Addiction Biology

SSA SOCIETY FOR THE STUDY OF ADDICTION

HUMAN NEUROIMAGING STUDY

doi:10.1111/adb.12243

Pavlovian-to-instrumental transfer effects in the nucleus accumbens relate to relapse in alcohol dependence

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ABSTRACT

In detoxified alcohol-dependent patients, alcohol-related stimuli can promote relapse. However, to date, the mechanisms by which contextual stimuli promote relapse have not been elucidated in detail. One hypothesis is that such contextual stimuli directly stimulate the motivation to drink via associated brain regions like the ventral striatum and thus promote alcohol seeking, intake and relapse. Pavlovian-to-Instrumental-Transfer (PIT) may be one of those behavioral phenomena contributing to relapse, capturing how Pavlovian conditioned (contextual) cues determine instrumental behavior (e.g. alcohol seeking and intake). We used a PIT paradigm during functional magnetic resonance imaging to examine the effects of classically conditioned Pavlovian stimuli on instrumental choices in $n = 31$ detoxified patients diagnosed with alcohol dependence and $n = 24$ healthy controls matched for age and gender. Patients were followed up over a period of 3 months. We observed that (1) there was a significant behavioral PIT effect for all participants, which was significantly more pronounced in alcohol-dependent patients; (2) PIT was significantly associated with blood oxygen level-dependent (BOLD) signals in the nucleus accumbens (NAcc) in subsequent relapsers only; and (3) PIT-related NAcc activation was associated with, and predictive of, critical outcomes (amount of alcohol intake and relapse during a 3 months follow-up period) in alcohol-dependent patients. These observations show for the first time that PIT-related BOLD signals, as a measure of the influence of Pavlovian cues on instrumental behavior, predict alcohol intake and relapse in alcohol dependence.

Keywords human Pavlovian-to-instrumental transfer, nucleus accumbens, relapse in alcohol use disorder.

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INTRODUCTION

Relapse in substance dependence can be triggered by positively valenced situations in which drug consumption has previously taken place (Robbins & Everitt 1999; Heinz *et al.* 2003; Sanchis-Segura & Spanagel 2006). A paradigm to model this effect experimentally is Pavlovian-to-instrumental transfer (PIT), which measures the influence of Pavlovian-conditioned cues on instrumental behavior (Everitt & Robbins 2005; Glasner, Overmier &

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Balleine 2005; Corbit & Janak 2007a). For example, affectively positive Pavlovian cues can promote approach, while negative Pavlovian cues can inhibit approach (Huys *et al.* 2011). In alcohol-dependent patients, confrontation with Pavlovian cues may interact with more complex effects of context and mood, which have been shown to interact with the relapse risk of detoxified patients (Heinz *et al.* 2003; Koob & Le Moal 2008). Nevertheless, the neural activation patterns underlying PIT effects are candidate mechanisms mediating or influencing drug seeking and relapse (Watson *et al.* 2012). A better understanding of PIT effects in substance dependence may thus help to explain how and why drug-related cues can induce craving and promote relapses even after prolonged periods of abstinence when drug intake is no longer desired (O'Brien *et al.* 1998; Grüsser *et al.* 2002; Robinson & Berridge 2008). Indeed, the strength of PIT effects may be an indicator of relapse risk.

In animal studies, it has been shown that non-drug-related PIT is enhanced in cocaine-dependent animals indicating that drug exposure causes alterations in reward learning that are not necessarily specific for drug-related reinforcers but concern more general mechanisms (Saddoris, Stamatakis & Carelli 2011; LeBlanc, Maidment & Ostlund 2013a,b; Ostlund *et al.* 2014). This has been studied in animal but not in human substance dependence so far.

Recently, PIT has been investigated in non-dependent humans both behaviorally (Paredes-Olay *et al.* 2002; Huys *et al.* 2011; Nadler, Delgado & Delamater 2011; Trick, Hogarth & Duka 2011; Lovibond & Colagiuri 2013) and with neuroimaging techniques (Bray *et al.* 2008; Talmi *et al.* 2008; Prevost *et al.* 2012; Geurts *et al.* 2013; Lewis *et al.* 2013; Mendelsohn, Pine & Schiller 2014). These studies point to the nucleus accumbens (NAcc) as an important mediating brain structure (Bray *et al.* 2008; Talmi *et al.* 2008; Prevost *et al.* 2012; Geurts *et al.* 2013; Lewis *et al.* 2013; Mendelsohn *et al.* 2014), hypothetically via cue-induced dopamine release (Robbins & Everitt 1999; Kienast & Heinz 2006). Findings in human subjects line up with lesion studies in animals where the NAcc has also been identified as a crucial neural substrate for PIT (Corbit, Muir & Balleine 2001; Everitt, Dickinson & Robbins 2001; Corbit & Balleine 2005; Corbit & Janak 2007b; Saddoris *et al.* 2011; Pecina & Berridge 2013; Ostlund *et al.* 2014). The NAcc as part of the ventral striatum is a core area of the so-called reward system (Volkow *et al.* 1996, 2009; Breiter *et al.* 2001) and has been implicated in mechanisms promoting cue reactivity, e.g. conditioned responses and relapse (Heinz *et al.* 2004; Myrick *et al.* 2004; Beck *et al.* 2012) and approach behavior to alcohol cues (Wiers *et al.* 2014). Indeed, animal experiments have shown substantial individual variance in cue reactivity. While some animals approach the conditioned stimulus (CS) that predicts reward (so-called 'sign-trackers'), other animals approach the place where the reward will be provided (so-called 'goal-trackers'; Robinson & Flagel 2009). Only sign-tracking animals show a shift of dopamine release in the NAcc from the unconditioned stimulus (US) to the CS as postulated by theories of phasic dopamine as teaching signals (Huys *et al.* 2014) and an addicted phenotype (Saunders & Robinson 2010; Flagel *et al.* 2011).

Here, we therefore examined the relationship between the NAcc activation, PIT, relapse and drinking behavior in alcohol-dependent patients after detoxification. We focused our imaging analysis on the NAcc as a predefined anatomical region of interest (ROI) because it has been associated with the reinforcement learning system (Flagel *et al.* 2011; Lesaint *et al.* 2014), human PIT (Talmi *et al.* 2008; Geurts *et al.* 2013), dopamine and alcohol dependence (Heinz *et al.* 2004, 2005), and has been reported to covary with relapse risk in alcohol dependence (Grüsser *et al.* 2004).

