Stimulus*CC Group interaction, was non-significant [Fs(1, 34) < 2.40, ps > .130, $\eta_p^2 s$ < .066].



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However, while the planned contrasts reported appetitive transfer in the Neutral CC group [t(17) = 2.27, p = .037, dz = 0.53], this same comparison was non-significant in the Aversive CC group [t(17) = 1.94, p = .069, dz = 0.46]. Additional independent groups t-tests compared each CC group's Response Rate within each Stimulus. For CS+ trials, the Aversive group Response Rate was significantly lower than the Neutral group [t(34) = 2.09, p = .045, d = 0.70], whereas for S± this group comparison was non-significant [t(34) = 0.67, p = .508, d = 0.22]. Therefore, CC appears to have suppressed appetitive PIT in the Aversive group, with any effects manifested solely in CS+ suppression, rather than changes in baseline responding.

*Post-transfer Awareness*

Both awareness measures indicated that propositional knowledge was unaffected by CC, and had been retained throughout the experiment. Table 2 displays mean hierarchical efficacy ratings for CS+ and S± by each CC group.

[insert Table 2]

For hierarchical efficacy ratings, a mixed ANOVA, with Stimulus (CS+, S±) and CC Group as factors, showed a significant effect of Stimulus (CS+ > S±) [F(1, 34) = 35.5, p < .001, $\eta_p^2$ = .511], and non-significant effects of CC Group, or Stimulus*CC Group [Fs < 1.32, ps > .259, $\eta_p^2 s$ < .037].

To explore changes in awareness from Pavlovian to post-transfer rating phases, CS+ expectancy ratings from the final block of Pavlovian training were converted to a percentage (i.e. [rating] / 9 * 100), to equilibrate the expectancy scale with the hierarchical scale. A mixed ANOVA, with Phase (Pavlovian, Post-transfer) and CC Group as factors, reported a main effect of Phase (Pavlovian *M* = 94.9, 95% CI 92.5 – 97.3; Post-transfer *M* = 78.0, 95% CI 72.2 – 83.8), but non-significant effects of CC Group or Phase*CC Group [Fs < 2.09, ps > .158, $\eta_p^2 s$ < .058]. Thus participant ratings descended over time, but the rate of decline was not affected by CC.

For Pavlovian contingency awareness, all participants remained able to correctly identify the CS+.

## Discussion

The current study tested whether reducing the positive emotional value of a reward-predictive CS would supress appetitive PIT. It employed a counter-conditioning procedure to reduce the hedonic value of a reward-paired CS, while leaving the predictive value of the CS unchanged, and measured PIT in terms of response initiation and response vigour. Results confirmed that reducing CS hedonic value did indeed supress appetitive PIT, but this PIT suppression was only displayed in response vigour. The data therefore support the necessary status of CS elicited emotion in cue-potentiated behaviour.

While some theories have championed the sufficiency of propositional discrimination in cue-potentiated reward seeking (Bolles, 1972; De Wit & Dickinson, 2009), and others have



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emphasised the sufficiency of subjective responses to cues (Bindra, 1974; Robinson & Berridge, 1993), the finding here provides experimental evidence of the bridge between the two (Toates, 1986). In combination with the extant literature supporting the necessary status of propositional knowledge in PIT (Hogarth et al., 2014; Jeffs & Duka, 2017), the finding here of PIT sensitivity to CS emotional value attests to the necessary status of both outcome expectancy and CS emotion in PIT.

Such a conclusion is currently limited to single-response PIT. It may hold for the general-PIT aspect of a full PIT paradigm, given that single-response PIT is argued to resemble general- more than specific-PIT (Cartoni et al., 2016). It will therefore be interesting to test the effects of counter-conditioning in a full PIT context. Such a manipulation would not be expected to alter specific-PIT, which is consistently insensitive to outcome value manipulations (Colwill & Rescorla, 1990; Rescorla, 1994; Watson et al., 2014) and has recently been shown to be insensitive to CS value (Jeffs & Duka, 2017).

