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Elevated Amygdala Responses During De Novo Pavlovian Conditioning in Alcohol Use Disorder Are Associated With Pavlovian-to-Instrumental Transfer and Relapse Latency

Claudia Ebrahimi, Maria Garbusow, Miriam Sebold, Ke Chen, Michael N. Smolka, Quentin J.M. Huys, Ulrich S. Zimmermann, Florian Schlagenhauf, and Andreas Heinz

ABSTRACT

BACKGROUND: Contemporary learning theories of drug addiction ascribe a key role to Pavlovian learning mechanisms in the development, maintenance, and relapse of addiction. In fact, cue-reactivity research has demonstrated the power of alcohol-associated cues to activate the brain's reward system, which has been linked to craving and subsequent relapse. However, whether de novo Pavlovian conditioning is altered in alcohol use disorder (AUD) has rarely been investigated.

METHODS: To characterize de novo Pavlovian conditioning in AUD, 62 detoxified patients with AUD and 63 matched healthy control participants completed a Pavlovian learning task as part of a Pavlovian-to-instrumental transfer paradigm during a functional magnetic resonance imaging session. Patients were followed up for 12 months to assess drinking behavior and relapse status.

RESULTS: While patients and healthy controls did not differ in their ability to explicitly acquire the contingencies between conditioned and unconditioned stimuli, patients with AUD displayed significantly stronger amygdala responses toward Pavlovian cues, an effect primarily driven by stronger blood oxygen level–dependent differentiation during learning from reward compared with punishment. Moreover, in patients compared with controls, differential amygdala responses during conditioning were positively related to the ability of Pavlovian stimuli to influence ongoing instrumental choice behavior measured during a subsequent Pavlovian-to-instrumental transfer test. Finally, patients who relapsed within the 12-month follow-up period showed an inverse association between amygdala activity during conditioning and relapse latency.

CONCLUSIONS: We provide evidence of altered neural correlates of de novo Pavlovian conditioning in patients with AUD, especially for appetitive stimuli. Thus, heightened processing of Pavlovian cues might constitute a behaviorally relevant mechanism in alcohol addiction.

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Alcohol use disorder (AUD) has been conceptualized as a disorder of maladaptive learning and memory (1–4). The incentive sensitization theory (1,5) highlights the motivational power of environmental stimuli to promote craving, drive recurrent drug use, and ultimately increase relapse risk. However, the underlying Pavlovian learning process whereby initially neutral stimuli (conditioned stimulus [CS]) acquire motivational properties through repeated pairings with the hedonic effects of a reinforcer like alcohol (unconditioned stimulus [US]) has rarely been investigated in AUD (6).

Human neuroimaging research has elucidated an extended network subserving Pavlovian threat and appetitive conditioning, including the amygdala, hippocampus, ventral striatum (VS) including the nucleus accumbens (NAcc), dorsal anterior cingulum, and orbitofrontal cortex (7–10). Surprisingly little is known about the underlying Pavlovian learning process in AUD, and we are unaware of any imaging studies investigating de novo Pavlovian conditioning with drug or nondrug rewards in this psychiatric condition. This may be partly due to methodological challenges that human appetitive conditioning research is facing (11). In contrast, 2 functional magnetic resonance imaging (fMRI) studies used a threat conditioning protocol in patients with AUD, providing the first evidence for attenuated blood oxygen level–dependent (BOLD) responses toward threat-predicting cues. Yang *et al.* (12) found attenuated neural differentiation in the pregenual anterior cingulate cortex, medial prefrontal cortex, and posterior cingulate cortex between a CS predicting a high- versus a low-heat US in men with alcohol dependence, while BOLD reactivity in the posterior insula toward the high- versus low-intensity US

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itself was increased in patients compared with control participants. Recently, Muench *et al.* (13) showed attenuated amygdala involvement during threat conditioning using mild electric stimulation as US in patients with AUD compared with healthy participants. In spite of general blunting, remaining amygdala activation scaled positively with dependence severity, as well as measures of depression, anxiety, and perceived stress (13). While subjective (12) or physiological conditioned responses (13) did not differ between patients with AUD and healthy controls in these imaging studies, 2 laboratory studies have shown blunted differential physiological responses during Pavlovian threat conditioning in high- compared with low-risk AUD populations (14,15). In line with these findings, reduced amygdala activation has also been observed in response to aversion-inducing alcohol-related cues in patients with AUD compared with control participants (16).

