EJN fens EUROPEAN JOURNAL OF NEUROSCIENCE

European Journal of Neuroscience, pp. 1–9, 2013 doi:10.1111/ejn.12377

Avoidance-based human Pavlovian-to-instrumental transfer

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Keywords: goal-directed, instrumental, motivation, negative reinforcement, striatum

Abstract

The Pavlovian-to-instrumental transfer (PIT) paradigm probes the influence of Pavlovian cues over instrumentally learned behavior. The paradigm has been used extensively to probe basic cognitive and motivational processes in studies of animal learning. More recently, PIT and its underlying neural basis have been extended to investigations in humans. These initial neuroimaging studies of PIT have focused on the influence of appetitively conditioned stimuli on instrumental responses maintained by positive reinforcement, and highlight the involvement of the striatum. In the current study, we sought to understand the neural correlates of PIT in an aversive Pavlovian learning situation when instrumental responding was maintained through negative reinforcement. Participants exhibited specific PIT, wherein selective increases in instrumental responding to conditioned stimuli occurred when the stimulus signaled a specific aversive outcome whose omission negatively reinforced the instrumental response. Additionally, a general PIT effect was observed such that when a stimulus was associated with a different aversive outcome than was used to negatively reinforce instrumental behavior, the presence of that stimulus caused a non-selective increase in overall instrumental responding. Both specific and general PIT behavioral effects correlated with increased activation in corticostriatal circuitry, particularly in the striatum, a region involved in cognitive and motivational processes. These results suggest that avoidance-based PIT utilizes a similar neural mechanism to that seen with PIT in an appetitive context, which has implications for understanding mechanisms of drug-seeking behavior during addiction and relapse.

Introduction

Goal-directed behaviors are often influenced by environmental cues associated with appetitive or aversive stimuli. This phenomenon, known as Pavlovian-to-instrumental transfer (PIT), can manifest as an increase in appetitive behaviors in response to a cue that, through conditioning, has attained appetitive properties (Balleine & Dickinson, 1998), or as a decrease in appetitive behaviors in the presence of an aversive conditioned cue (Estes & Skinner, 1941). It is thought that PIT can explain maladaptive behaviors maintained by positive reinforcement, such as reinstatement of drug-seeking behaviors after presentation of drug-related cues (Cardinal & Everitt, 2004). Modulation by Pavlovian cues upon instrumental responding, however, can also be assessed when that responding is maintained by negative reinforcement (e.g. avoidance learning; Rescorla & Solomon, 1967), a potentially important factor in continued or renewed drug-seeking behavior (Baker *et al.*, 2004). In the case of drug-seeking behavior, drug-related Pavlovian cues can lead to the experience of negative withdrawal symptoms; subsequently, these cues motivate avoidance behaviors. Yet, little is known about the mechanisms underlying the maintenance of instrumental avoidance behavior by aversive Pavlovian stimuli. Given its ability to function as a model of avoidance-based drug-seeking behavior, it is important to understand both the behavioral representation and neural correlates associated with avoidance-based PIT.

Previous research has characterized two qualitatively distinct forms of PIT. In 'specific PIT', reinforced instrumental responding is selectively increased in response to a conditioned Pavlovian cue with which the instrumental response once shared a reinforcing outcome. In 'general PIT', a conditioned Pavlovian cue motivates non-selective increases in reinforced instrumental responding, even when the cue and responses never shared a reinforcing outcome. Using appetitive PIT tasks, work in rodents demonstrates the necessity of the nucleus accumbens shell (Corbit & Balleine, 2011) and the basolateral amygdala (Corbit & Balleine, 2005) in specific PIT, and the nucleus accumbens core (Corbit & Balleine, 2011) and central nucleus of the amygdala (Corbit & Balleine, 2005) in general PIT. This is corroborated by human neuroimaging studies, which suggest the involvement of the striatum in specific PIT (Bray *et al.*, 2008; Talmi *et al.*, 2008; Prevost *et al.*, 2012), and the amygdala in general PIT (Prevost *et al.*, 2012). However, the aforementioned neuroimaging studies were conducted in the appetitive domain, examining approach behaviors maintained by positive reinforcement. Furthermore, animal studies that have examined PIT in the aversive domain have rarely attempted to distinguish between specific and

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Received 18 June 2013, revised 28 August 2013, accepted 1 September 2013

general PIT effects (Rescorla & LoLordo, 1965; LoLordo, 1967). Therefore, it is unclear if similar neural mechanisms are involved when PIT occurs in an aversive context, and if the motivation to actively avoid aversive events would promote both specific and general PIT effects (as suggested by Nadler *et al.*, 2011).

The current study sought to investigate the neural correlates of both specific and general PIT using an avoidance learning task with aversive conditioned stimuli. Specifically, we adapted a behavioral PIT paradigm (Corbit & Balleine, 2005) to examine how aversive Pavlovian stimuli motivate instrumental avoidance behavior. We expected to observe specific and general PIT effects using negative reinforcement. We further hypothesized that the striatum would be engaged during specific PIT with negative reinforcement, in accordance with previous studies of PIT with positive reinforcement. Additionally, we predicted that striatal activation would correlate with general PIT, highlighting the general motivational properties of this region in an avoidance learning context.

Materials and methods

Participants

Twenty-four right-handed volunteers were recruited via flyers posted on the Rutgers campus (12 female, 12 male). The final analysis included 20 participants (12 female, eight male, mean age = 20.84 years, SD = 2.99), as three participants were excluded due to failure to meet instrumental learning criteria (see Procedure description) and one participant was excluded due to excessive head motion during the scanning session. All participants gave informed written consent prior to the experiment. The study was approved by the Rutgers University Institutional Review Board for the Protection of Human Subjects in Research, and was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Procedure

The current study examined both specific and general PIT using a computer game paradigm modified from that used by Nadler *et al.* (2011). At the start of the experiment, participants were told that they would be playing a simple computer game wherein their goal was to defend a fictional kingdom against attacks by various creatures. Participants proceeded to perform three phases of the PIT task: (1) instrumental phase; (2) Pavlovian phase; and (3) transfer test phase (outlined in Table 1).