Indeed, Quail, Morris, & Balleine (2017) showed that trait anxiety moderated general-PIT, but did not moderate specific-PIT, using a full PIT paradigm. At first glance, the current experimental results could be misinterpreted as further evidence of this trait anxiety effect, to the extent that trait anxiety is correlated with state anxiety. The CC phase may simply have raised state anxiety, and so abolished general-PIT in the same way as the high trait-anxious participants of Quail et al. However, scrutiny of the two experiment's data reveals a dissociation; whereas the high trait-anxious group of Quail et al abolished general-PIT through a CS- response *elevation*, the CC manipulation in the present experiment abolished general-PIT through a CS+ response *suppression* (CS- responding was unaffected by CC; see Supplementary Results, Section 2.1). Thus, while Quail et al showed that trait anxiety *increased* reward-seeking, the present results showed that CC *reduced* reward-seeking. This may have important implications for people displaying pathologically high reward-seeking, e.g. alcoholic (Garbusow et al., 2016) or obese (Watson et al., 2017) individuals. Whereas chronic anxiety may exacerbate their symptoms, acute anxiety targeted at alcohol- or food-paired cues may attenuate consumption.

The confirmation that the manipulation of cued emotion affected only the proximal behaviour of response rate, and did not influence the comparatively distal behaviour of response initiation, provides support for the finding that CSs have their greatest effect on responses closest to reward delivery (Balleine, 1992; Balleine, Garner, Gonzalez, & Dickinson, 1995; Corbit & Balleine, 2003). More specifically, Balleine and colleagues showed that proximal behaviours are sensitive to CS *presentation* but not outcome devaluation, whereas the reverse holds for distal behaviours. These previous experiments had not specified the necessary psychological qualities that allowed the CS to influence responding, and so the current data may add that CS *emotional value* usurps control later in the response sequence.

At a methodological level, the dissociation between response initiation and vigour in the current experiment warrants further investigation in future PIT experiments. For example, previous research into the sensitivity of specific-PIT to outcome devaluation has returned equivocal results. Some experiments find little effect of devaluation on specific-PIT (e.g. Hogarth, 2012; Hogarth & Chase, 2011; Seabrooke, Le Pelley, Hogarth, & Mitchell, 2017, exp. 1), while others report an attenuation of the specific-PIT effect (Allman, DeLeon, Cataldo, Holland, & Johnson, 2010; Eder & Dignath, 2016). While multiple methodological



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differences exist between these sets of studies, one consistent difference is that the *insensitivity* findings are based on a response *initiation* (percentage choice) measure, whereas the *sensitivity* findings are based on response *rate* (although see (van Steenbergen, Watson, Wiers, Hommel, & de Wit, 2017), and (Watson et al., 2014), for examples of insensitivity via both initiation and rate). The current results accord with this synopsis, in that response initiation was insensitive to CS value, whereas response rate was sensitive. Therefore, it may be worthwhile for future PIT studies to investigate response initiation alongside response vigour, as the latter may be more sensitive to value manipulations.

However, before the results of the present experiment are assimilated with this extant literature, limitations in the method should be explored. Firstly, participants did not rate their liking of the instrumental S±. However, given that the CS± and S± shared the same contingency with reward (50%), and given that contingency is argued to dictate the strength of Pavlovian conditioning (Rescorla, 1987), it would be expected that CS± and S± would share similar evaluative qualities. Therefore, the omission of S± liking ratings should not unduly influence conclusions drawn from the present data.

Secondly, we did not collect liking ratings following the transfer phase, which was itself conducted under extinction. However, given the relative stability of subjective conditioned responses in spite of extinction (Hofmann, De Houwer, Perugini, Baeyens, & Crombez, 2010; Kerkhof et al., 2011; Van Gucht et al., 2010), it is unlikely that the pattern of liking ratings for each CS would have changed across the transfer phase.