In contrast, generic or idiosyncratically appetitive conditioned cues like the sight or smell of an alcoholic beverage have been shown to bias attention and approach tendencies, induce physiological arousal, and often increase subjective craving in AUD [e.g., (17,18)], [for review, see (19)]. BOLD responses elicited by such alcohol-associated cues were predictive of subsequent relapse, most consistently in the VS (20–22). At the same time, there have been relatively few systematic investigations of the underlying acquisition process of drug conditioning in AUD. Mayo and de Wit (23) showed that a novel cue paired with alcohol elicited increased orienting responses that correlated with subjective liking of alcohol in social drinkers. In another study, only participants scoring low on self-reported alcohol sensitivity—a proposed risk phenotype for AUD (24)—demonstrated conditioned neurophysiological responses during second-order conditioning with an alcoholic olfactory cue, suggesting that this group may be more susceptible to attributing incentive salience to novel, alcohol-associated cues (25). In addition, we previously showed an increased ability of de novo–conditioned Pavlovian cues to bias instrumental choice behavior in recently detoxified patients with alcohol dependence compared with healthy participants, measured using a Pavlovian-to-instrumental transfer (PIT) task (26–28). Moreover, PIT-related neural activity in the NAcc was increased in prospective relapsers (26,28).

Altogether, impaired threat conditioning in combination with increased cue reactivity could point toward a unique pattern of associative learning alterations in AUD. On the one hand, reward-associated Pavlovian conditioning may be exaggerated, resulting in elevated reactivity toward drug-associated cues. On the other, a reduction in threat conditioning could make people more vulnerable to engaging in drug-taking behaviors despite severe negative consequences (29). To test this hypothesis, we investigated appetitive and aversive de novo conditioning for the first time as part of a PIT paradigm during fMRI in a large sample of 62 recently detoxified patients with AUD and 63 matched control participants. We further explored the behavioral and clinical relevance of these associative learning processes by linking differential BOLD responses during Pavlovian learning to the instrumental choice bias in the subsequent PIT phase and prospective relapse risk during a 12-month follow-up period.

METHODS AND MATERIALS

Participants

As a part of the LeAD study (Learning and Relapse Risk in Alcohol Dependence) (clinical trial preregistration identifier: NCT01679145), 62 recently detoxified patients with alcohol dependence (referred to hereafter as AUD) and 63 healthy control participants (HCs) matched for age, gender, and smoking status were included at 2 German study sites in Berlin and Dresden (Table 1; see the Supplement for exclusion criteria including Figure S1 and Table S1 for participant flowchart). Only participants showing a significant degree of CS-US contingency knowledge postlearning were included in the final analyses (Figure 1B). After detoxification, patients were followed up for 12 months to assess relapse status (see the Supplement for details on follow-up assessments). Follow-up information was available for 44 patients with AUD (27 relapsers vs. 17 abstainers).

PIT Paradigm

The paradigm consists of 4 parts: instrumental conditioning, Pavlovian conditioning, PIT, and a forced-choice task to assess CS-US contingency awareness (see the Supplement and Figure S2 for task details).

Instrumental Conditioning. Participants learned to collect "good" shells and to leave "bad" shells via probabilistic monetary feedback. Shells could be collected via repeated button presses, and participants completed up to 120 trials depending on their task performance.