Instrumental phase

Instrumental training was modeled after a Sidman avoidance task (Sidman, 1953a,b), used extensively to study negative reinforcement processes in rodents (Mackintosh, 1974), but more rarely used with humans. In the instrumental phase, associations between two distinct instrumental responses (R1 and R2) and the avoidance of two distinct aversive outcomes (O1 and O2) were acquired. Prior to the start of the instrumental phase, participants were instructed that they would be attacked by two different creatures (e.g. goblin, troll or ogre, counterbalanced across participants) and that they could utilize two available button presses, each of which yielded a different type of imaginary shield. Participants were told that each shield may or may not be effective at defending against a particular type of attack, and that they had to learn through trial and error which button press would protect them from a specific attack (e.g. button 1 yielded an imaginary shield that was effective at protecting against goblin attacks). Participants underwent two sessions of instrumental conditioning during which they were to learn the avoidance contingency in effect. In one of these sessions the R1–O1 avoidance contingency was in effect, and during the second session the R2–O2 avoidance contingency was in effect. During a single session, only one outcome was presented (either O1 or O2). Each session lasted for 180 s, and during this time an aversive outcome was scheduled to occur 1 s after the termination of the previous outcome, unless the participant made the appropriate button press response within this time period. If the correct button was pressed, this delayed the onset of the subsequent aversive outcome by an additional 3 s. Therefore, this schedule should favor participants learning that one R could lead them to avoid getting attacked by a particular O. To discourage participants from randomly responding at all times, any button presses that occurred while the aversive outcome was on the screen were without any consequences.

When an aversive outcome (O1 or O2) was scheduled to occur it was shown on the center of the screen for 1 s. A fixation cross was presented on the screen at other times (Fig. 1A). Participants were allowed to perform instrumental responses R1 and R2 at will in order to prevent the aversive outcomes (O1 and O2) in each training phase, but a different one of these responses was operational during each phase. Thus, R1 prevented O1 in the first session and R2 prevented O2 during the second. In this schedule, participants could prevent the aversive outcome from occurring by continually performing the correct response during the fixation period. At the end of the second instrumental session, participants were asked to rate the efficacy of each R–O contingency on a scale from 1 to 10. For each outcome, the rating for the incorrect response was subtracted from the rating for the correct response. Participants were excluded from further analysis if this calculation resulted in a value ≤ 0 for either outcome, because this would indicate that the participant had not learned both of the instrumental contingencies. Based on this criterion, three participants were excluded from the remainder of the study, given that it would have been impossible to obtain an explicit PIT effect without learning the initial R–O contingencies. Imaging data was not collected during this phase of the study.

TABLE 1. Contingencies present in experimental paradigm

Instrumental phase	Pavlovian phase	Transfer test
R1–O1	CS1–O1	CS1: R1 vs. R2
R2–O1	CS2–O2	CS2: R1 vs. R2
	CS3–O3	CS3: R1 vs. R2
	CS4–O4	CS4: R1 vs. R2
	CS5–O5	CS5: R1 vs. R2

Pavlovian phase

During the Pavlovian phase, participants were asked to learn five stimulus–outcome (S–O) contingencies. In the spirit of the game, participants were told that a wizard would teach them about various colored signals, representative of different types of attacks, and that it was necessary to pay attention in order to learn what each colored signal represented. On every trial, one of five stimulus–outcome pairings was presented, such that each visual stimulus (S1–S5) was paired with either one of the previously viewed aversive outcomes (O1 and O2; e.g. goblin attack, troll attack), a novel aversive

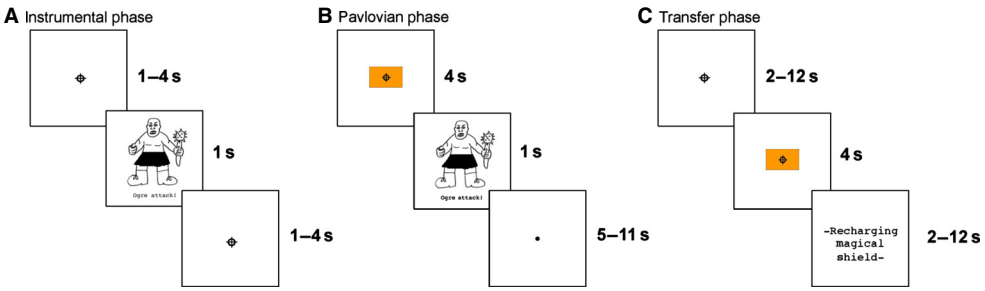

FIG. 1. Experimental design. (A) Instrumental phase. An aversive outcome, with a duration of 1 s, occurred after each 1-s fixation. Participants were free to respond using R1 and R2. The correct instrumental response, when made during the fixation period, prolonged the onset of the subsequent aversive event by an additional 3 s. Participants underwent two blocks of instrumental conditioning, each with a separate R–O contingency. (B) Pavlovian phase. Participants passively viewed five S–O contingencies, in random order, and were explicitly told to remember the contingencies presented. (C) PIT test. Participants were shown S1–S5, in random order, each preceded by a fixation and followed by a 'recharge' period. Participants were explicitly told to not perform instrumental responses during the recharge period, but were free to perform R1 and R2 as they saw fit at any other period in time.

outcome (O3; e.g. ogre attack), or one of two different neutral outcomes (O4 and O5; i.e. a screen that read 'malfunction' or the presentation of a fixation dot). Stimuli appeared on the screen for 4 s and outcomes were subsequently presented at stimulus offset for 1 s (Fig. 1B). A jittered inter-trial interval with a duration of either 7 s, 9 s or 11 s separated the trials. Stimulus–outcome pairs were shown nine times each, in random order, for a total of 45 trials. Participants were instructed to refrain from instrumental responding during the Pavlovian phase. At the end of this phase, participants viewed S1–S5 one at a time while the text 'What did this signal represent?' appeared on the screen along with a list of the five potential options. In order to check for explicit knowledge of each S–O contingency, participants were asked to respond verbally with the correctly paired outcome. This was meant to be used as an exclusionary criterion, given that the inability to learn S–O contingencies would have prevented an explicit PIT effect from being obtained in the transfer phase. However, all participants correctly reported all S–O contingencies; therefore, no participants were excluded based on the Pavlovian learning criterion.