Other known risk factors for relapse include craving (Bottlender & Soyka 2004; Adamson, Sellman & Frampton 2009), severity of alcohol dependence (McLellan *et al.* 1994; Langenbucher *et al.* 1996; Staines *et al.* 2003; Adamson *et al.* 2009) and smoking (Gulliver *et al.* 1995). To assess whether neural activation associated with PIT might be clinically valuable, we compared the predictability of relapse through the PIT signal in the NAcc to the predictability based on severity of alcohol dependence, craving and smoking.

We designed and implemented a PIT paradigm according to Huys *et al.* (2011) and Geurts *et al.* (2013). We hypothesized a stronger behavioral PIT effect, i.e. a higher number of button presses for positive and a lower number of button presses for negative background pictures, and a higher neural PIT activation in the NAcc in alcohol-dependent patients compared with healthy controls. Secondly, we also hypothesized that behavioral and neural PIT effects are stronger in relapsers compared with abstainers and that they are positively associated with the amount of alcohol intake during relapse.

METHODS AND MATERIALS

Participants

The bicentric study was conducted in Berlin and Dresden, Germany. We assessed $n = 31$ patients [age in years mean = 45.3, standard deviation (SD) = 11.4; $n = 4$ females] suffering from alcohol dependence according to DSM-IV-TR (American Psychiatric Association 2000) as well as $n = 24$ age and gender-matched healthy controls (age in years mean = 42.2, SD = 11.2; $n = 3$ females). The

Table 1 Sample characteristics (alcohol-dependent patients and healthy controls).

	Alcohol-dependent patients		*Healthy controls*		χ^2/t-*test*
Gender	Female: 4;male: 27		Female: 3; male: 21		0.96[b]
	Mean	*SD*	*Mean*	*SD*	P
Age in years	45.29	11.43	42.17	11.16	0.31
SES	−0.32	1.93	0.32	1.87	0.31
Lifetime alcohol intake in kg (pure alcohol)[c]	2006.73	1035.40	179.70	142.20	< 0.001
ADS	14.50	7.48	2.83	3.87	< 0.001
OCDS-G total score	13.03	9.42	3.21	3.23	< 0.001
Smokers	87%		67%		0.21[b]
MWT-B	105.87	10.79	102.27	10.02	0.22
BIS-15 total score	31.28	7.11	28.17	6.11	0.09
Behavioral PIT	0.77	1.30	0.40	0.69	0.09[a]

[a]One-tailed testing; [b]p-value of χ^2 test; [c]Prior to detoxification in alcohol-dependent patients. Socioeconomic status (SES) was computed as the sum of *z*-transformed social status, household income and inverse personal debt scores (Schmidt *et al.* 2006). ADS = Alcohol Dependence Scale (Skinner & Horn 1984); BIS-15 = Barratt Impulsiveness Scale 15, German version (Meule *et al.* 2011); MWT-B = Mehrfachwahl-Wortschatz-Intelligenztest (verbal intelligence, Lehrl 2005); OCDS-G = Obsessive Compulsive Drinking Scale, German version (Mann & Ackermann 2000); PIT = Pavlovian-to-instrumental transfer.

data were collected as a part of the LeAD study (www.lead-studie.de; clinical trial number: NCT01679145). Here, we analyzed all subjects for which data were available at the time of analysis.

Exclusion criteria for all subjects were left-handedness, a history of any substance dependence or current substance use (assessed by breath and drug urine testing) except for nicotine dependence in healthy controls and nicotine and alcohol dependence in patients; other major psychiatric disorders (DSM-IV axis one was assessed by the computer-based Composite International Diagnostic Interview, CIDI; Wittchen & Pfister 1997) and neurologic disorders. Alcohol-dependent patients had been detoxified on a ward. Patients were alcohol dependent for a minimum of 3 years and were recruited during acute detoxification. The severity of alcohol dependence was assessed using the Alcohol Dependence Scale (ADS; Skinner & Horn 1984), the amount of lifetime alcohol intake was measured by the CIDI (Wittchen & Pfister 1997) and current alcohol craving by the Obsessive Compulsive Drinking Scale (OCDS-G; Mann & Ackermann 2000) and smoking status was assessed using the Fagerström Test for Nicotine Dependence (FTND, Heatherton et al. 1991). To assess trait impulsivity, we used the Barratt Impulsiveness Scale (Meule, Vögele & Kübler 2011). The socioeconomic status (SES) was computed as the sum of *z*-transformed self-ratings of social status, household income and inverse personal debt scores (Schmidt *et al.* 2006). Verbal intelligence was assessed by a standardized vocabulary test (Mehrfachwahl-Wortschatztest-Intelligenztest; Lehrl, 2005). For sample characteristics, see Table 1. The group of patients suffering from alcohol dependence had gone through detoxification procedures on average 3.6 times (SD 3.77; range 1–15). All patients had been abstinent for at least 5 days (in days: mean = 20.38, SD = 10.86), were free of any psychotropic medication or drugs known to interact with the central nervous system (more than four half-lives post last intake) including detoxification treatment and showed no significant alcohol withdrawal (CIWA-Ar score below 3; Stuppäck *et al.* 1995) before functional magnetic resonance imaging (fMRI). Current substance or alcohol abuse was checked by breath and urine testing in all subjects. All participants gave written informed consent to participate. Ethical approval for the study was obtained from the ethics committee of Charité-Universitätsmedizin Berlin (EA1/157/11) and Universitätsklinikum Dresden (EK228072012). Participants received a monetary compensation of 10 €/hour for study participation.

Patients were contacted every 2 weeks for 3 months after detoxification. We assessed relapse rates [with relapse defined as ≥60/48 (male/female) gram of alcohol per occasion] and the amount of alcohol consumption using the timeline follow back method (Sobell & Sobell 1992). Breath tests were performed on each personal appointment (every 4 weeks) and urine drug tests before MRI scanning. Relatives were sporadically contacted to verify patient's abstinence status. Assessors active in the follow-up procedures were blinded for the behavioral and imaging data analysis. During the follow-up period, we lost seven patients because of dropout or technical problems. Thus, relapse rates and alcohol consumption during

the follow-up period were available in 24 patients (11 relapsers and 13 abstainers).