Thirdly, because we used different awareness measures during different experimental phases, it may be difficult to assess the stability of participant awareness pre- versus post-CC. On the one hand, Hogarth et al (2014) found similar rates of awareness (~90% of participants) when using expectancy and hierarchical awareness measures, suggesting that our different measures assessed a shared awareness construct. On the other hand, comparing Pavlovian to post-transfer CS+ expectancy/hierarchical ratings showed a reduction from the earlier to later rating. This is perhaps unsurprising, given that the transfer phase was conducted in extinction, and so may represent contingency re-appraisal. Alternatively, Seabrooke et al (2017) report an awareness *confidence* rating reduction from pre- to post-transfer, thus the current rating data may reflect participant *confidence* in their contingency knowledge, rather than their contingency knowledge per se. However, as there were no group effects across any of the three awareness measures, we consider it unlikely that any temporal changes in awareness influenced the differential group pattern of evaluative and motoric results.

Relatedly, while different awareness measures appear to provide similar awareness rates *within* studies, different Pavlovian training procedures may be responsible for the discordant awareness rates *across* studies. The current experiment's percentage of 59% is similar to some (e.g. Jeffs & Duka, 2017; Talmi et al., 2008; Trick et al., 2011), but lower than others, where 85% (e.g. Allman et al., 2010; Eder & Dignath, 2016; Seabrooke, Le Pelley, Porter, & Mitchell, 2018) or even 98% (Watson et al., 2014) awareness rates are reported. One methodological distinction that may explain these varying rates is the use of simultaneously presented stimuli during Pavlovian conditioning. While those studies reporting lower awareness rates presented multiple stimuli at once, those reporting higher rates presented only a single stimulus at any one time. For example, our method included two non-predictive



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stimuli (CS±) that were co-displayed with the CS+, whereas other PIT studies present the CS+ alone. Thus, this added complexity may interact with participant characteristics such that only those with a relatively analytical approach to the task become aware within the finite time window of Pavlovian training. This potential interaction of Pavlovian method with participant individual differences may limit the applicability of the present results to the wider population. But as we did not collect information on participants' analytical traits, we can only speculate on the individual differences that may explain awareness rates. Nevertheless, given that PIT has only been demonstrated in aware participants so far, it may be fruitful for future studies to collect individual differences data with a view to predicting awareness.

Finally, the effect of CC on evaluative ratings, and in turn on transfer behaviour, was relatively small. While the relevant CS+ suppression effects were detected by planned contrasts, they were not of sufficient magnitude to be shown as an ANOVA interaction. This may have been because the current CC method employed secondary reinforcers, e.g. unpleasant *pictures*, whereas previous CC studies employed primary reinforcers, e.g. unpleasant taste (Kerkhof et al., 2011; Van Gucht et al., 2010). This use of secondary reinforcers may have allowed for greater individual differences in the effectiveness of the CC manipulation. While the aversiveness of the images used has been previously validated (Lang et al., 2008), our participants did not explicitly rate the aversiveness of the CC procedure itself (although most described the experience as 'unpleasant'). Future studies may therefore wish to incorporate primary reinforcers into their CC procedure, and/or explicitly measure participant subjective experience.

Nevertheless, future research may wish to explore the validity of the present results under more naturalistic conditions, i.e. conditions of continued outcome availability. Indeed, Colagiuri & Lovibond (2015) report that PIT was maintained when the food outcome remained available during the transfer phase, but that the direction of PIT (excitatory versus inhibitory) was affected by satiation; high satiation produced only excitatory PIT, whereas low satiation produced only inhibitory PIT. It should be noted that these PIT effects occurred against a changing instrumental baseline, with high satiation suppressing, and low satiation enhancing, baseline responding. Nevertheless, if the direction of naturalistic PIT is indeed influenced by outcome value, it will be interesting to assess the effects of CS value as well.

Therefore, it may be prudent for future studies to make use of counter-conditioning that has already occurred in the natural environment. An example of this may be found in the addition of health warnings to cigarette packets. Smokers will have experienced numerous acquisition pairings of the packet itself with nicotine reward, but will more recently have had counter-conditioning pairings of the packet with unpleasant images. To the extent that smokers now find the packet less pleasant, or even unpleasant, they may present a germane population in which to investigate the effects of counter-conditioning.