Transfer phase

Participants were instructed that the wizard would now send out the colored signals about which they had just learned, and that they would be free to utilize the available button presses (i.e. shields) as they saw fit during this phase. The transfer phase included presentation of the five previously seen visual Pavlovian stimuli (S1–S5) in the absence of reinforcement. That is, the entire transfer phase was performed under extinction conditions. During this phase, participants were free to respond using R1 and R2, or to not respond at all, in response to the presentation of S1–S5. Each trial began with a 2–12-s jittered fixation period. A stimulus (S1–S5) was then presented on the screen for 4 s, followed by a jittered 2–12 s screen that said 'Recharging Magical Shield' during which participants were explicitly told not to make instrumental responses (Fig. 1C). However, participants were free to make responses during either the pre-stimulus fixation period or during stimulus presentation. Both the pre-stimulus fixation period and the post-stimulus 'recharge' period were included in order to have: (i) a baseline measure that allowed for instrumental responding; and (ii) a baseline measure wherein no responding occurred with which to compare the behavioral and blood oxygen level-dependent responses from the stimulus presentation period of each trial. Stimuli S1–S5 were shown 12 times each in random order for a total of 60 trials.

Behavioral analysis

As in our previous study (Nadler *et al.*, 2011), we measured specific and general forms of PIT by comparing the number of instrumental responses (R1 and R2) made: (i) across stimulus types (S1–S5); and (ii) during presentation of stimuli S1–S5, as compared with the pre-stimulus fixation period. All behavioral analyses consisting of more than two *t*-tests within a family of comparisons were corrected for multiple comparisons with the sequential Bonferroni correction (Holm, 1979; Rice, 1989).

Functional magnetic resonance imaging acquisition and analysis

Images were acquired using a 3T Siemens TRIO scanner at the Rutgers University Brain Imaging Center (RUBIC). Structural images were collected using a T1-weighted MPRAGE sequence (256 × 256 matrix; FOV = 256 mm; 176 1-mm sagittal slices). Functional images were acquired using a single-shot gradient echo EPI sequence (TR = 2000 ms, TE = 30 ms, FOV = 192, flip angle = 90 °, bandwidth = 2232 Hz/Px, echo spacing = 0.51) and comprised 32 contiguous oblique-axial slices (3 × 3 × 3 mm voxels) parallel to the anterior commissure-posterior commissure line. Functional images were collected during both the Pavlovian and transfer phases of the task. BrainVoyager QX software (version 2.3; Brain Innovation) was used to pre-process and analyse the imaging data. Pre-processing consisted of 3D motion correction (six parameters), slice scan time correction (trilinear/sinc interpolation), spatial smoothing with a 3D Gaussian filter (4 mm FWHM), voxel-wise linear detrending, and high-pass filtering of frequencies (3 cycles per time course). One participant was excluded from analysis due to excessive head motion during functional runs. Structural and functional data from each participant were then transformed to standard Talairach stereotaxic space (Talairach & Tournoux, 1988).

In modeling the transfer phase, a random-effects general linear model was conducted using each of the five stimulus types (S1–S5) as regressors of interest. We also included six regressors of no interest (six motion parameters). Regressors were convolved with a 2-gamma hemodynamic response function and z-transformed at the single participant level. Transfer phase analyses were performed at a threshold of $P < 0.001$, FDR corrected. All *post hoc* analyses consisting of more than two *t*-tests within a family of comparisons were corrected for multiple comparisons with the sequential Bonferroni correction (Holm, 1979; Rice, 1989).

Results

Behavioral results

Instrumental conditioning

To measure instrumental learning, we assessed the number of times that participants experienced aversive outcomes during the instrumental phase – a measure commonly employed to determine successful Sidman avoidance learning (Sidman, 1962; Ulrich *et al.*, 1964; Klein & Rilling, 1972). To obtain an estimate of learning over time, we broke up each 180-s block into six 30-s bins. We observed a significant decrease in the number of experienced aversive outcomes from the first 30 s to the last 30 s of each 180-s block (one-tailed paired *t*-test, $t_{18} = 9.179$; $P < 0.001$), indicating that the correct R–O contingencies were learned over time (Fig. 2A). Importantly, this decrease happened irrespective of outcome (O1: $t_{18} = 8.286$; $P < 0.001$; O2: $t_{18} = 6.229$; $P < 0.001$), suggesting that both R–O contingencies were acquired successfully. To confirm, participants were asked at the end of the instrumental phase to verbally report, on a scale of 1–10, how effective each response (R1 and R2) was at preventing each outcome (O1 and O2). For those participants who met the instrumental learning criterion, verbal ratings were as follows: R1–O1 (correct contingency), mean = 9.684, SD = 0.749; R2–O1 (incorrect contingency), mean = 1.842, SD = 1.344; R2–O2 (correct contingency), mean = 9.684, SD = 0.820; R1–O2 (incorrect contingency), mean = 1.947, SD = 1.682.