Pavlovian Conditioning. The task comprises 2 appetitive conditions (CS paired with monetary win +1€ or +2€, respectively), 2 aversive conditions (CS followed by monetary loss −1€ or −2€, respectively), and a neutral control condition without monetary feedback (0€), using 5 different multimodal cues as CSs (Figure 1A). Each CS was presented 16 times, resulting in a total of 80 trials. Participants were instructed to attend to the relations between CS and US and to memorize the pairs. They were further informed that they would receive the displayed accumulated amount of money after the session.

Pavlovian-to-Instrumental Transfer. During the PIT phase, the influence of the learned Pavlovian CSs on instrumental choice behavior was measured. Participants performed the instrumental task without receiving feedback while one of the Pavlovian CSs tiled the background.

Forced-Choice Task. Finally, CS-US contingency knowledge of Pavlovian learning was assessed. Participants had to choose the higher-valued CS out of 2 CSs presented on the left and right side of the screen. Each CS combination was presented 3 times in pseudorandomized order. Only participants performing significantly above chance (83% of AUD and 91% of control participants) were considered contingency aware and included in the final analyses because contingency awareness seems necessary for Pavlovian trace conditioning to occur (30–32). Likewise, PIT effects can only be meaningfully analyzed in contingency-aware participants (26–28)

Table 1. Sample Characteristics

Characteristics	Patients With AUD		Healthy Control Participants		*p* Value
	n	Mean (SD)	*n*	Mean (SD)	
Sociodemographic Variables					
Gender, Female/Male, *n*	62	13/49	63	10/53	.61[a]
Age, Years	62	43.98 (11.59)	63	42.86 (11.19)	.59
Smokers, %	62	75.8%	63	73.0%	.88[a]
Education, Years	62	14.37 (3.12)	61	15.9 (3.89)	.02
SES	54	−0.41 (1.88)	42	0.49 (1.81)	.02
Neurocognitive Functioning					
Verbal Intelligence (MWT-B)	60	104.52 (9.43)	62	104.66 (9.53)	.93
TMT-A, Seconds	60	29.42 (8.7)	62	28.31 (9.39)	.50
TMT-B, Seconds	60	69.98 (26.46)	62	60.16 (22.54)	.03
AUD Severity					
Years With Diagnosis (DSM-IV)	57	11.35 (10.24)	–	–	
Number of DSM-IV Symptoms	58	5.71 (1.24)	63	0.51 (1.03)	<.001
Severity of AUD (ADS)	62	15.31 (7.06)	63	1.94 (2.93)	<.001
Lifetime Alcohol Consumption (Pure Alcohol)[b], kg	62	1717.26 (1180.3)	63	303.67 (988.74)	<.001
Craving (OCDS-G Total Score)	61	12.84 (8.33)	62	2.87 (2.89)	<.01
Days of Abstinence Before Scanning	62	20.31 (11.65)	63	88.89 (342.16)	.12
Personality					
Impulsivity (BIS-15 Total Score)	59	30.47 (6.4)	62	29.15 (5.55)	.23

Data are presented as mean (SD) unless specified otherwise.

ADS, Alcohol Dependence Scale; AUD, alcohol use disorder; BIS-15, Barratt Impulsiveness Scale 15; MWT-B, Mehrfachwahl-Wortschatz-Intelligenztest; OCDS, Obsessive Compulsive Drinking Scale; SES, socioeconomic status; TMT, Trail Making Test.

SES was computed as the sum of self-rated z-transformed scores of social status, household income, and inverse personal debt scores (74). Verbal intelligence was assessed with the MWT-B (German Multiple-Choice Vocabulary Intelligence Test) (75) and executive functioning by the TMT-A and TMT-B (76). Amount of lifetime alcohol intake was measured by the Composite International Diagnostic Interview (77), current craving by the OCDS-G (German version) (78), and trait impulsivity using the BIS-15 (German version) (79).

[a]*p* Value of χ^2 test, independent *t* test otherwise.

[b]Prior to detoxification in patients with AUD.

(Figure 1B; see the Supplement and Table S2 for sample characteristics of aware vs. unaware participants).

Data Analysis

Behavioral data were analyzed using MATLAB R2019b (The MathWorks, Inc.) and R version 3.6.1 (R Core Team) (33). The alpha level was set at $p < .05$ for all analyses.