Indeed, Hogarth, Maynard, & Munafò (2015) report that cigarette-seeking within a PIT paradigm was attenuated by 'plain' cigarette packs (i.e. packs with health warnings but no branding) relative to traditionally branded packs. However, this attenuation of lab-based cigarette-seeking was not translated into real-world smoking cessation. Maynard, Leonards, et al (2015) compared the smoking topography of smokers randomly assigned to use either plain or branded packs, but showed non-significant differences between the two groups. This null result may have been due to smokers' reduced emotional response to cigarette health warnings (Stothart, Maynard, Lavis, & Munafò, 2016), yet data from Wang and colleagues



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(Wang, Romer, et al., 2015; Wang, Lowen, Romer, Giorno, & Langleben, 2015) suggest that smoking urges can be reduced if a sufficiently graphic warning is used. Thus, the negative emotional content of health warnings may yet prove to be a viable target for real-world smoking cessation.

However, the effectiveness of this counter-conditioning as a smoking cessation intervention may further depend on the degree to which cigarette packets engage specific- or general-PIT. For example, Hogarth & Chase (2011) found that specific-PIT was unattenuated by nicotine devaluation via health warnings. Notwithstanding the equivocal data regarding specific-PIT sensitivity to devaluation, discussed earlier, specific-PIT may be less sensitive than general-PIT to health warnings on cigarette packets. Future research may therefore wish to test the effects of cigarette health warnings using a general-PIT paradigm, e.g. one using a single outcome, as per the current method, or using a 'full' PIT paradigm where specific- and general-PIT can be assessed within the same experiment (e.g. Corbit & Balleine, 2005).

In summary, the current experiment finds that reducing the appetitive emotional reaction to a reward-predictive cue causes a reduction in response rate when the cue is encountered in an instrumental situation. It therefore appears that both expectations of reward and appetitive emotional responses are necessary for cues to potentiate reward-seeking behaviour in a general-PIT context. Such processes may be applicable to nicotine seeking, where reward-predictive cues such as cigarette packets have become aversive due to their superposition with health warnings. Thus whether the current results generalise to such a naturalistic situation should be investigated.

**Declaration of conflicting interests**

The Authors declare that there is no conflict of interest.

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**Supplementary Material**

The Supplementary Material is available at: qjep.sagepub.com

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**Figure Captions**

Fig. 1. Stimuli used as CSs in Pavlovian training (left) and example images used as aversive (middle) or neutral (right) counter-conditioning stimuli.

Fig. 1. Simplified diagram of procedure, depicting counter-conditioning (CC) embedded within PIT paradigm.

Fig. 2. Instrumental training event sequence, indicating screen durations for response-positive and response-negative trials, and response requirements during 18.5s blank screen.

In effect, to maximise their probability of reinforcement, participants needed to press at a rate > 1Hz throughout the 18.5s blank screen. To elicit this repeated pressing, the 18.5s included a hidden 1s reinforcement window of random onset (minimum 1.5s from screen onset, so as not to penalise slow responders), within which participants needed to press twice.

This schedule was adapted from the single-response PIT task of Talmi et al, (2008).

Fig. 4. Mean Expectancy of winning 50p after viewing CS+ across blocks of Pavlovian training, separated by counter-conditioning group.

Dashed line at ‘5’ represents rating of ‘don’t know’; error bars represent 95% CI.

Fig. 5. Mean Pleasantness rating for CS+ and CS± for Neutral and Aversive counter-conditioning group, following Pavlovian training (a) and counter-conditioning (b).

* CS+ > CS±, ps < .036; error bars represent 95% CI.

Fig. 6. Mean Response Initiation (a) and Response Rate (b) for each counter-conditioning group across blocks of instrumental training.

