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The Association of Non–Drug-Related Pavlovian-to-Instrumental Transfer Effect in Nucleus Accumbens With Relapse in Alcohol Dependence: A Replication

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ABSTRACT

BACKGROUND: The Pavlovian-to-instrumental transfer (PIT) paradigm measures the effects of Pavlovian conditioned cues on instrumental behavior in the laboratory. A previous study conducted by our research group observed activity in the left nucleus accumbens (NAcc) elicited by a non–drug-related PIT task across patients with alcohol dependence (AD) and healthy control subjects, and the left NAcc PIT effect differentiated patients who subsequently relapsed from those who remained abstinent. In this study, we aimed to examine whether such effects were present in a larger sample collected at a later date.

METHODS: A total of 129 recently detoxified patients with AD (21 females) and 74 healthy, age- and gender-matched control subjects (12 females) performing a PIT task during functional magnetic resonance imaging were examined. After task assessments, patients were followed for 6 months. Forty-seven patients relapsed and 37 remained abstinent.

RESULTS: We found a significant behavioral non–drug-related PIT effect and PIT-related activity in the NAcc across all participants. Moreover, subsequent relapsers showed stronger behavioral and left NAcc PIT effects than abstainers. These findings are consistent with our previous findings.

CONCLUSIONS: Behavioral non–drug-related PIT and neural PIT correlates are associated with prospective relapse risk in AD. This study replicated previous findings and provides evidence for the clinical relevance of PIT mechanisms to treatment outcome in AD. The observed difference between prospective relapsers and abstainers in the NAcc PIT effect in our study is small overall. Future studies are needed to further elucidate the mechanisms and the possible modulators of neural PIT in relapse in AD.

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Alcohol dependence (AD) is a prevalent disorder characterized by a high relapse rate (1,2). The impact of cues on drug-seeking and drug-taking behavior has been hypothesized to be an important mechanism underlying relapse (3). According to the incentive salience sensitization theory of addiction, alcohol can induce the sensitization of incentive salience attribution to alcohol-predictive cues, promoting alcohol seeking and consumption despite a person's intention to remain abstinent (4). The Pavlovian-to-instrumental transfer (PIT) paradigm has been established to experimentally measure the effects of reward-predictive cues on instrumental behaviors (5–8). In a PIT task, Pavlovian and instrumental training are first conducted separately, and then instrumental performance is assessed in the presence of Pavlovian conditioned stimuli (CS) (9). The PIT effect refers to the promotion or inhibition effect of Pavlovian CS on instrumental behavior (10). Studies further found that PIT effects come in 2 neurobiologically distinct forms (11–13). In outcome-specific PIT, the Pavlovian CS associated with a reward enhances instrumental behavior leading to the same reward, whereas general PIT refers to a situation in which a CS enhances instrumental behavior regardless of the identity of the reward (11,13).

Studies conducted with rodents have found that drug-related cues enhance PIT effects in drug-treated animals (5,14). For example, in one study, ethanol-related cues promoted not only ethanol seeking but also nonethanol reward seeking (5). In addition to drug-related PIT, PIT tasks applying non–drug-related cues allow for studying a more general impact of drug use or addiction on cue-guided behavior. Enhanced non–drug-related PIT effects have been observed in cocaine-exposed rats (15–18). Similarly, mice under chronic ethanol exposure showed enhanced non–ethanol-related PIT effects in one study (19). These findings suggest

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a general alteration in motivational processes in drug-exposed animals. Comparably, our research group previously observed a more pronounced behavioral non–drug-related PIT effect in detoxified patients with AD compared with healthy control subjects (HCs) (20–22). Furthermore, the behavioral non–drug-related PIT effect was predictive of future relapse, with prospective relapsers being less able to inhibit instrumental approach behavior when positive Pavlovian CS were present (23).

Using neural imaging techniques, the nucleus accumbens (NAcc) has been identified as an essential neural substrate for PIT (18,24–27). The NAcc is a part of the ventral striatum and is a core area of the human brain reward system (28). A low availability of dopamine D_2 receptors in the NAcc has been associated with AD (29). However, to date, few studies have explored the neural mechanism of PIT in patients with AD. To our knowledge, a study by Garbusow *et al.* (21) was the first to investigate neural non–drug-related PIT in a clinical sample of patients with AD after detoxification. In that study, functional activation of the left NAcc ($NAcc_L$) elicited by PIT was observed in both patients with AD and HCs. Moreover, the $NAcc_L$ PIT effect was stronger in subsequent relapsers (n = 13) than in abstainers (n = 11) (21).