CS-US Contingency Awareness. Contingency awareness was measured as the percentage of higher-valued CS choices during the forced-choice task, and group differences were examined via Mann-Whitney *U* test (see the Supplement for more detailed analyses).

Pleasantness and Arousal Ratings. Subjective ratings of CS pleasantness and arousal, obtained at the end of the PIT paradigm, were analyzed in separate linear mixed-effects models including CS value, group, and study site (see Supplement for details). Aversive and appetitive conditioning were investigated separately, given first evidence of deficits in Pavlovian threat conditioning in high-risk samples (14,15) and attenuated neural differentiation in AUD (12,13), while lacking systematic investigations on appetitive conditioning in AUD.

Behavioral PIT Effect. The behavioral PIT effect was analyzed as previously described (26) (see the Supplement).

Functional MRI. After standardized preprocessing (see the Supplement), an event-related analysis was applied using the generalized linear model approach within SPM 12 (Wellcome Department of Imaging Neuroscience; http://www.fil.ion.ucl.ac.uk/spm/) on 2 levels. For each participant, onset regressors for each CS and US type were modeled as stick functions and convolved with the canonical hemodynamic response function. Additional nuisance regressors included an eye-tracker recalibration period after half of the trials (mean duration 71.6 seconds), modeled as box-car function, and the 6 movement parameters to account for movement-related variance. Baseline contrasts for each CS were computed and entered into a random-effects flexible factorial model on the second level together with the group factor (AUD/HC). We investigated main effects across participants as well as group differences for the following 3 contrasts: Pavlovian learning was probed by contrasting CSs across valence conditions with

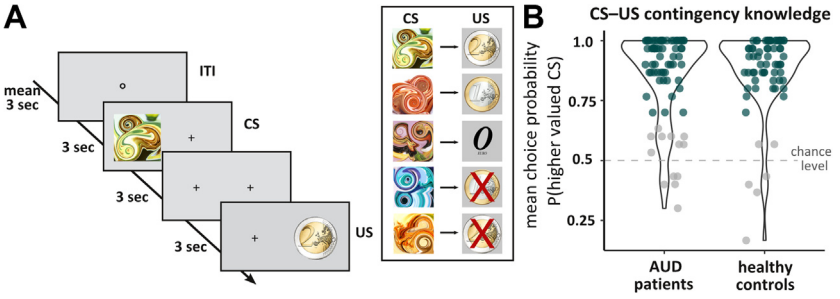

Figure 1. **(A)** Exemplary appetitive conditioning trial. In each trial, a conditioned stimulus (CS) (fractal image combined with 1 out of 5 pure tones) was presented either on the right or left side of the screen for 3 seconds. After a fixed 3-second trace interval, the associated monetary unconditioned stimulus (US) (or neutral outcome [0 Cent]) appeared on the opposite side for 3 seconds (100% reinforcement schedule). Trials were separated by a jittered intertrial interval (ITI) (exponentially distributed; range: 2–6 seconds; mean = 3 seconds). The paradigm comprised 5 different conditions (2 appetitive, 2 aversive, and 1 neutral condition). CS assignment to conditions was counterbalanced across participants. **(B)** CS-US contingency knowledge. Mean probability of choosing the higher-valued CS during the postconditioning forced-choice task did not significantly differ between patients with alcohol use disorder (AUD) and healthy control participants (HCs) (W = 2515.5, p = .77; AUD: n = 75, mean [SD] = 85.6 [18.1]; HC: n = 69, mean [SD] = 87.8 [16.7]). Only participants performing significantly above chance (teal color-coded participants, i.e., over 50% correct choices, as confirmed by a binomial test) were considered contingency aware (83% of patients with AUD, 91% of HCs) and included in the final sample (for participant characteristics, see Table 1; for sample characteristics of aware vs. unaware participants, see Table S2).

the control condition (0€), taking into account the grading within appetitive and aversive conditions (i.e., Pavlovian CSs: −2€ −1€ 0€ +1€ +2€; contrast for Pavlovian learning: [+2 +1 −6 +1 +2]). Then, we separately investigated appetitive and aversive Pavlovian learning (contrast for aversive Pavlovian learning: [+2 +1 −3 0 0]; contrast for appetitive Pavlovian learning: [0 0 −3 +1 +2]). Group differences were investigated by testing the group × contrast interaction followed by post hoc t tests in case of a significant effect.