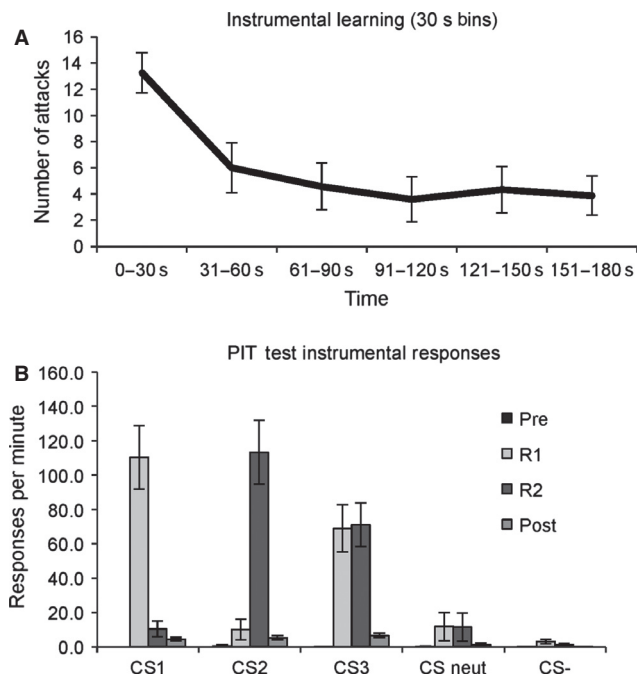

FIG. 2. Behavioral results. (A) Number of attacks per 30-s bin during the instrumental phase. Participants experienced significantly fewer attacks during the last 30 s as compared with the first 30 s, indicating that learning of the correct R–O contingencies had occurred ($P < 0.001$). (B) Number of responses per minute, by trial type, during the PIT test. Specific transfer effects occurred in response to S1 and S2, wherein responding increased selectively for one of the instrumental responses (R1 or R2), but not the other, as compared with the pre-stimulus period (all $P < 0.001$). In contrast, a general transfer effect was seen in response to S3, wherein responding with R1 and R2 increased non-selectively as compared with the pre-stimulus period (all $P < 0.001$). The post-stimulus 'recharge' period is also graphed. Error bars represent standard error of the mean (SEM).

Pavlovian conditioning

Following the Pavlovian phase, all participants were asked to explicitly verbalize the outcomes associated with S1–S5 by answering the question 'What did this signal represent?' All participants had correctly learned all five S–O contingencies by the end of this phase, as indicated by success in explicitly matching each stimulus to its associated outcome.

PIT

To measure specific and general PIT, we compared instrumental responding (R1 and R2) made: (1) across all five stimulus types; and (2) during stimulus presentations, as compared with the pre-stimulus fixation period (Fig. 2B). A three-way repeated-measures ANOVA examining the effects of stimulus (S1–S5), interval (pre-stimulus and stimulus) and response (R1 and R2) revealed a significant main effect of stimulus ($F_{4,76} = 22.627$; $P < 0.001$), and a significant main effect of interval ($F_{1,19} = 34.898$; $P < 0.001$). *Post hoc t*-tests revealed that the amount of instrumental responding was elevated during presentation of S1–S3 as compared with the neutral stimulus, S5 (all $P < 0.001$). No significant differences in the amount of instrumental responding were present amongst S1–S3 (e.g. S1 vs. S2; all $P > 0.05$). There was also no difference between instrumental responding during presentation of S4 and S5 ($P = 0.218$). Regarding the main effect of interval, a significantly greater number of instrumental responses were made during the stimulus period as compared to the pre-stimulus period. A stimulus × response interaction was also observed ($F_{4,76} = 26.447$; $P < 0.001$), as was a stimulus × interval interaction ($F_{4,76} = 22.982$; $P < 0.001$). Finally, a three-way stimulus × interval × response interaction was observed ($F_{4,76} = 25.480$; $P < 0.001$). This three-way interaction was further analysed via one-way ANOVAS across the four levels of responding (pre-stimulus, stimulus, R1 and R2) for each stimulus using a pooled mean square error term (MSerr = 5464.632). Significant main effects were obtained for S1–S3 (all $P < 0.01$), but not for S4–S5 (all $P > 0.05$). *Post hoc t*-tests supported the finding that S1 selectively elevated R1 ($P < 0.001$) but not R2 responding ($P > 0.05$) relative to the pre-stimulus period, and that S2 selectively elevated R2 ($P < 0.001$) but not R1 ($P > 0.05$) responding relative to the pre-stimulus period. *Post hoc t*-tests for S3 revealed that R1 and R2 responding did not differ ($P = 0.401$), but were both significantly greater than responding during the pre-stimulus period (all $P < 0.001$). Therefore, a specific PIT effect was found, wherein a selective increase in R1 and R2 occurred during presentation of S1 and S2, respectively, that is, when both the stimulus and response shared a learned Pavlovian outcome. Additionally, a general transfer effect was observed such that the stimulus (S3) associated with the novel aversive outcome (O3) elicited a non-selective increase in both available responses (R1 and R2) as compared with the pre-stimulus baseline. No increases in instrumental responding from pre-stimulus period to stimulus presentation occurred for S4 or S5.

We divided the PIT test into 5 bins of 12 trials each in order to examine potential changes in number of responses made across time, as has been done in previous studies (Bray *et al.*, 2008; Prevost *et al.*, 2012). Importantly, the PIT test was performed in extinction and without reinforcement, suggesting that any instrumental responding in this phase is an actual behavioral expression of PIT. We performed a two-way repeated-measures ANOVA, examining the effects of both stimulus type (S1–S5) and bin (1–5) on total number

of instrumental responses made during stimulus presentation. This ANOVA revealed a main effect of stimulus ($F_{4,28} = 9.355$; $P < 0.001$), as expected, and no main effect of bin ($F_{4,28} = 0.487$; $P = 0.745$) or stimulus × bin interaction ($F_{16,112} = 1.006$; $P = 0.456$). Importantly, although the PIT test was performed under extinction conditions, participants maintained R1 and R2 responding in the presence of the Pavlovian stimuli throughout the duration of the PIT test.

Neuroimaging results

PIT

To identify brain regions involved in specific and general PIT, we performed a one-way ANOVA comparing activation during presentation of all five stimuli (S1–S5; Fig. 3A). We then examined the overall *F*-test, FDR-corrected to a threshold of $q < 0.001$. All significant clusters are reported in Table 2. Of particular interest was bilateral activation in the putamen (left, *x, y, z* = −22, 4, 6; right, *x, y, z* = 17, 7, 3), cingulate cortex (*x, y, z* = 2, 13, 42) and bilateral insula (left, *x, y, z* = −43, −2, 6; right, *x, y, z* = 35, 1, 6). We focused on these regions given their role in human conditioning, avoidance learning and PIT (Kim *et al.*, 2006; Bray *et al.*, 2008; Delgado *et al.*, 2008; Delgado *et al.*, 2011; Prevost *et al.*, 2012; Talmi *et al.*, 2008). To understand directionality, parameter estimates were extracted from these regions and *post hoc* two-tailed *t*-tests were run. Bilateral putamen (Fig. 3B), cingulate cortex (Fig. 3C) and bilateral insula (Fig. 3D) all exhibited increased activation during presentation of specific transfer stimuli (S1 and S2) and the general transfer stimulus (S3) compared with the neutral stimulus, S5 (all $P < 0.05$). The cingulate showed increased activation in response to specific transfer stimulus S2 compared with the general transfer stimulus ($P < 0.05$), but there was no difference between S1 and the general transfer stimulus ($P > 0.05$). Within all of these regions, there were no differences in activation during presentation of S4 and S5 (all $P > 0.131$).