Most patients indicated abstinence as their therapeutic goal, whereas only two patients (one subsequent relapser and one abstainer) were aiming for controlled alcohol intake.

PIT paradigm

The PIT paradigm consisted of four parts: (1) instrumental training, (2) Pavlovian training, (3) PIT and (4) a forced choice task (see Fig. 1). Instrumental training was conducted before and the forced choice task after the scanning session; the Pavlovian and PIT part were assessed during fMRI scanning. The task was programmed using Matlab 2011 (MATLAB version 7.12.0, 2011; MathWorks, Natick, MA, USA) with the Psychophysics Toolbox Version 3 (PTB-3) extension (Brainard 1997; Pelli 1997). It was presented on a computer screen (instrumental training, forced choice) and on a projector via a mirror system (Pavlovian training and PIT). For further details regarding the paradigm, see Garbusow *et al.* (2014).

Instrumental training

Subjects were instructed to collect shells by repeated button presses and received probabilistic feedback. In go trials, a shell was monetarily rewarded in 80% and punished in 20% of trials if collected and vice versa if not collected. In no-go trials, if a shell was collected, this was monetarily punished in 80% and rewarded in 20% of the trials, and vice versa if not collected (see Fig. 1a). Participants performed 60–120 trials, depending on their performance: in order to ensure that all subjects were at comparable performance levels before advancing to the PIT part, a learning criterion was enforced (80% correct choices over 16 trials).

Pavlovian conditioning

At the beginning of each trial, a compound CS consisting of fractal-like pictures and pure tones (henceforth referred to as 'fractal CSs') was presented for 3 seconds. This was followed by a delay of 3 seconds with two fixation crosses at the two potential CS locations (left and right), a US was presented for a further 3 seconds (see Fig. 1b). We separated the CS and US presentation in time by including a 3-second interstimulus interval (i.e. effectively creating a trace conditioning paradigm) to exactly disentangle the blood oxygen level-dependent (BOLD) response of both stimuli. Subjects were instructed to observe the CSs and USs and to memorize the pairings. The set of stimulus pairings consisted of two positive CSs paired with images of +2 EUR and +1 EUR coins, one

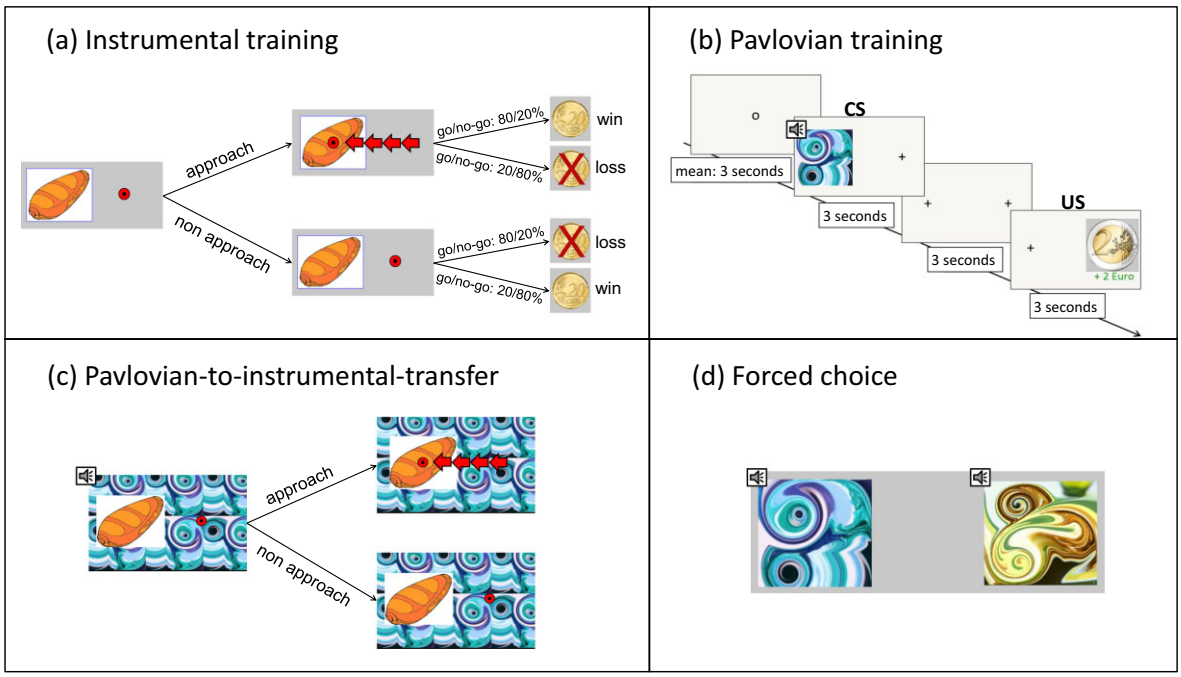

Figure 1 The PIT paradigm consisted of four parts: (a) instrumental training. In order to collect a shell, subjects had to move the red dot onto the selected shell by repeated button presses. Each response moved the button a fraction of the way toward the shell. (b) Pavlovian training. Audiovisual compound cues ('fractal CSs') were deterministically associated with one of five outcomes (two negative, one neutral, two positive). (c) Pavlovian-to-instrumental transfer. Subjects performed the instrumental task in nominal extinction (i.e. no explicit outcomes were presented). The background was tiled with the conditioned fractal CSs. (d) In order to assess Pavlovian conditioning, subjects were faced with a choice between two fractal CSs and asked to choose the better one

neutral CS paired with 0 EUR and two negative CSs paired with −1 EUR and −2 EUR (coins with a superimposed red cross, see also Fig. 1a). All subjects completed 80 trials.

Pavlovian-to-instrumental transfer

Subjects performed the instrumental task again with fractal CSs tiling the background (see Fig. 1c). No outcomes were presented, but subjects were instructed that their choices still counted toward the final monetary outcome (so-called nominal extinction). Participants completed 90 trials with fractal CSs tiling the background. Patients also completed trials with drink and water stimuli tiling the background. These data will be reported elsewhere.