These findings indicate a role of the non–drug-related PIT in predicting treatment outcomes in patients with AD after detoxification. However, studies conducted with humans have yielded heterogeneous findings in this regard. In a recent study using a different PIT paradigm, the researchers did not find differences between relapsing and abstaining patients with AD in either behavioral or neural PIT effects (30). Another human study also did not find significant differences in non–drug-related PIT between treatment-seeking drug users and HCs (31). In one animal study, the investigators reported no categorical difference in non–drug-related PIT between cocaine-addicted and nonaddicted rats but did find an association between the strength of the PIT effect and amount of drug intake (32). These inconsistent findings underscore a need for more studies aimed at elucidating the clinical relevance of PIT in AD.

The aim of this study was to examine whether the initial findings of non–drug-related PIT-induced activity in the NAcc and a stronger NAcc-PIT effect in prospective relapsers than abstainers in Garbusow *et al.*'s study (21) could be replicated in a new, larger sample. We analyzed a replication sample of 129 recently detoxified patients with AD and 74 HCs who performed the PIT task in a functional magnetic resonance imaging scanner. On the behavioral level, following the finding of a group difference involving relapse in behavioral PIT by Sommer *et al.* (23), we asked whether there would be a stronger behavioral PIT effect in relapsers than abstainers in a study with a 6-month follow-up period. At the neural level, we hypothesized a stronger NAcc-PIT effect in relapsers than abstainers.

In addition to the replication sample, we conducted the analyses again with the full sample containing all of the assessed participants [regardless of whether the data were reported in Garbusow *et al.* (21)] (see S12 and S13 in the Supplement) to examine whether the findings were comparable in this larger, combined sample.

METHODS AND MATERIALS

Participants

The data were collected as part of the LeAD (Learning and Relapse Risk in Alcohol Dependence) study (https://ssl.psych.tu-dresden.de/lead/; clinical trial numbers NCT01679145 and NCT02615977). The replication sample described in this article is a subsample that was assessed after the initial sample reported in Garbusow *et al.* (21). This study was conducted in Berlin and Dresden, Germany, with approval from local ethics committees of the Charité Universitätsmedizin Berlin (EA/1/157/11 and EA1/268/14) and the Technische Universität Dresden (EK 228072012 and EK 300082014).

Participants performed the PIT task and other tasks (see clinical trial registration). The present study was focused on replication of the non–drug-related PIT task. Other data from the study are reported elsewhere (see https://ssl.psych.tu-dresden.de/lead/node/7 for an overview of data that have been reported as of 2019). Nevertheless, for comparison purposes, we also report the results of a drug (alcohol) versus water cue experiment in S15 and S16 in the Supplement. A total of 129 patients who fulfilled the criteria for a diagnosis of AD according to the DSM-IV-Text Revision (33,34) and 74 HCs were included in the final analyses of neural PIT as a replication sample after data cleaning (see S1 in the Supplement for study inclusion and exclusion procedures). Sample characteristics are presented in Table 1. Patients with AD were followed for 6 months and had 6 or 7 in-person or telephone interviews. Alcohol use was assessed using the timeline follow-back as part of each follow-up interview (35). Relapse was defined as at least 4 or 5 standard drinks (e.g., 1 standard drink = 0.33-L beer) that were consumed on one drinking occasion for men and women, respectively. Forty-seven patients relapsed, while 37 remained abstinent; the remaining 45 patients had missing follow-up information and so could not be classified as abstainers or relapsers. Sample characteristics of patients with known versus unknown relapse status as well as participants who had successful versus unsuccessful Pavlovian conditioning (see S2 and S7 in the Supplement) are reported in S3 in the Supplement.

PIT Paradigm

The PIT paradigm was described in our first study (21) and has been described in other previous publications (20,22,23). Participants completed an instrumental task (i.e., collecting "good" shells via repeated button pressing or leaving "bad" shells via omitting a reaction) while monetary Pavlovian CS were presented in the background. In addition, participants performed trials in which alcohol or water cues were used instead of Pavlovian CS (results are reported in S15 and S16 in the Supplement). For a detailed description of the task, see S2 in the Supplement.

MRI Acquisition

At both study centers, scanning was performed using a Siemens Trio 3T MRI scanner (Siemens Healthineers). Details of MRI acquisition are reported in S4 in the Supplement.