We focused our analyses on 3 predefined regions of interest (ROIs): the amygdala and hippocampus, due to their central role in appetitive and aversive Pavlovian (trace) conditioning (8,34–36), as well as the VS (10,37), which is critically involved in reward processing (38) and has previously been shown to modulate PIT effects in AUD (26,28,39). Bilateral ROIs for the amygdala and hippocampus were derived using the WFU PickAtlas (http://www.fmri.wfubmc.edu/download.html) and the VS as a functionally defined mask using the BrainMap database (40), similar to previous publications (41,42). ROI analyses were performed with the cutoff for statistical significance set at a familywise error (FWE)–corrected $p < .05$, complemented by exploratory whole-brain analyses at $p < .05$ with FWE correction at the cluster level, using a cluster-forming threshold of $p < .001$ uncorrected and cluster extend of 10 contiguous voxels. To account for multiple comparisons across ROIs, p values were additionally adjusted for the number of ROIs using Bonferroni correction.

Brain-Behavior Associations. Individual PIT effects (see the Supplement) were entered as a covariate within SPM in a separate second-level generalized linear model with the Pavlovian learning contrast and the group factor (AUD/HC), allowing for an interaction between group and covariate. We focused our ROI analyses on the amygdala and VS, which have been shown to modulate neural PIT effects (43–45).

To investigate whether neural signatures during Pavlovian learning were predictive of subsequent relapse, we reran the flexible factorial model and informed the group factor by patients' prospective relapse status (relapsers vs. abstainers vs. HCs). We further explored whether neural responses during Pavlovian learning correlated with relapse latency in prospective relapsers using simple regression analysis with the Pavlovian learning contrast and the number of abstinence days until relapse as a covariate. Study site was included as an additional covariate in all analyses.

RESULTS

Explicit Learning of CS-US Associations: Contingency Awareness

Contingency awareness was assessed postlearning in a forced-choice task using data from all participants providing high-quality fMRI data (75 patients with AUD vs. 69 HCs) (Figure 1B; see Figure S1 for participant flowchart). Overall performance was at 86.6% correct choices (SD = 17.4; range: 16.7–100), with no differences between groups (W = 2515.5, p = .77), indicating equal levels of contingency awareness (Figure 1B; see also Figure S4). All subsequent analyses were based on participants who performed significantly above chance (i.e., Pavlovian learner as confirmed by binomial test).

Subjective Measures of Pavlovian Learning: Pleasantness and Arousal Ratings

Subjective CS pleasantness and arousal ratings, acquired postconditioning, were significantly influenced by the conditioning protocol, which was evident in a linear effect of CS value on pleasantness ratings (b = 0.15, SE = 0.06, $t_{447.56}$ = 2.78, p = .006) and a linear and quadratic effect on subjective arousal (b_{linear} = 0.09, SE = 0.04, $t_{448.0}$ = 2.22, p = .027; $b_{quadratic}$ = 0.08, SE = 0.04, $t_{448.0}$ = 2.24, p = .026) (Figure S5). This indicated that participants' pleasantness and arousal ratings reflected Pavlovian value after conditioning. Arousal ratings were higher in patients with AUD than healthy controls across cues (b = −0.58, SE = 0.26, t = −2.22, p = .028), but we did not observe a group × value interaction, indicating that the groups did not differ in conditioned responses (pleasantness: p = .358; arousal: $p \geq .158$). Separate investigation of appetitive and aversive conditioning revealed that the observed behavioral effects were driven by appetitive CSs (pleasantness: b = 0.26, SE = 0.12, $t_{223.03}$ = 2.21, p = .028; arousal: b = 0.22, SE = 0.09, $t_{224.0}$ = 2.46, p = .015) rather than aversive CSs (pleasantness and arousal $p \geq .386$), without significant effects of group or group × CS value interaction in either analysis ($p \geq .118$).