* Block 1 < Blocks 2 – 4, ps < .05; error bars represent 95% CI.

Fig. 7. Mean Response Initiation (a) and Response Rate (b) during transfer phase for CS+ and S± trials, and each counter-conditioning group.

* CS+ > S±, ps < .037; error bars represent 95% CI.



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Fig. 1. Stimuli used as CSs in Pavlovian training (left) and example images used as aversive (middle) or neutral (right) counter-conditioning stimuli.

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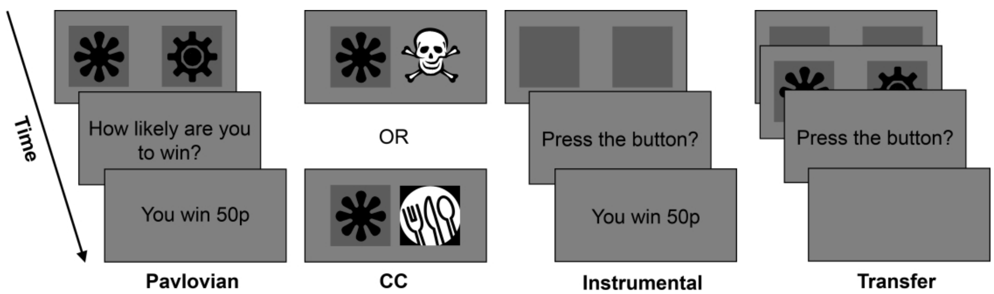


Fig. 2. Simplified diagram of procedure, depicting counter-conditioning (CC) embedded within PIT paradigm.

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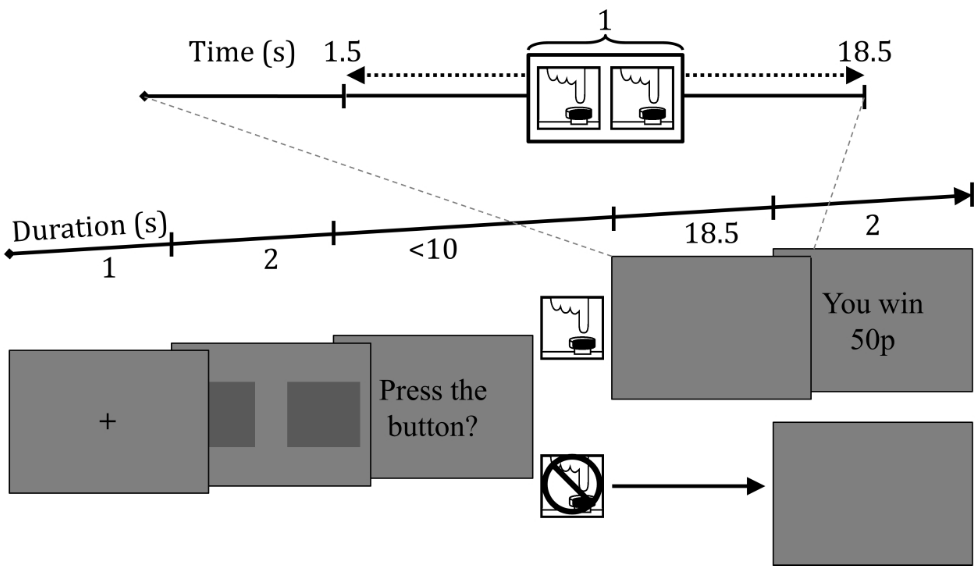


Fig. 3. Instrumental training event sequence, indicating screen durations for response-positive and response-negative trials, and response requirements during 18.5s blank screen.
In effect, to maximise their probability of reinforcement, participants needed to press at a rate > 1Hz throughout the 18.5s blank screen. To elicit this repeated pressing, the 18.5s included a hidden 1s reinforcement window of random onset (minimum 1.5s from screen onset, so as not to penalise slow responders), within which participants needed to press twice.
This schedule was adapted from the single-response PIT task of Talmi et al, (2008).