Table 1. Sample Characteristics and Test Statistics Comparing Patients With AD and HCs and Comparing Relapsers and Abstainers

	AD vs. HC			Relapsers vs. Abstainers		
Characteristics	Patients With AD, n = 129	HCs, n = 74	p	Relapsers, n = 47	Abstainers, n = 37	p
Gender, Female	21/129 (16%)	12/74 (16%)	.99[a]	7/47 (15%)	6/37 (16%)	.87[a]
Age, Years	44.3 (9.9)	44.1 (10.8)	.92[b]	44.8 (9.9)	44.9 (10.5)	.97[c]
Education, Years	15.3 (4.1)	15.9 (3.4)	.09[b]	15.7 (4.3)	14.7 (3.8)	.27[b]
Smokers	93/121 (77%)	53/74 (72%)	.41[a]	30/45 (67%)	28/34 (82%)	.12[a]
AD Severity (ADS)	16.7 (7.4)	1.9 (2.9)	<.001[b]	16.2 (7.0)	16.9 (7.4)	.66[c]
With Family History of AD (FHAM)	47/122 (39%)	8/67 (12%)	<.001[a]	14/43 (33%)	17/36 (47%)	.18[a]
Time Since Last Alcoholic Drink, Days	22.1 (12.4)	75.0 (310.2)[d]	<.001[b]	22.1 (13.7)	18.9 (7.2)	.68[b]
Alcohol Intake per Day in Past Year, g of Pure Ethanol	162 (134)	9.6 (10.6)	<.001[b]	148 (94.4)	149 (121)	.76[b]
Lifetime Alcohol Intake, kg of Pure Ethanol	1728 (1291)	314 (936)	<.001[b]	1834 (1390)	1859 (1265)	.80[b]
Craving for Alcohol (OCDS-G)	12.6 (7.9)	2.8 (2.6)	<.001[b]	12.0 (7.3)	12.9 (8.9)	.78[b]
Trait Impulsivity (BIS-15)	31.5 (6.2)	29.8 (5.3)	.03[c]	31.6 (6.4)	32.2 (5.9)	.68[b]
Current Anxiety (HADS)	4.3 (3.2)	1.9 (1.9)	<.001[b]	4.7 (2.9)	4.2 (3.4)	.29[b]
Current Depressivity (HADS)	3.6 (3.7)	1.2 (1.8)	<.001[b]	3.8 (3.3)	4.3 (4.5)	.74[b]

Values are presented as n/total n (%) or mean (SD). ADS provides a sum score with greater values indicating more severe AD (50); FHAM (51); BIS-15 provides a sum score with greater values indicating stronger trait impulsivity (52); OCDS-G provides a sum score with greater values indicating stronger craving for alcohol within 7 days before assessment (53); HADS provides sum scores with greater values indicating stronger anxiety/depressivity within 7 days before assessment (54).

AD, alcohol dependence; ADS, Alcohol Dependence Scale; BIS-15, short German version of the Barratt Impulsivity Scale-15; FHAM, Family History Assessment Module; HADS, Hospital Anxiety and Depression Scale; HC, healthy control subject; OCDS-G, German version of the Obsessive Compulsive Drinking Scale.

[a]χ^2.

[b]Wilcoxon rank-sum test.

[c]t test.

[d]Three control subjects abstained from alcohol longer than 2 SD above the mean. The median (1st quartile–3rd quartile) of abstinence from alcohol in the control group is 4 (2–14) days.

Data Analysis

Data were analyzed using MATLAB (version 9.9, R2020a; The MathWorks, Inc.) and the R System for Statistical Computing version 4.0.3. Functional MRI data were analyzed using SPM12 (36).

Behavioral Analyses

Patients with AD (n = 129) and HCs (n = 74) were included in analyses as a replication sample, with some of the participants (56 patients with AD and 50 HCs) having already been reported in another study of behavioral non–drug-related PIT in Sommer *et al.* (23) using the measurement of accuracy. In this article, we report analyses of the behavioral data with the measurement of number of button presses following Garbusow *et al.* (21) to compute behavioral PIT effects in our sample.