Forced choice task

Finally, subjects chose one of two sequentially presented CSs (Fig. 1d). All possible CS pairings were presented three times in an interleaved, randomized order and stimuli were presented one at a time for 2 seconds. Slow responses led to a reminder requesting faster responses. We used these data to verify acquisition of Pavlovian expectations.

MRI acquisition

Functional imaging was performed on a Siemens Trio 3 Tesla MRI scanner with an echo planar imaging (EPI) sequences (repetition time: 2410 ms; echo time: 25 ms; flip angle: 80°; field of view: 192×192 mm^2; voxel size: $3 \times 3 \times 2$ mm^3) comprising 42 slices approximately −25° to the bicommissural plane. For co-registration and normalization during preprocessing, a three-dimensional magnetization-prepared rapid gradient echo image was acquired (repetition time: 1900 ms; echo time: 5.25 ms; flip angle: 9°; field of view: 256×256 mm^2; 192 sagittal slices; voxel size: $1 \times 1 \times 1$ mm^3). Prior to functional scanning, a field map was collected to account for individual homogeneity differences of the magnetic field.

Participants wore MR-compatible Siemens headphones; the sound volume of each tone was adapted individually. Responses were made on a 1×4 current design MR-compatible response box button using the right index finger (instrumental response in training and transfer) or two buttons using the left and the right index finger (forced choice).

Data analysis

Data were analyzed using Matlab 2011 (MATLAB version 7.12.0, 2011; MathWorks, Natick, MA, USA) and the R System for Statistical Computing Version 3.0.0 (R Development Core Team 2013). fMRI data were analysed using Statistical Parametric Mapping 8 (SPM8) software package (http://www.fil.ion.ucl.ac.uk/spm/; Wellcome Department of Imaging Neuroscience).

Behavioral analyses

We calculated individual PIT effects by regressing the mean number of button presses on the negative, neutral and positive value of the five CSs (−2, −1, 0, +1, +2). The regression slope reflects a measure of the strength of the individual PIT effect (from −2 to +2). As Shapiro–Wilk tests of normality indicated that the regression slopes were not normally distributed, simple group comparisons were performed using the Wilcoxon rank sum tests. We performed one-tailed statistical tests on the a priori hypotheses that PIT effects are stronger in alcohol-dependent patients compared with healthy controls and stronger in relapsers versus abstainers.

Imaging analyses

The PIT fMRI was pre-processed using Nipype (Gorgolewski *et al.* 2011). First, correction for differences in slice time acquisition to the middle slice was performed. Voxel-displacement maps were estimated based on acquired field maps. All images were realigned to correct for head motion, distortion and their interaction. After co-registration of the individual structural T1 images to the individual mean EPI, the structural image was spatially normalized with a resampling solution of $2 \times 2 \times 2$ mm^3 and the normalization parameters were applied to all EPI images. Finally, images were spatially smoothed with a Gaussian kernel of 8 mm full width at half maximum. Prior to statistical analysis, data were high-pass filtered with a cut-off of 128 seconds.

Data were analyzed using the general linear model approach as implemented in SPM8 at two levels.

On the single-subject level, the fractal CSs shown in the background were modeled as separate events each parametrically modeled by the number of trial-by-trial button presses. The neural CS effect was assessed by a linear contrast, which weighted the event regressors for each of the CSs by their associated Pavlovian values (−2, −1, 0, +1, +2). Similarly, the neural PIT effect, i.e. the influence of Pavlovian stimulus values on instrumental response rate was measured by constructing a linear contrast, which weighted the parametric modulator of each condition (i.e. trial-by-trial number of button presses) by their associated Pavlovian values (i.e. −2, −1, 0, +1, +2), such that for positive Pavlovian values, a high number of button presses indicated a higher numerical value of the PIT regressor, while for negative Pavlovian values, a high number of button presses indicated a lower numerical value of the PIT regressor. To account for variance caused by motor responses associated with button presses, individual button presses (for go and no-go conditions) were

modeled as an additional regressor. Trials involving drink stimuli were modeled as separate regressors of no interest. Regressors were convolved with the canonical hemodynamic response function to account for the expected delayed increase of the BOLD signal. As additional regressors, the realignment parameters with derivatives were included (Iglesias *et al.* 2013). Linear contrast images coding neural CS value and neural PIT effects were taken to the second level.

To test for neural PIT effects at the group level, individual contrast images were subjected to a second-level random-effects analysis including study site, age and gender as covariates. As our main hypothesis concerned the NAcc, a ROI analysis was conducted for the neural PIT effects by extracting the average effect sizes per subject for a priori-defined ROIs in the right and the left NAcc [derived from the wake Forest University (WFU) PickAtlas software; http://www.fmri.wfubmc.edu/download.htm], which we will refer to as the left and right NAcc PIT effect. A similar analysis was conducted for the neural CS effect in the NAcc.

We first tested whether significant PIT effects were present across all subjects in the left or the right NAcc ROIs separately using one-sample Welch's *t*-test (an adaptation of Student's *t*-test, which can also handle unequal variances; Welch 1947). We followed up on significant PIT effects (for the left or right NAcc ROI) by comparing the NAcc PIT effect between groups (alcohol-dependent patients versus healthy controls, and relapsers versus abstainers) using two-sample Welch's *t*-tests.

We tested whether the NAcc PIT at initial assessment related to the amount of alcohol intake and relapse after the 3 months follow-up period using multiple Poisson and logistic regressions, respectively. We controlled for other a priori-defined variables [current smoking status, craving (OCDS score) and severity of alcohol dependence (ADS score)] and the behavioral PIT effect. To avoid outlier-driven effects, we performed outlier detection (median of amount of drinking during relapse +2SD). Moreover, we performed explorative whole-brain analyses for the neural PIT effect on a significance level of $P_{uncorr} < 0.001$ and with a minimum of $k = 20$ activated voxels per cluster (see Supporting Information Table S1).