Relationship between Pavlovian striatal activation and behavioral PIT

We were interested in the relationship between striatal activation during the Pavlovian learning phase and subsequent motivated responding in the transfer phase. Specifically, we were interested in how the striatal response to S1–S3 while learning S–O contingencies would later impact R1 and R2 instrumental responses while viewing S1–S3 in extinction. We hypothesized that greater Pavlovian phase activation in the striatum while viewing S1–S3, potentially reflecting increased motivation during learning, would subsequently lead to increases in motivation during the transfer phase, as demonstrated by more vigorous instrumental responding. Thus, the peaks of activation in both left (*x, y, z* = −22, 4, 6) and right putamen (*x, y, z* = 17, 7, 3) from the transfer phase ANOVA were used to create regions of interest (ROIs) in the Pavlovian phase. Parameter estimates from the Pavlovian phase using these ROIs were extracted and correlated with subsequent behavior in the transfer phase. Left Pavlovian putamen activation during presentation of S1 (Fig. 4A) and S2 (Fig. 4B) positively correlated with the number of responses made during specific PIT (S1: $r = 0.523$, $P = 0.018$; S2: $r = 0.495$, $P = 0.027$). A trend for a positive correlation between left putamen activation during presentation of S3 and number of subsequent general PIT responses was observed (left, $r = 0.390$, $P = 0.089$; Fig. 4C). In the right putamen, activation in response to S1, S2 and S3 was positively correlated with instrumental responding, but was only significant for S2 ($r = 0.620$, $P = 0.004$) and not for S1 ($r = 0.225$, $P = 0.340$) or S3 ($r = 0.051$, $P = 0.831$). For S4 and S5, no correlations between Pavlovian phase putamen activation and subsequent responding during the transfer phase were found (all $P > 0.216$). Thus, greater putamen activation toward aversive stimuli in the Pavlovian phase was associ-

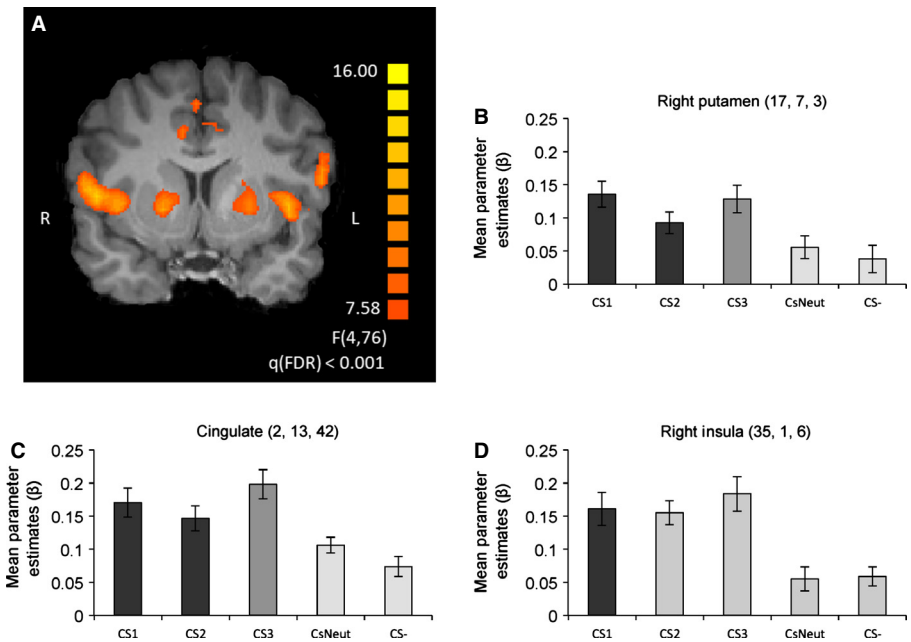

FIG. 3. (A) A one-way ANOVA during the PIT test examined potential differences across S1–S5 and identified ROIs in the bilateral putamen, cingulate cortex and bilateral insula ($y = 8$). Graphs depict mean parameter estimates (β) for ROIs in (B) right putamen, (C) cingulate cortex and (D) right insula, all of which exhibited increased activation during presentation of specific and general transfer stimuli (S1–S3) as compared with the neutral stimulus, S5 (all $P < 0.05$). Error bars represent SEM.

TABLE 2. Regions of activation in a one-way ANOVA during the PIT test, q (FDR) < 0.001

Region of activation	BA	Laterality	Talairach coordinates			Voxels (mm^3)	F
			x	y	z		
Medial frontal gyrus	6	L	−4	−8	51	3041	15.43
Cingulate cortex	32	R	2	13	42	679	11.75
Inferior parietal cortex	40	L	−49	−32	42	20493	32.45
Inferior parietal cortex	40	R	44	−35	42	3234	15.94
Occipital cortex	19	R	17	−83	30	481	16.40
Superior frontal gyrus	9	L	−37	37	30	150	10.10
Occipital cortex	18	L	−16	−83	27	1083	12.00
Postcentral gyrus	3	L	56	−17	24	140	9.71
Inferior frontal gyrus	44	L	−52	4	21	276	21.01
Thalamus		R	11	−14	9	525	12.77
Thalamus		L	−16	−17	9	917	27.70
Insula		R	35	1	6	4404	19.00
Putamen		L	−22	4	6	842	16.95
Putamen		R	17	7	3	1116	12.77
Putamen		L	−31	−11	3	811	16.15
Insula		L	−40	3	3	787	20.15
Inferior frontal gyrus	47	L	−40	31	−3	474	13.86
Cerebellum		R	11	−47	−21	6280	29.47
Cerebellum		L	−40	−47	−27	319	11.55

BA, Brodmann's area; FDR, false discovery rate; L, left; R, right.

ated with greater instrumental responding toward those same stimuli in the transfer phase, but this was only significant for specific transfer stimuli (S1 and S2).