We established Poisson-distributed generalized linear mixed-effects models (GLMMs) with predictors of the associated monetary value of Pavlovian CS (Pavlovian CS value: −2€, −1€, 0€, +1€, +2€) and the trial type of the instrumental condition (instrumental condition: +0.5 = go trial vs. −0.5 = no-go trial) to predict the trial-by-trial number of button presses in the transfer part. Participant IDs, instrumental stimuli (shells), and Pavlovian CS (fractals combined with pure tones) were taken for random intercept effects to control for potential participant and item effects. For group comparison between patients with AD and HCs, a group factor (+0.5 = patient with AD vs. −0.5 = HC) as well as its interaction with other predictors were included as fixed effects. For the 3-group comparisons between abstainers, relapsers, and HCs, another GLMM was conducted with a 3-level group factor. The analysis method used in the study being reported here is different from that used in the first study, in which the individual PIT effects were first calculated by regressing the number of button presses on Pavlovian CS value and then subjected to group comparisons (21). We used the new analysis method because it is more sensitive for detecting small effects. We additionally explored whether the behavioral PIT effect was correlated with the severity of AD, current alcohol craving, and family history of AD (see S8 in the Supplement).

Imaging Analyses

Details of the imaging data preprocessing are reported in S5 in the Supplement. After preprocessing, individual general linear models (GLMs) were established for single-participant analyses (see Figure S3 for the GLM design matrix). Non–drug-related PIT trials were modeled as 1 condition with onset as the main regressor. The following 3 parametric modulators for the main regressor were used: 1) Pavlovian CS value (−2€, −1€, 0€, +1€, +2€); 2) the number of button presses (log-transformed calculated with ln [original number of button presses + e]); and 3) the PIT parameter (transformed number of button presses × Pavlovian CS value). A higher number of button presses in response to a higher CS value would then lead to a higher numerical value in the PIT parameter. This

GLM was adapted from the original one used in Garbusow *et al.* (21) in which button presses corresponding to each Pavlovian CS were put into separate regressors. The new model congregated the PIT parameters corresponding to all Pavlovian CS into one regressor so that the model would not easily fail if there was no behavioral response variability.

Individual contrasts were calculated for the parametric modulator of non–drug-related PIT. To measure the neural PIT effect across participants, individual contrast images were subjected to a one-sample *t* test in the second-level analysis in SPM, with participants' age, gender, study center, and Pavlovian training version (early vs. later version; see S2 in the Supplement) as covariates. Following the previous study, a region-of-interest (ROI) analysis was conducted with an a priori–defined compound ROI comprising the bilateral NAcc ($NAcc_L$ and $NAcc_R$) derived from the Wake Forest University PickAtlas software (http://www.fmri.wfubmc.edu/download.htm). In addition, explorative whole-brain analyses for the neural PIT effect using a significance level of $p_{unc} < .001$ and with $k \geq$ at least 20 activated voxels per cluster were performed (see S9 in the Supplement). Moreover, we examined whether the behavioral PIT effect correlated with the neural PIT effect in the NAcc by adding the extracted individual behavioral PIT slopes from another GLMM without the group factor as an additional covariate in the second-level analysis in SPM. For group comparisons, the mean beta values in the predefined NAcc ROIs were extracted separately for the $NAcc_L$ and the $NAcc_R$. For non-normally distributed data, Wilcoxon rank-sum tests were conducted for 2-group comparisons, and Kruskal-Wallis tests were conducted for 3-group comparisons. We further explored the effects of excitatory and inhibitory Pavlovian CS separately (see S14 in the Supplement). Analyses were also conducted for drug-related PIT trials (see S15 and S16 in the Supplement).

In addition, we explored whether the neural PIT effect was correlated with AD severity, current alcohol craving, and family history of AD using the Spearman correlation test or Wilcoxon rank-sum test (see S10 in the Supplement). Finally, similar to the initial study (21), we conducted a logistic regression to examine whether the behavioral and neural PIT effects were associated with relapse status in patients when controlling for AD severity, alcohol craving, and smoking status (see S11 in the Supplement).

RESULTS

Behavioral PIT

A significant behavioral PIT effect was present across groups, indicated by more button presses during trials with higher-valued Pavlovian CS (Pavlovian CS value: estimate = 0.28, z = 108.27, $p < .001$) (Table 2). Patients with AD displayed a stronger PIT effect than HCs (Pavlovian CS value × group interaction: estimate = 0.03, z = 5.21, $p < .001$).