Finally, we performed explorative analyses using support vector machine (SVM) classification (Vapnik 1995) to assess whether the individual NAcc PIT effect can predict relapse and alcohol intake, and conducted leave-one-out cross-validation in the R System for Statistical Computing (R Development Core Team 2013). For relapse, we trained an SVM using the e1071-package (Meyer *et al.* 2014) to all but one subject and used the resulting parameters to predict the relapse status for the excluded subject (leave-one-out cross-validation), iterating over all subjects.

We tested the prediction accuracy (i.e. the fraction of correctly predicted subjects among all subjects) against a chance level of 50% using a binomial test. We also used leave-one-out cross-validation to assess the ability to predict drinking amount via Poisson regression; we report Spearman correlation coefficients between the predicted drinking amounts and the true drinking amounts.

We assessed whether the average NAcc beta values allow improved predictions by running the models three times—once with NAcc BOLD data only, once with a priori-defined questionnaire/behavioral measures only and once with all measures combined.

Moreover, in an additional analysis (see Supporting Information Appendix S1), we performed automatized selection of behavioral predictor variables. This selection was nested within each leave-one-out iteration (i.e. based on the respective training data) to avoid optimism (Whelan & Garavan 2014).

RESULTS

Behavioral results

Pavlovian CSs influenced the rate of instrumental responding (behavioral PIT effect). Collapsing across groups, there was a significant linear main effect of Pavlovian CS value (rank sum = 1310, $P < 0.001$, see Fig. 2), with positive values of CSs in the background promoting approach (i.e. a higher rate of button presses) and negative values of CSs in the background promoting non-approach (i.e. a lower rate of button presses). See Supporting Information Appendix S1 for functional activation associated with affectively positive and aversive Pavlovian cues independent of button presses, reflecting cue reactivity effects. On the query trials, where subjects had to choose the better of two fractal CSs, not all subjects performed above chance (see Supporting Information Appendix S1). Among subjects who did show evidence of Pavlovian conditioning, we observed a significant group difference with a stronger PIT effect (i.e. increased rate of button presses elicited by positive Pavlovian background cues and decreased rate of button presses by negative Pavlovian background cues) in alcohol-dependent patients compared with healthy controls (rank sum = 342, $P = 0.03$, see Fig. 3). There were no group differences when considering only appetitive or aversive PIT effects (see Supporting Information Fig. S1). Patients with and without relapse (see Supporting Information Table S2 for group details) in the 3-months follow-up period did not differ in terms of behavioral PIT effect (rank sum = 74, $P = 0.45$).

NAcc BOLD signal covaries with PIT effect in relapsers

We next examined the neural PIT effect, i.e. the parametric modulators with the number of button presses per trial for

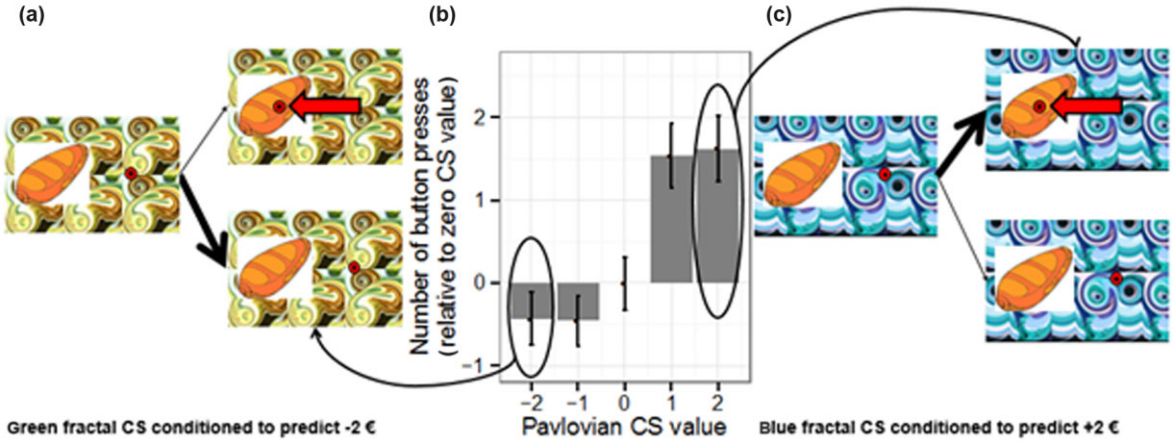

Figure 2 Behavioral PIT effect across groups (alcohol-dependent patients and healthy controls collapsed). (a) Approach was inhibited by the negatively valued Pavlovian background stimulus (e.g. a green fractal CS conditioned to predict –2 EUR). (b) PIT effect across all subjects. Reduction in button presses with negative Pavlovian CSs in the background and increase in button presses with positive Pavlovian background CSs. Bars represent subject-based SEM. (c) Approach was promoted by positively valued background stimulus (e.g. a blue fractal CS conditioned to predict +2 EUR)

each CS (five Pavlovian cues, –2, –1, 0, +1, +2) on NAcc functional activation. Collapsing across groups, there was a significant neural PIT effect in the left NAcc ($x = -12$, $y = 4$, $z = -10$ $t_{(54)} = 3.13$, $P_{SVC} = 0.011$; right NAcc: $t_{(54)} = 1.13$, $P_{SVC} > 0.4$; voxel-based analysis; see Fig. 4a), which replicates previous findings (Bray *et al.* 2008; Talmi *et al.* 2008; Geurts *et al.* 2013; Mendelsohn *et al.* 2014). Further analyses were based on average PIT effect sizes in the predefined NAcc ROIs. The left NAcc PIT effect did not significantly differ between all alcohol-dependent patients (relapsers and abstainers) and healthy controls ($t_{(52)} = 0.78$, $P = 0.22$). Critically, however, testing our second hypotheses revealed that the left NAcc PIT effect was stronger in relapsers compared with abstainers ($t_{(18)} = 1.78$, $P = 0.05$; see Fig. 4b), with a significant PIT effect seen only in relapsers (*post hoc* $t_{(10)} = 3.34$, $P = 0.008$), but neither in abstainers ($t_{(12)} = -0.29$, $P = 0.77$) nor in healthy controls alone ($t_{(23)} = 0.74$, $P = 0.23$). Furthermore, the PIT effect was also significantly stronger in relapsers compared with the healthy controls ($t_{(29)} = 1.7$, $P = 0.05$), while healthy controls and abstainers did not differ significantly ($t_{(19.262)} = 0.61$, $P = 0.73$).