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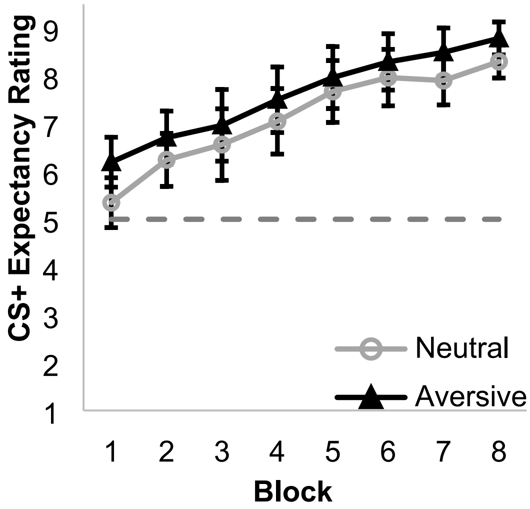


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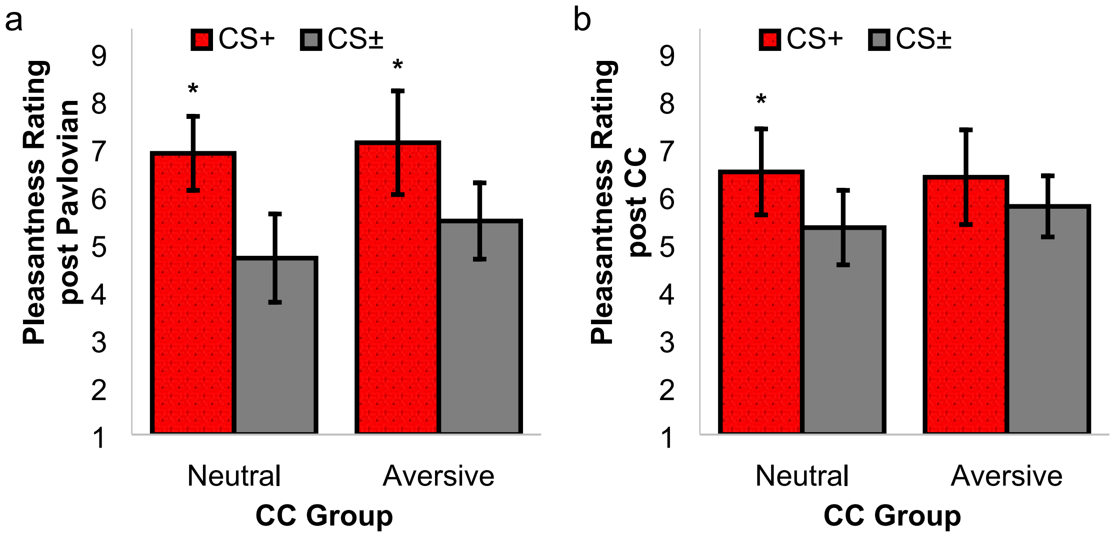


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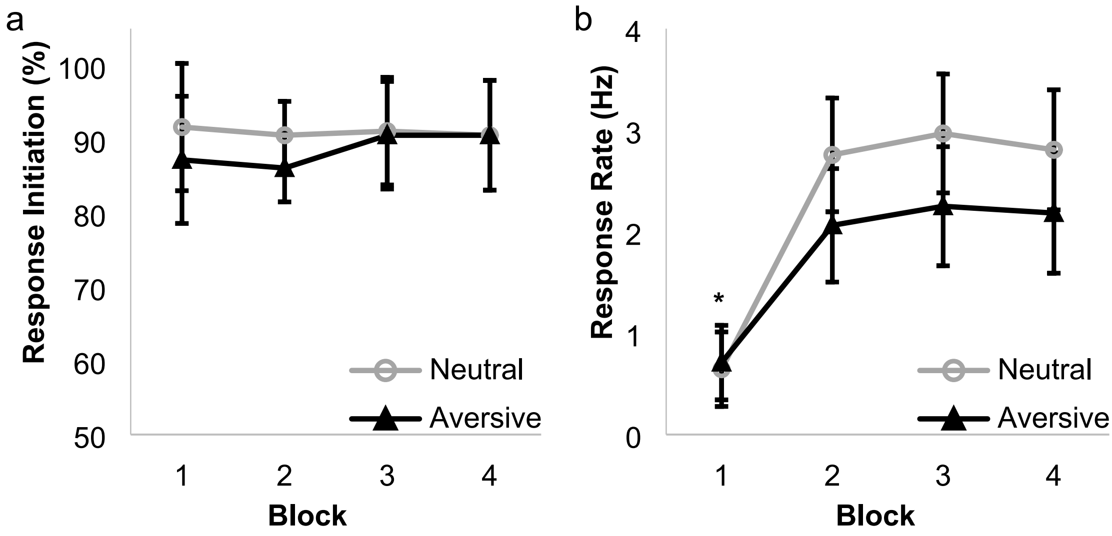