When comparing the behavioral PIT effect among abstainers, relapsers, and HCs (Table 3), we observed a significant interaction of group and Pavlovian CS value (χ^2 = 434.32, $p < .001$; type II Wald χ^2 tests for the GLMM). Post hoc analyses showed that the PIT effect was the strongest in relapsers followed by HCs and was smallest in abstainers (relapsers > abstainers: estimate = 0.15, z = 20.24, $p < .001$; relapsers > HCs: estimate = 0.10, z = 15.39, $p < .001$; HCs > abstainers: estimate = 0.05, z = 7.24, $p < .001$) (Figure 1).

Table 2. Behavioral PIT in Patients With AD (n = 129) and HCs (n = 74)

Parameter	Estimate (SE)	z	p
Intercept	1.42 (0.04)	37.07	<.001
Pavlovian CS Value	0.28 (0.003)	108.27	<.001
Instrumental Condition, Go vs. No-Go	0.59 (0.05)	10.91	<.001
Group, AD Patient vs. HC	0.10 (0.05)	1.87	.061
Pavlovian CS Value × Group	0.03 (0.005)	5.21	<.001
Instrumental Condition × Group	−0.23 (0.01)	−15.88	<.001

AD, alcohol dependence; CS, conditioned stimulus; HC, healthy control subject; PIT, Pavlovian-to-instrumental transfer.

NAcc Blood Oxygen Level–Dependent Signal Elicited by PIT

Across the 2 groups (patients with AD and HCs) combined, we observed a significant neural PIT effect in the $NAcc_L$ (x = −10, y = 6, z = −8, t_{198} = 3.34, $p_{FWE\text{-}SVC}$ = .009, voxel-based analysis) (Figure 2) but only a marginally significant trend in the $NAcc_R$ (x = 6, y = 10, z = −10, t_{198} = 2.66, $p_{FWE\text{-}SVC}$ = .058, voxel-based analysis). Furthermore, when including the individual behavioral PIT slopes into the second-level GLM analysis, we found a significant association between the behavioral PIT and PIT-related activation in the $NAcc_L$ (x = −8, y = 8, z = −12, t_{197} = 3.60, $p_{FWE\text{-}SVC}$ = .004) but not in the $NAcc_R$ (x = 6, y = 10, z = −10, t_{197} = 2.44, $p_{FWE\text{-}SVC}$ = .095).

The individually extracted mean beta values in the predefined ROI of the $NAcc_L$ were then subjected to group comparisons. Patients with AD did not show a different $NAcc_L$ PIT effect compared with HCs (Wilcoxon rank-sum test; W = 4813, p = .922). When comparing abstainers, relapsers, and HCs, a significant effect of group was observed (Kruskal-Wallis rank-sum test; χ^2 = 6.27, p = .044, $\eta^2[H]$ = 0.03). Post hoc comparisons showed a stronger effect in relapsers than abstainers (Dunn test with Bonferroni correction: z = 2.50, p = .037), while no difference was found between abstainers and

Table 3. Behavioral PIT in Relapsers (n = 47), Abstainers (n = 37), and HCs (n = 74)

Parameter	Estimate (SE)	z	p
Intercept	1.59 (0.06)	24.58	<.001
Pavlovian CS Value	0.22 (0.005)	40.29	<.001
Instrumental Condition, Go vs. No-Go	0.50 (0.06)	8.86	<.001
Group, Relapser vs. Abstainer	−0.20 (0.08)	−2.56	.010
Group, HC vs. Abstainer	−0.22 (0.07)	−3.14	.002
Pavlovian CS Value × Group, Relapser vs. Abstainer	0.15 (0.008)	20.24	<.001
Pavlovian CS Value × Group, HC vs. Abstainer	0.05 (0.007)	7.24	<.001
Instrumental Condition × Group, Relapser vs. Abstainer	−0.05 (0.02)	−2.52	.012
Instrumental Condition × Group, HC vs. Abstainer	0.21 (0.02)	10.78	<.001

CS, conditioned stimulus; HC, healthy control subject; PIT, Pavlovian-to-instrumental transfer.