We next asked whether the overall strength of the behavioral PIT effect (the linear regression; see Fig. 3) correlates with the NAcc activity across subjects. We found this to be the case only in relapsers ($r = 0.72$, $t = 3.08$, $P = 0.01$), but not in abstainers ($r = -0.14$, $t = -0.47$, $P = 0.65$), or healthy controls ($r = -0.17$, $t = -0.82$, $P = 0.42$).

Predicting treatment outcome

Next, we examined the predictive aspects of the NAcc PIT signal. The left NAcc PIT effect continued to be significantly associated with relapse ($b = 1.17$, SE = 0.69, $z = 1.68$, $P = 0.05$, $n = 22$; see Table 2) and with amount of alcohol intake during the follow-up period ($b = 0.66$, SE = 0.08, $t = 8.40$, $P < 0.001$, $n = 21$; see Table 3) after correcting for behavioral PIT effect size, smoking status (FTND sum score), alcohol dependence severity (ADS sum score) and craving (OCDS-G sum score) scores. The same remained true when correcting for the BOLD effect elicited by CS value (i.e. from strongly positive to negative) in the NAcc (see Supporting Information Appendix S1). SES did not differ between relapsers or abstainers (see Supporting Information Table S2).

In further exploratory analyses, we tested whether the individual NAcc PIT activation can predict relapse and alcohol intake during relapse (Whelan & Garavan 2014). Based on NAcc PIT activation alone, SVMs leave-one-out cross-validation predicted relapse status correctly in 17/24 = 71% of the patients (accuracy significantly above chance level, $P = 0.03$). NAcc PIT activation did not, however, improve relapse predictions based on OCDS-G, ADS, smoking and behavioral PIT effects significantly [relapse status correct classification: 17/22 (77%) with NAcc PIT activation versus 15/22 (68%) without NAcc PIT activation, $P = 0.25$, binomial test, two subjects with some missing data in questionnaires were excluded]. Similarly, NAcc PIT activation did not improve prediction of drinking amounts (correlation between predicted and observed drinking amounts of 0.56 without versus 0.50 with NAcc PIT activation). Similar to these findings, in additional analyses based on automatic variable selection, predictions based on NAcc PIT activation were not significantly better compared with predictions only including questionnaire/behavioral measures (see Supporting Information Appendix S1).

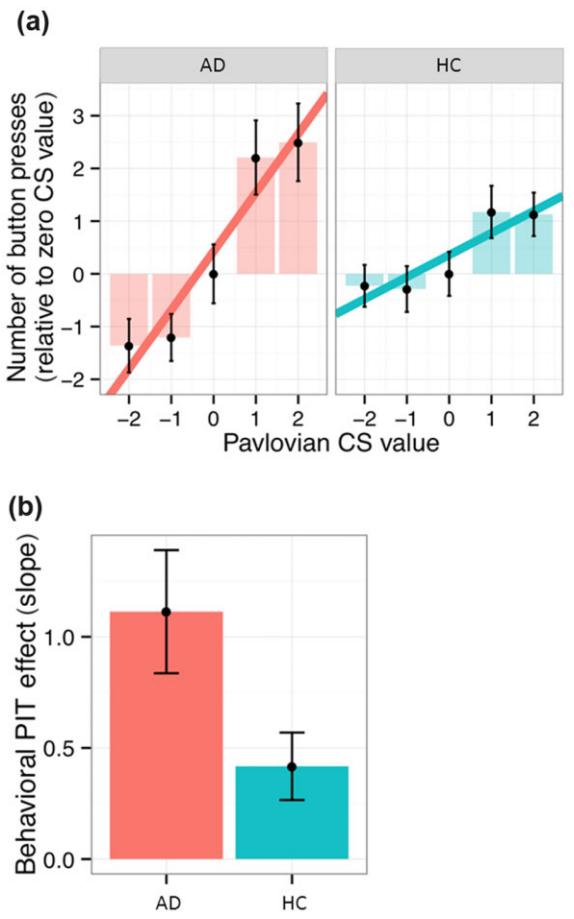

Figure 3 Alcohol-dependent patients (AD) showed a stronger behavioral PIT effect than healthy controls (HC). (a) Number of button presses (relative to the zero CS value condition) as a function of background Pavlovian CS value for alcohol-dependent patients (left, light red bars) and healthy controls (right, light green bars). Solid lines are linear regressions. (b) Linear regression coefficients for alcohol-dependent patients (left, red bar) and healthy controls (right, turquoise bar) how showed evidence of Pavlovian learning. The linear PIT effect for alcohol-dependent patients was significantly stronger than for healthy controls (rank sum = 342, $P = 0.03$). Bars represent standard error of the mean (SEM)