Discussion

In the current study, our aim was to understand the behavioral and neural manifestation of avoidance-based PIT in humans. Behaviorally, the ability of stimuli associated with aversive outcomes to motivate instrumental responses paralleled a prior version of this task (Nadler *et al.*, 2011), and extended it by using a pure avoidance procedure (as opposed to the quasi-avoidance procedure previously used). A specific PIT effect was found, wherein an instrumental response that previously signaled the omission of a specific aversive outcome was selectively increased in the presence of a conditioned stimulus that signaled that same aversive outcome. A general PIT effect was also observed, as responding for both R1 and R2 increased above baseline in the presence of a conditioned stimulus that signaled a novel aversive outcome for which participants had never learned an avoidance response. Investigating avoidance-based PIT in the human brain, we observed increased activation in corticostriatal circuits, including the striatum (bilateral putamen) and the cingulate cortex during specific and general forms of PIT. Furthermore, activity in the putamen ROI during Pavlovian conditioning correlated with the vigor of instrumental responding during specific PIT. Our findings support previous research suggesting that corticostriatal regions are involved in PIT in humans (Bray *et al.*, 2008; Talmi *et al.*, 2008; Prevost *et al.*, 2012), and further suggest this involvement occurs

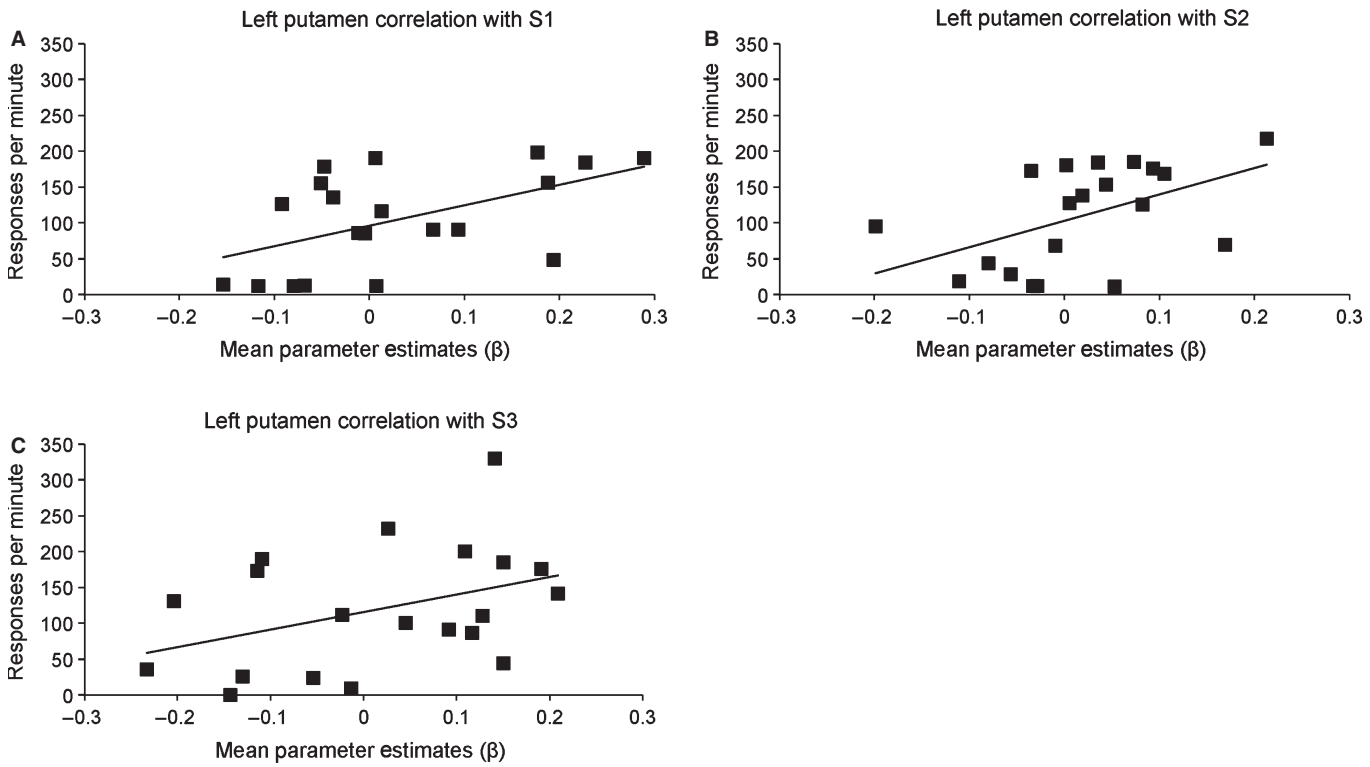

FIG. 4. Correlation between left putamen activation in the Pavlovian phase and number of specific transfer instrumental responses made during the transfer phase. Significant correlations were present between left putamen activation (x, y, z = −22, 4, 3) in the Pavlovian phase and subsequent number of instrumental responses made during the PIT test in the presence of both specific stimuli: (A) S1 and (B) S2. A trend for a positive correlation was present between left putamen activation in the Pavlovian phase and subsequent number of instrumental responses made in the presence of the general stimulus (C) S3 during the PIT test.

when the context is aversive. That corticostriatal activation was present during motivated responding to avoid negative outcomes fits with the claim made by Dickinson & Dearing (1979) that there should be a convergence between neural circuits for 'rewarding' outcomes across motivational classes.