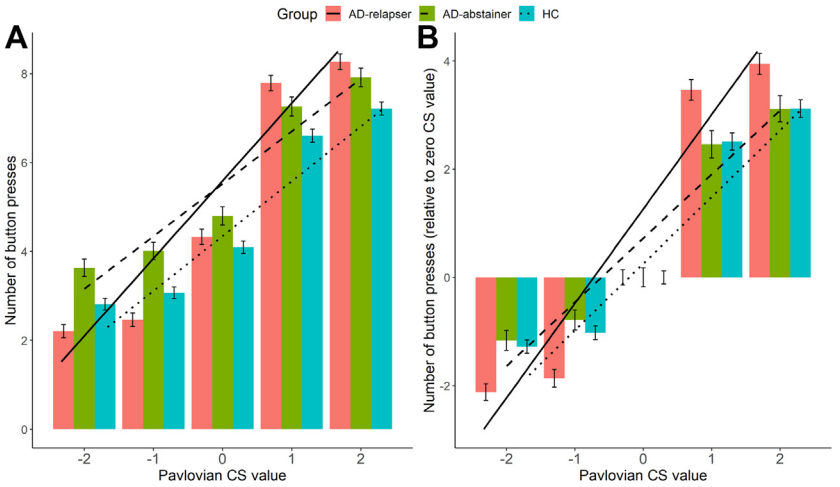

Figure 1. Behavioral Pavlovian-to-instrumental transfer effect in relapsers (n = 47), abstainers (n = 37), and healthy control subjects (HCs) (n = 74). The behavioral Pavlovian-to-instrumental transfer effect was the strongest (steepest slope) in subsequently relapsed patients (alcohol dependence [AD]–relapsers), followed by HCs, and the smallest in abstinent patients (AD-abstainers). **(A)** shows the original number of button presses to each Pavlovian conditioned stimulus (CS) value; **(B)** shows the number of button presses relative to 0 CS value. Group mean and standard error of the mean are shown with bars and error bars.

HCs (z = −1.65, p = .299) or between relapsers and HCs (z = 1.17, p = .731) (see Figure 3). In the full (combined) sample, there was no significant group difference when comparing the 3 groups. However, an additional Wilcoxon rank-sum test that replicated the 2-group comparison analysis strategy used in the Garbusow *et al.* study (21) still showed a significantly stronger $NAcc_L$ PIT effect in relapsers than abstainers (see S13 in the Supplement).

DISCUSSION

We observed a stronger behavioral non–drug-related PIT effect in prospective relapsers than abstainers and HCs, which is consistent with the findings of Sommer *et al.* (23). More importantly, with a larger sample than in the first study that we conducted (21), we observed a neural PIT effect in the NAcc across participants in this study, and the $NAcc_L$ PIT effect was stronger in subsequent relapsers than in abstainers. Overall, this study basically replicated the neural PIT findings of Garbusow *et al.* (21).

Given the abundant evidence implying altered NAcc functioning in AD, which could be due to drug effects on monoaminergic neurotransmission in this brain area (29), one would also expect different PIT-related NAcc activation to be induced by excessive alcohol intake. Indeed, it was found that young, male, nonclinical adults with a high-risk drinking pattern displayed a nonsignificant trend in increased neural responses to PIT in the ventral striatum (37). However, with individuals in

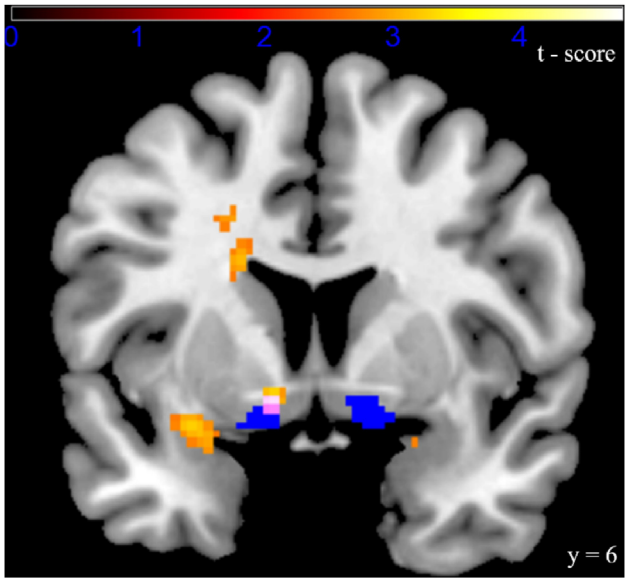

Figure 2. Neural Pavlovian-to-instrumental transfer effect across all study participants (N = 203). Bilateral nucleus accumbens region of interest (blue) and functional Pavlovian-to-instrumental transfer activation (yellow; p_{unc} < .005 was used for illustration in the figure, while $p_{FWE\text{-}SVC}$ < .05 was used for data analyses).