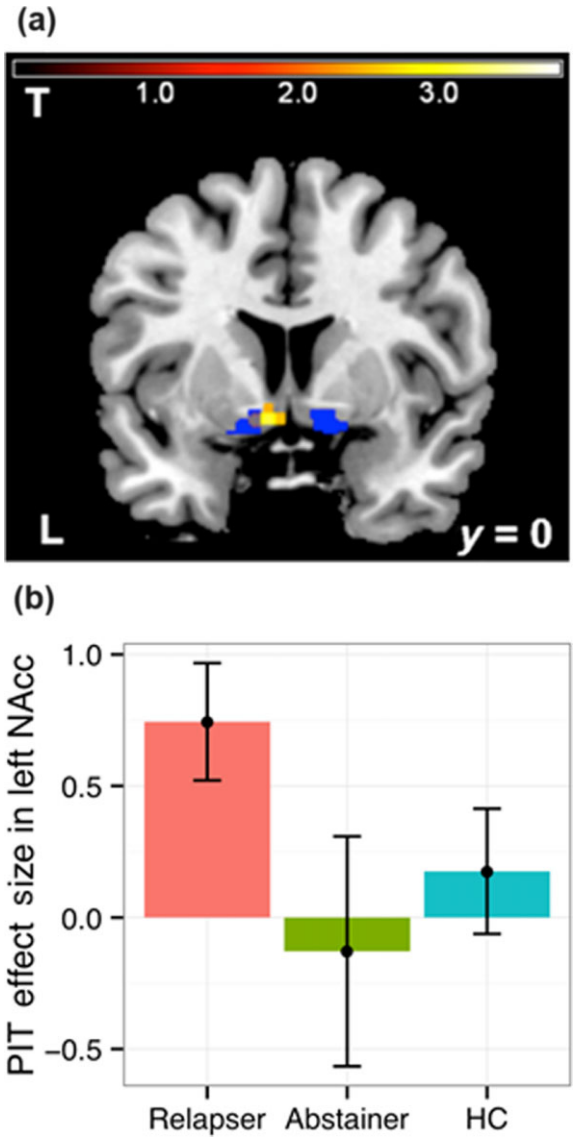

Figure 4 Neural PIT effect in the left NAcc. (a) NAcc ROI (blue) and functional PIT activation (yellow) for all subjects. (b) Bars represent average effect size of PIT activation in the left NAcc ROI for relapsers, abstainers and healthy controls (HC). PIT activation in left NAcc in relapsers was significantly higher than in abstainers ($t = 1.78$, $P = 0.05$) or in healthy controls ($t = 1.70$, $P = 0.05$). Bars represent SEM

DISCUSSION

The data suggest that the functional activation of the NAcc elicited by the PIT effect is increased in relapsers compared with abstainers or healthy controls and that this increase in activation might be predictive of relapse. The present work thus suggests that the strength of the PIT effect in the NAcc is an important risk factor for treatment outcomes in alcohol dependence. Animal research on PIT (Corbit & Balleine 2005; Lex & Hauber 2008; Holmes, Marchand & Coutureau 2010; Wassum *et al.* 2013) has shown that both drug-related and non-drug-related appetitive Pavlovian cues can promote drug seeking and intake (Parkinson *et al.* 1999; Everitt & Robbins 2005; Corbit & Janak 2007a; Saddoris *et al.* 2011; LeBlanc, Ostlund & Maidment 2012; LeBlanc *et al.* 2013b; Shiflett, Riccie & Dimatteo 2013; Depoy *et al.* 2014; Ostlund *et al.* 2014). The present results indeed suggest that PIT effects are associated with subsequent alcohol intake not only in animals but also in humans. To our knowledge, this is the first time that neural PIT effects have been investigated in a clinical sample of substance-dependent patients after detoxification and associated with treatment outcome.

Three key findings support the role of PIT-associated NAcc activation on drinking behavior among alcohol-dependent patients: the NAcc (1) was activated during

Table 2 Association of relapse with left NAcc, craving, alcohol dependence severity, smoking and behavioral PIT.

	β	*SE*	z	P[a]
Left NAcc PIT	1.17	0.69	1.68	0.05
OCDS-G	0.19	0.95	0.20	0.844
ADS	1.37	1.05	1.31	0.19
Smoking	1.50	0.87	1.71	0.085
Behavioral PIT	0.47	0.86	0.54	0.59

[a]One-tailed testing. ADS = Alcohol Severity Scale measuring severity of alcohol dependence; OCDS-G = Obsessive Compulsive Drinking Scale measuring craving; PIT = Pavlovian-to-instrumental transfer.

Table 3 Association of amount of alcohol intake during relapse with left NAcc, OCDS, ADS, smoking and behavioral PIT.

	β	*SE*	t	P[a]
Left NAcc PIT	0.66	0.08	8.40	< 0.001
OCDS-G	1.48	0.06	26.41	< 0.001
ADS	0.33	0.04	8.58	< 0.001
Smoking	1.67	0.11	14.89	< 0.001
Behavioral PIT	−1.2	0.08	−14.87	< 0.001

[a]One-tailed testing. ADS = Alcohol Severity Scale measuring severity of alcohol dependence; OCDS-G = Obsessive Compulsive Drinking Scale measuring craving.

PIT only in relapsers; (2) was correlated with the strength of the individual PIT effect; and (3) was associated with—and potentially predictive of—treatment outcome.

First, we found that the trial-by-trial variation in response rate interacting with Pavlovian value (+2, +1, 0, −1, −2) correlated with NAcc BOLD activity (reflecting the neural PIT effect) in relapsers, but not in abstinent alcohol-dependent patients or controls. Notably, this correlation arose after having corrected for the CS value effect itself, i.e. the activation only elicited by Pavlovian CS excluding the behavioral effect on response rates. A PIT effect in the NAcc is consistent with a large body of literature, which indeed was the basis for our choice of ROI (Corbit & Balleine 2005; Lex & Hauber 2008; Talmi *et al.* 2008; Holmes *et al.* 2010; Geurts *et al.* 2013; Wassum *et al.* 2013). However, the present data suggest that the NAcc is involved in mediating PIT only in patients experiencing relapse after detoxification. Positron emission tomography studies in humans suggest an important role of NAcc dopamine dysfunction for cue reactivity, craving and relapse in alcohol-dependent patients (Volkow *et al.* 1996; Heinz *et al.* 2004), though alterations of functional activation of the NAcc may be triggered by dopaminergic effects on glutamatergic and GABAergic neurotransmission in striatal networks (Brown *et al.* 2012; Luthi & Lüscher 2014), which may explain the observed alterations in the BOLD signal (Knutson & Gibbs 2007). Recent work in rodents has shown that animals displaying a tendency to learn through dopaminergic prediction-error mechanisms in the NAcc core are more attracted by Pavlovian CSs (e.g. sign-trackers) and—critically—are at high risk for developing dependent behavior (Flagel *et al.* 2011; Huys *et al.* 2014). NAcc activation by Pavlovian cues has been shown to involve dopaminergic neurotransmission (Everitt & Robbins 2005; Kienast & Heinz 2006; Di Chiara & Bassareo 2007; Flagel *et al.* 2011) with phasic dopamine release in the NAcc during PIT (Pecina & Berridge 2013; Wassum *et al.* 2013). Dopamine antagonists (Everitt *et al.* 2001; Wassum *et al.* 2011) and lesions of the NAcc (Hall *et al.* 2001) interfere with PIT effects.