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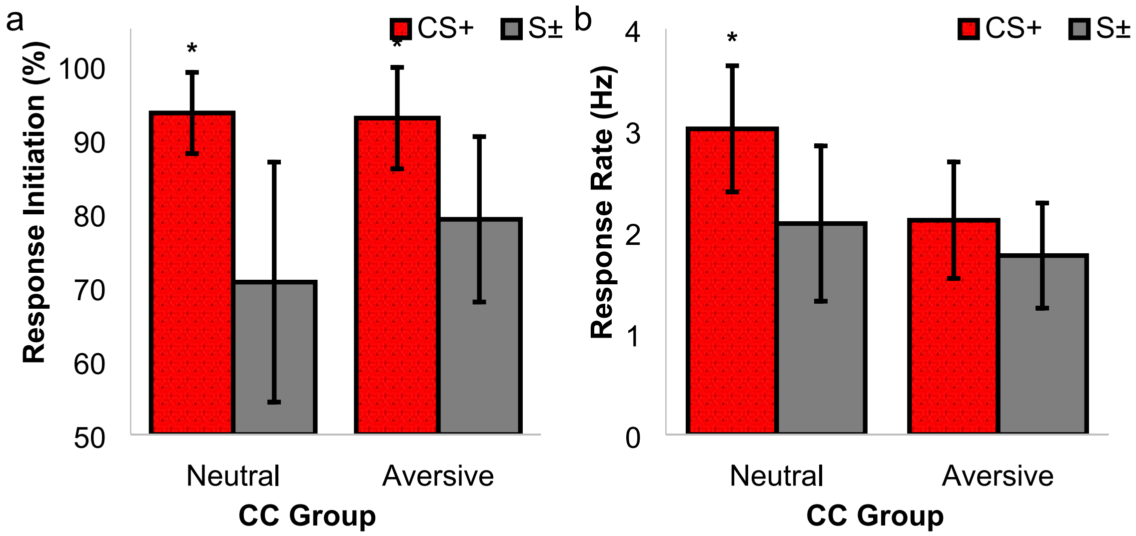


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*Table 1. Mean (95% CI) anxiety rating for CS+ and CS± for Neutral and Aversive counter-conditioning group, following Pavlovian training and counter-conditioning (CC).*

| | Post-Pavlovian | | Post-CC | |
|---|---|---|---|---|
| | CS+ | CS± | CS+ | CS± |
| Neutral | 2.39 (0.93) | 3.14 (0.87) | 3.06 (0.94) | 3.00 (0.83) |
| Aversive | 2.56 (1.05) | 2.61 (0.73) | 3.83 (1.26) | 3.28 (0.83) |

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*Table 2. Mean (95 + CI) estimated chance of winning (%) during transfer phase, for CS+ and S± by each CC group.*

** CS+ > S±, p < .001.*

| | CS+ * | S± |
|---|---|---|
| Neutral | 79.0 (8.16) | 49.3 (13.0) |
| Aversive | 77.0 (8.15) | 39.8 (13.0) |

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