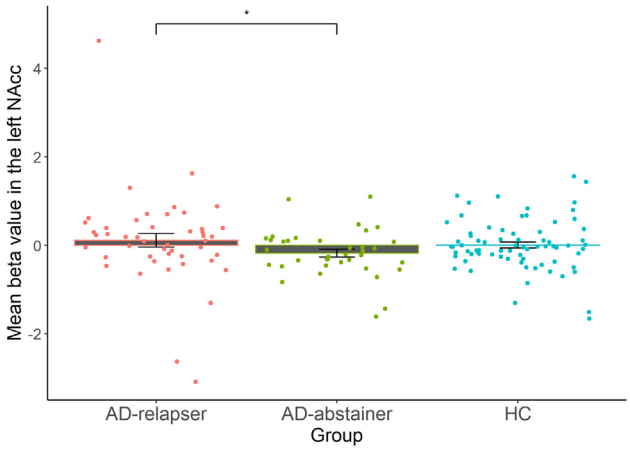

Figure 3. Mean beta values in the left nucleus accumbens (NAcc) in subsequent relapsers (n = 47), abstainers (n = 37), and healthy control subjects (HCs) (n = 74). Group mean and standard error of the mean are shown with bars and error bars, and individual values are represented by colored dots. Relapsers showed a higher left NAcc Pavlovian-to-instrumental transfer effect than abstainers. *p < .05. AD, alcohol dependence.

clinical settings who met diagnostic criteria for AD, we did not observe a different NAcc PIT effect compared with HCs in either the first study (21) or this one unless we distinguished between prospective relapsers and abstainers. This indicates that instead of being a marker of AD, the NAcc PIT effect perhaps has more importance in predicting an aspect of clinical severity, that is, the propensity to relapse. The underlying mechanism could be that patients who are prone to the influence of environmental cues (i.e., a stronger PIT effect) may be inclined to alcohol intake in environments that are associated with alcohol intake or certain mood states (38). Indeed, an animal study observed that mice with higher food-related behavioral PIT effects had stronger subsequent cue-induced reinstatement of alcohol seeking (39). Consistently, the behavioral PIT effect differentiated relapsers and abstainers in Sommer *et al.*'s study (23) and in this study. Accordingly, and in line with the findings of Garbusow *et al.* (21), we observed a higher $NAcc_L$ PIT effect in relapsers compared with abstainers across different follow-up periods (3 months in the first study and 6 months in this one). The group difference in neural PIT effect was not as stable as expected when examining the effect in the full sample (see S13 in the Supplement). However, another 2-group comparison still indicated a stronger $NAcc_L$ PIT effect in relapsers compared with abstainers. We suspect that the nonsignificant 3-group difference in the full sample may be partly due to a sample effect in PIT performance among control group participants. Indeed, the variance among HCs in behavioral PIT differed, with stronger behavioral PIT effects observed in HCs in the replication sample compared with HCs who had been reported on previously (see S12 in the Supplement).

The difference in functional activation elicited by non–drug-related PIT between relapsers and abstainers was consistently shown in the $NAcc_L$, both in the first study and in this one. Previous research suggested lateralized dopamine release to unconditioned stimulus (US) and CS in the NAcc, with dopamine release in the $NAcc_L$ mostly reflecting alcohol intake (US, intoxication) while dopamine release in the $NAcc_R$ mostly reflects the drink-related CSs (i.e., beer flavor) in male heavy drinkers (40). The finding in our study may underline the significance of $NAcc_L$ in relapse to alcohol intake (21). Nevertheless, our study was not designed to investigate the hemispheric difference of the NAcc. Group differences between relapsers and abstainers in drug-related PIT were conversely found in the $NAcc_R$ rather than the $NAcc_L$ [(41) and S16 in the Supplement)]. Further research is needed to elucidate the roles of the $NAcc_L$ and the $NAcc_R$ in different PIT tasks.

Findings from several earlier studies did not support an association between PIT and addiction (30,31,32,42). The inconsistent findings question categorical differences in cue reactivity. Therefore, replication studies are important and should include assessments of clinical severity that could reflect differences in drug effects on the ventral and dorsal striatum (43). The inconsistent findings in human studies may be explained by differences in sample characteristics, PIT manipulations, and sample sizes. For example, findings in one study were based on social drinkers rather than patients with AD and with different types of reward (42). There, no association between hazardous drinking and PIT was observed using beer points as the outcome (no beer was provided after the PIT session) (42). Another study investigating diagnosed patients with AD found no association of behavioral or neural PIT either with AD status or with treatment outcome (30). That study used food outcomes (participants were allowed to eat the earned snack at the end of the experiment) rather than monetary outcomes as in our task. We speculate that the type of reward used for conditioning and the approach to providing the reward has an impact on the resulting experimental behavior.