Second, we observed that the strength of the individual behavioral PIT effect (the slope of the linear regression of CS value onto response rate) correlated with the NAcc activity (functional activation betas for the response rate × CS value regressors), but again, this correlation was present only in relapsers. Hence, while behavioral PIT effects were present in all groups, with a stronger PIT effect in alcohol-dependent patients, the NAcc activation appears to be directly correlated with the overall strength of the behavioral PIT effect only in relapsers. In the other groups, the PIT effect may involve different neural substrates outside of the NAcc, e.g. in prefrontal areas that can act as goal-directed control systems during PIT and have been shown to be specifically involved in modulating approach behavior (Geurts *et al.* 2013), which needs to be explored in independent and bigger samples with enhanced statistical power.

Another possible interpretation for a stronger neural PIT effect in relapsers is that money may have a higher value (and thus, a higher incentive salience) for relapsers either because of economic difficulties in this group, or maybe because of a generally increased sensitivity to rewards and punishments (Bechara, Dolan & Hindes 2002). However, relapsers and abstainers did not differ in SES (see Supporting Information Table S2), and we also failed to observe differences in Pavlovian query trials (see Supporting Information Appendix S1), which were reinforced by monetary outcomes and should likely have been even more proximal measures of changes in reinforcement sensitivity than PIT effects. Indeed, on a neural level, we observed that functional activation by Pavlovian cues independent of the PIT effect was significantly increased in prospective relapsers; but, functional activation elicited by PIT effects were still predictive of relapse after controlling for this potential confound (see Supporting Information Appendix S1).

Third, in the group of alcohol-dependent patients, the NAcc PIT activation was predictive of both relapse status and the amount of alcohol intake during relapse. These

promising exploratory findings await future investigation in larger samples. Yet, they emphasize the involvement of the NAcc as a neural correlate of PIT in the process of relapse with implications for preventive and therapeutic interventions.

In the present paradigm, both Pavlovian and instrumental CSs were associated with monetary outcomes, and we therefore cannot differentiate between outcome-general and outcome-specific PIT. As animal experiments point to different neural substrates involved in general versus specific PIT effects (Corbit & Balleine 2005; Hogarth *et al*. 2013), the involvement of other areas ranging from the ventromedial orbital prefrontal cortices to the amygdala, putamen and caudate (Bray *et al*. 2008; Talmi *et al*. 2008; Prevost *et al*. 2012; Geurts *et al*. 2013; Lewis *et al*. 2013; Mendelsohn *et al*. 2014) should be examined in larger samples. While we cannot differentiate between outcome-general versus outcome-specific PIT, the present findings suggest that PIT effects are generally increased in relapsers. After detoxification, alcohol-dependent patients with a poor treatment outcome may thus be specifically prone toward Pavlovian transfer effects on instrumental behavior.

It is interesting to consider PIT more broadly: Pavlovian effects may directly influence behavior (e.g. Guitart-Masip *et al*. 2012), or influence more complex and cognition-based, goal-directed decision mechanisms (e.g. by pruning decision trees and facilitating rapid exclusion of whole branches in this decision trees; Huys *et al*. 2012). In the context of substance dependence, salient stimuli that have previously predicted reward might thus facilitate approach behavior toward drugs when these are available, or bias higher level cognitive processes even in their absence (Robinson & Berridge 1993; Grüsser *et al*. 2002).

Our study has several limitations. Seven patients dropped out during follow-up. Next, the number of female patients was substantially smaller than the number of male patients; however, we did not observe any gender effects. The weakness might be mitigated by the rigorously performed prediction analyses on a priori-defined, previously described risk factors and using cross-validation. Interestingly, the NAcc effect was lateralized on the left side. This is consistent with earlier findings that in heavy drinkers, increase of DA transmission was located in left NAcc when self-administrating ethanol (US, intoxication) and in right NAcc when confronted with the flavor of alcohol (CS); there was a bilateral NAcc dopamine release during combined CS and US presentation (Oberlin *et al*. 2014). Our results suggest a lateralization of NAcc function and that the left NAcc may be more relevant for alcohol seeking and consumption.

In conclusion, our findings indicate that during PIT task, patients at high risk of relapse recruit the NAcc to a higher degree than both patients at low relapse risk and healthy controls, and that the NAcc activation in the group of alcohol-dependent patients was predictive of relapse status. This provides a path for Pavlovian cues to exert a potentially harmful influence on patients attempting to withstand the temptations of consumption and a potential target for therapeutic interventions.

Acknowledgements

We thank the LeAD study teams in Dresden and Berlin for data acquisition. This work was supported by the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG, FOR 1617 (grants HE 2597/13-1, HE 2597/14-1, HE 2597/15-1, SCHL 1969/2-1, SM 80/7-1, STE 1430/6-1, RA 1047/2-1, WI 709/10-1, ZI 1119/3-1) and EXC 257 NeuroCure). D.E.G. acknowledges the Netherlands Organisation for Healthy Research and Development (grant AGIKO 92003576).

Authors Contribution

AH, MAR, MNS, QJMH, USZ were responsible for the study concept and design. CS, DEMG, DJS, HW, MG, MS, NB, PS, QJMH, SPK implemented and piloted the PIT paradigm (behaviorally and inside the scanner). AH, BS, CS, EF, MS and USZ were responsible for recruitment of alcohol-dependent patients. HUW and SP were responsible for the assessment of questionnaires. DKM and SN set up a preprocessing pipeline for the imaging data. DJS, EF, FS and MG did the imaging analyses on first and second level. DJS and MG were responsible for further statistical analyses with support of AH, MAR and QJMH. DJS and QJMH implemented the leave-one out cross validation with methodological support of MAR and PS. AH, DJS, EF, FS, MG and MS drafted the manuscript. CS, DEMG, DKM, HUW, HW, MAR, MNS, NB, NK, PS, QJMH, SN, SP and USZ provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved final version for publication. (Authors contributions are sorted alphabetically.)

Disclosure

All authors have no competing interests of financial or other nature.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1 PIT effects for appetitive versus aversive Pavlovian cues. Mean and individual regression coefficients for negative versus neutral and positive versus neutral CSs, respectively. No significant group differences. Bars represent SEM

Table S1 Explorative whole-brain analyses: Activations for the PIT effect at $P_{unc} < 0.001$

Table S2 Sample characteristics (abstainers and relapsers)

Appendix S1 Supplement