As was pointed out by Rescorla & Solomon (1967), aversive conditioned stimuli can influence instrumental responding by either facilitating or suppressing behavior through their activation of a 'central motivational state' that interacts with the motivation to respond. For instance, it has been long known in research with rats that stimuli signaling electric foot shock will suppress food-reinforced lever pressing (Estes & Skinner, 1941) but increase lever pressing maintained on a shock avoidance schedule (Rescorla & LoLordo, 1965). The first effect, conditioned suppression, is generally understood to reflect a motivational conflict between food seeking and the anticipation of danger (Rescorla & Solomon, 1967). However, the second effect, conditioned facilitation, is thought to reflect a motivational synergy between the anticipation of danger and the knowledge of how to avoid that danger (Seligman & Johnston, 1973). It is by no means obvious that the neural substrates mediating these two effects should partially overlap. While this analysis is only in its infancy, both with humans and with non-human animals, the present data implicate corticostriatal regions in the facilitative effect of such stimuli on negatively reinforced avoidance responding. This is complimentary with recent work suggesting striatal involvement in the association between aversive stimuli and the inhibition of behavioral responses during PIT (Guerts *et al.*, 2013).

One goal of our avoidance-based PIT procedure for humans was to follow closely the methodologies of PIT studies conducted with non-human animals (Corbit & Balleine, 2005). Corbit & Balleine (2005) found that rodents selectively increased responding toward conditioned stimuli when both the specific instrumental response and the stimulus shared an outcome, an effect we replicate and extend with humans. Both humans and rodents also show a non-selective increase in behavior (general PIT) in the presence of a conditioned stimulus that was never seen during instrumental conditioning and, therefore, did not share an outcome with any available instrumental responses. However, unlike Corbit & Balleine (2005) and other previous animal studies of PIT, the current study examined PIT with negative reinforcement, specifically in an avoidance learning context. Rescorla & Solomon (1967) noted that while Pavlovian modulation by conditioned stimuli upon instrumental responding occurs when instrumental responding is maintained by positive reinforcement, it can also occur when responding is maintained by negative reinforcement. To our knowledge, this study is the first to examine PIT with negative reinforcement in the human brain. The current study suggests that negatively reinforced conditioned stimuli are successful at motivating behavior that is aimed at preventing specific negative outcomes as well as increasing a more general avoidance behavior. Given that negative reinforcement yields a powerful influence on behavior (perhaps greater than positive reinforcement in some contexts; Niznikiewicz & Delgado, 2011), and given that past research suggests differences in the ability of appetitive and aversive Pavlovian stimuli to modulate active instrumental behaviors (Huys *et al.*, 2011), the role of negative reinforcement in the maintenance of behavior, particularly under extinction conditions, is a topic of great interest for future research. In particular, direct comparisons of both the behavioral and neural manifestation of PIT when behavior is motivated by positive or negative reinforcement should be considered.

An important point about the Rescorla & Solomon (1967) approach is that it does not adequately anticipate the distinction between specific and general PIT effects. That is, it does not distinguish between the effects of different stimuli that both signal qualitatively distinct outcomes from the same motivational class. In order to explain specific PIT, then, another mechanism must be assumed, and the typical one is that such stimuli activate a specific representation of the outcome with which it was paired (Kruse *et al.*, 1983). Our behavioral data support this distinction between general motivational and specific expectancy influences of Pavlovian stimuli upon instrumental avoidance responding. Here, we present evidence that such a distinction also applies to PIT in avoidance learning contexts.

Similar specific and general PIT effects upon instrumental behaviors in positive and negative reinforcement (avoidance) contexts do not necessarily entail similar underlying neural mechanisms. Consider how specific PIT is generally assumed to work in a positive reinforcement setting. Separate response–outcome (R–O) and stimulus–outcome (S–O) associations are assumed to be learned in the instrumental and Pavlovian learning phases, respectively. During the PIT test, the S is assumed to activate a representation of the specific O with which it was paired, and this in turn is assumed to directly activate the particular R that was also associated with that O through a backward action on the R–O link (Pavlov, 1932; Mackintosh & Dickinson, 1979). In an avoidance learning situation, on the other hand, the instrumental response signals the absence of the aversive outcome, generating an R–no O association (Seligman & Johnston, 1973). The present data are interesting in suggesting that the neural substrates recruited in specific PIT in an avoidance learning context may be similar to those seen in appetitive positive reinforcement learning contexts (Bray *et al.*, 2008; Talmi *et al.*, 2008; Prevost *et al.*, 2012). How are we to reconcile these differences in underlying learning with similar results in the two domains? If the avoidance response itself is supported by an anticipation that a specific aversive outcome will occur unless a response is made, then this could result in the formation of a direct O–R associative link during the instrumental learning phase (perhaps in addition to an R–no O link). Specific PIT can be mediated by these S–O and O–R links in avoidance learning. The main difference may be that in avoidance learning the O–R link is established directly, but in positive reinforcement the R–O link is used in the backward direction (Pavlov, 1932). Nevertheless, the present data point more to similarities than differences in the way in which specific PIT effects occur in appetitive and aversive domains, but additional work will be needed to more clearly identify underlying neural circuits.

It is noteworthy that a recent interpretation of specific and general PIT effects (Cohen-Hatton *et al.*, 2013) suggests that if Pavlovian training follows instrumental training, the presentation of an O during the Pavlovian phase can activate the associated R and, if this R occurs contiguously with S, an S–R link can be acquired. Therefore, specific PIT may be a reflection of these learned S–R associations. We believe that this sort of mechanism is unlikely to apply to the present situation, as each O was not embedded within the corresponding S during our Pavlovian training phase. Given that the outcomes only occurred after the Pavlovian signals were turned off in the present study, this would mean that the S was more contiguous with the O than the presumed O–activated R motor program that would follow the O. Our imaging data do not fully capture any presumed underlying neural differences between general (central motivational state mediated) and specific (expectancy mediated) forms of PIT, for example, which have been previously reported (Prevost *et al.*, 2012). In our study, the presence of a strong correlation between putamen activity during Pavlovian training and specific, but not general, PIT may suggest that this structure is chiefly involved in coding specific expectancy effects, rather than more general moti-

vational effects of stimuli upon behavior. Given the known involvement of the striatum in the acquisition of aversive S–O contingencies with both primary and secondary reinforcers (Delgado *et al.*, 2011), perhaps it is not surprising that greater striatal engagement during the acquisition of the S1 and S2 contingencies correlated with increased behavioral responding during PIT. However, it would be interesting for future research to examine in greater detail the properties of the specific and general transfer stimuli that lead to differences in the importance of striatal engagement during Pavlovian conditioning for the maintenance of a vigorous behavioral PIT response. Our results point more strongly to a role in specific PIT, though, and this is consistent with prior animal work demonstrating that specific, but not general, PIT effects were abolished by inactivation at the time of Pavlovian training of the dorsomedial or dorsolateral striatum (Corbit & Janak, 2010). These authors suggested that the dorsomedial striatum is more involved in acquisition of specific R–O associations, while the dorsolateral striatum is more involved in acquisition of specific S–O associations.