The observed group difference in the activation of the NAcc associated with our non–drug-related PIT task is small overall ($\eta^2[H] = 0.03$ for the 3-group comparison in the replication sample and $r = 0.21$ for the relapsers vs. abstainers comparison in the full sample), indicating that neural PIT cannot thoroughly elucidate the mechanisms of relapse in AD. Indeed, relapse in AD has multifactorial causes that vary from person to person and within each individual (44), and other mechanisms might interact with PIT process and relapse (45). It is also worth noting that behavioral PIT could be more efficient in relapse prediction than neural PIT in our study, given that the logistic regression comprising multiple predictors of $NAcc_L$ PIT, behavioral PIT, alcohol craving, AD severity, and smoking status yielded the only significant effect of behavioral PIT in predicting relapse (see S11 in the Supplement). Future studies are warranted to further elucidate the mechanisms and possible modulators of neural PIT in relapse in alcohol dependence and to translate neurobiological findings to the treatment of AD.

This study has limitations. First, although we used the identical procedure as in the first study, the replication study was not preregistered except for clinical trials registration. Second, patients compared with HCs and relapsers compared with abstainers showed less changed button pressing responses to instrumental go versus no-go trials during the instrumental training (see S6 in the Supplement) in our study. The group difference in behavioral PIT may be partly explained by differences in learned instrumental response–outcome contingency given that previous research observed a larger PIT effect when the instrumental response–outcome contingency was less reliable (46). However, adding the instrumental learning slope as a covariate in the behavioral PIT GLMM model does not change the significance of group differences in PIT effects (see S6 in the Supplement), indicating that the observed group differences in PIT cannot be fully explained by differences in instrumental learning. Third, a subset of participants in our study (60 patients and 20 HCs) completed cognitive bias modification training after the PIT task reported here, which we hypothesized would be effective in reducing relapse risk. However, we did not observe such an effect (results will be reported elsewhere). In fact, the proportion of relapsers did not differ significantly between patients in this sample who underwent verum training (14/21; 67%), placebo training (9/19; 47%), or no training (24/44; 55%) ($\chi^2 = 1.58$, $p = .453$; Cramer's $V = 0.14$). Therefore, we believe that the training did not confound our findings. Fourth, we slightly changed the design of Pavlovian training during the study (an interstimulus interval between the presentation of CS and US was used in the first but not in the second version of training; see S2 in the Supplement). Although it can lead to different training efficacy, we argue that it did not affect the PIT findings because

participants who did not successfully learn the association of CS and US (11% of patients with AD and 9% of HCs) were not included in the analyses. Moreover, the version of Pavlovian training was included as a covariate in the neural PIT analysis to eliminate a potential confounding effect. However, patients who had unsuccessful Pavlovian conditioning also had more lifetime drinking than those who had successful Pavlovian conditioning (see S3 in the Supplement), indicating altered associative learning induced by prolonged alcohol intake (47,48). Excluding patients with unsuccessful Pavlovian conditioning potentially limits the PIT assessment of patients with less alcohol intake in our study. Fifth, the non–drug-related PIT task in our study could be contributed to by both outcome-specific and general PIT because monetary outcomes were used in both instrumental and Pavlovian training, but with different values. Research has suggested that outcome-specific and general PIT effects depend on the NAcc shell and core, respectively (12). Due to limitations in spatial resolution and smoothing, we cannot distinguish between the NAcc core and shell in this study. Finally, this study was conducted by the same research group that conducted the first study. Replication studies conducted by independent investigators and institutions are needed to further reduce potential biases.

In conclusion, this study replicated previous findings of a stronger behavioral non–drug-related PIT and PIT-related activation in the $NAcc_L$ in relapsing patients with AD than abstaining patients with AD. These findings suggest that behavioral and $NAcc_L$ PIT may be related to vulnerability to relapse in patients with AD after detoxification. Future studies are needed to further elucidate the mechanisms and possible modulators of neural PIT in relapse in AD. In addition, to confirm the clinical relevance of the findings, further research in addition to replication studies is required to test the generalizability of the findings across different samples and measurement conditions (49).

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