Additionally, the relative contributions of specific PIT and general PIT effects will very likely differ in different settings. In one previous attempt to demonstrate PIT in rats using alcohol rewards, general, but not specific, PIT was attained (Glasner *et al.*, 2005). The authors concluded that the more cognitive specific PIT, which involved encoding individual stimulus–outcome and response–outcome relationships, was less influential than the non-specific motivational arousal generated by the appetitive conditioned stimuli. It may be that if aversive stimuli are more salient than appetitive stimuli in certain contexts, these general PIT effects will dominate to an even greater extent over specific PIT effects.

Another noteworthy difference between the current study and previous investigations of PIT in humans (Bray *et al.*, 2008; Talmi *et al.*, 2008; Prevost *et al.*, 2012) is that we obtained successful specific and general PIT using instructed reinforcers. Unlike more typically used primary reinforcers (such as food or shock) that are inherently appetitive or aversive, or secondary monetary reinforcers, the reinforcers used in the current study acquired their value through instruction at the onset of the task. In utilizing aversive outcomes with which participants have no real-world experience, we hoped to minimize individual variability in perception of the outcomes. While the reinforcers used in the current study were not biologically relevant, our task still mirrored Pavlovian learning with biologically relevant outcomes in that it assessed control by associative relationships among multiple stimuli. We were able to observe whether the specific sensory properties or the more general features of these reinforcers predict the manner in which such stimuli affect instrumental performance. Given that we were able to obtain both specific and general behavioral PIT effects, our data speak to the strength of this type of reinforcement in associative learning studies.

Interestingly, the use of instructed, non-primary reinforcers may explain why we did not see correlations between general PIT and amygdala activation, as has been found previously in studies with both humans (Prevost *et al.*, 2012) and non-human animals (Corbit & Balleine, 2005). The human amygdala has been implicated in the acquisition of a conditioned response to aversive primary reinforcers (for review, see Phelps & LeDoux, 2005), but its involvement in the acquisition of a conditioned response to aversive secondary reinforcers in humans is less clear (e.g. monetary loss; Delgado *et al.*, 2011). Therefore, it may be possible that the lack of amygdala activation seen during conditioning with aversive secondary reinforcers extends to PIT. An important question for future studies, therefore, will be to directly compare the PIT phenomenon with primary, secondary and instructed reinforcers in order to delineate potential differences in the maintenance of behavior brought about by these distinct types of reinforcement.

While our study differs from previous studies of PIT in its use of both instructed reinforcement and an avoidance learning context, it is nonetheless an examination of the same basic phenomenon. Thus, our results in some part overlap with those obtained in past examinations of PIT. Human (Bray *et al.*, 2008; Talmi *et al.*, 2008; Prevost *et al.*, 2012) and animal studies (Corbit & Janak, 2007; Corbit & Balleine, 2011) have found a correlation between PIT and the striatum. As in the current study, previous human studies of PIT (Bray *et al.*, 2008; Prevost *et al.*, 2012) also found activation in the putamen, a lateral region of the striatum, during specific PIT. In contrast, research by Talmi *et al.* (2008) has implicated the more medial region of nucleus accumbens in PIT. Like the current study, Bray *et al.* (2008) and Prevost *et al.* (2012) separately examined specific and general PIT (though a general PIT effect was not found by Bray *et al.*, 2008). In contrast, the procedure used by Talmi *et al.* (2008) did not delineate between specific and general PIT, which may explain why their striatum ROI was in a more medial location than that found in the current study. Of note, we also found activation in the cingulate cortex, a region with projections to the striatum (for review, see Haber & Knutson, 2010), during both specific and general instances of PIT. While this region has not been found in previous human studies of PIT, it has been implicated, along with the insula, in studies of aversive conditioning (Büchel *et al.*, 1998; Jensen *et al.*, 2003; Delgado *et al.*, 2008, 2011).

Understanding the basic behavioral and neural mechanisms underlying PIT in humans with both positive and negative reinforcement will allow for PIT to be used as a model for a variety of non-normative behavioral responses toward real-world stimuli. The ability of positively reinforced Pavlovian conditioned stimuli to motivate behavior can be applied to real-world maladaptive behavior, such as instances of drug addiction wherein drug-related stimuli in the environment trigger drug-seeking behavior (Cardinal & Everitt, 2004). Behavioral research has already been successful in obtaining a specific PIT effect in nicotine-dependent individuals using smoking-related stimuli (Hogarth *et al.*, 2007). Evidence suggests that negative affect leads to drug craving and increases the likelihood of relapse (for review, see Sinha, 2007). Moreover, it has been found that addictive drugs are effective at reducing many negative symptoms of withdrawal (for review, see Baker *et al.*, 2004); thus it may be the case that attempts to avoid withdrawal symptoms can lead to relapse as well. Therefore, the current study, which sheds light on PIT in an avoidance learning context, might be used in the future as a model for drug relapse, wherein drug-related stimuli seem to motivate drug seeking through negative reinforcement (for review, see Baker *et al.*, 2004) and/or incentive sensitization mechanisms (Robinson & Berridge, 2001). Avoidance-based PIT can also be a useful model for gaining an understanding of other disorders involving avoidance of aversive stimuli, such as phobias and post-traumatic stress disorder.

Conflict of interest

The authors declare no competing financial interests.

Acknowledgements

This research was supported by funding from the National Institute on Drug Abuse to M.R.D. (DA027764) and A.R.D. (DA034995). The authors thank Natasha Nadler for her work with the initial version of the paradigm used in this article.

Abbreviations

PIT, Pavlovian-to-instrumental transfer; ROI, region of interest.

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