Table 2. ROI Analyses of Pavlovian Conditioning

Analysis	Contrast	Region	Side	Peak Voxel MNI x	y	z	Z_{max}	p_{FWE}
All Participants								
	Pavlovian conditioning ($CS_{-2€} > CS_{-1€} > CS_{0€} < CS_{+1€} < CS_{+2€}$)	Amygdala	R	28	−2	−14	3.08	.033
		VS	L	−4	12	−8	3.3	.037
	Appetitive conditioning ($CS_{+2€} > CS_{+1€} > CS_{0€}$)	Amygdala	R	28	−2	−14	3.09	.032
		VS	L	−4	12	−8	3.55	.017[a]
			R	14	6	−12	3.33	.034
	Aversive conditioning ($CS_{-2€} > CS_{-1€} > CS_{0€}$)	–						
Group Differences								
AUD > HC	Pavlovian conditioning ($CS_{-2€} > CS_{-1€} > CS_{0€} < CS_{+1€} < CS_{+2€}$)	Amygdala	R	28	−4	−22	3.74	.004[a]
			L	−24	−8	−22	3.35	.014[a]
AUD > HC	Appetitive conditioning ($CS_{+2€} > CS_{+1€} > CS_{0€}$)	Amygdala	R	26	−6	−22	4.06	.001[a]
			L	−24	−8	−22	3.56	.007[a]
		Hippocampus	R	26	−10	−22	3.76	.004[a]
			L	−24	−10	−24	3.5	.011[a]

p_{FWE} indicates familywise error–corrected $p < .05$ for bilateral anatomical region.

AUD, alcohol use disorder; CS, conditioned stimulus; HC, healthy control participants; L, left hemisphere; MNI, Montreal Neurological Institute; R, left hemisphere; ROI, region of interest; VS, ventral striatum.

[a]Denotes statistical significance after Bonferroni correction for number of ROI comparisons.

Neural Representation of Pavlovian Learning: BOLD Signals Toward Appetitive and Aversive Pavlovian Cues

Across participants, Pavlovian learning induced marginally increased BOLD responses in the right amygdala ($p_{FWE\ ROI}$ = .099) (Table 2). Separate investigation of appetitive and aversive Pavlovian conditioning revealed significantly increased BOLD responses toward reward-predicting cues in the left VS ($p_{FWE\ ROI}$ = .05) (Table 2), while aversive Pavlovian conditioning showed no significant differential BOLD responses. No additional activated clusters survived in the whole-brain analyses.

Group comparison revealed significant different engagement of the right amygdala during Pavlovian conditioning (amygdala right: [x:28, y:−4, z:−22], $F_{1,492}$ = 14.65, $p_{FWE\ ROI}$ = .029). Post hoc analysis showed that patients with AUD exhibited significantly stronger differential BOLD responses in the bilateral amygdala toward Pavlovian cues relative to HCs (Figure 2; Table 2; complementary analyses are provided in the Supplement). Investigating differential BOLD responses for appetitive and aversive Pavlovian conditioning separately revealed that the observed group difference was specific to reward-predicting cues assessed with the appetitive Pavlovian conditioning contrast (amygdala right: [x:26, y:−6, z:−22], $F_{1,492}$ = 16.75, $p_{FWE\ ROI}$ = .006; amygdala left: [x:–24, y:−8, z:−22], $F_{1,492}$ = 12.84, $p_{FWE\ ROI}$ = .045). Here, patients with AUD also showed stronger recruitment of an anterior cluster within the hippocampus ([x:26, y:−10, z:−22], $F_{1,492}$ = 14.38, $p_{FWE\ ROI}$ = .027) (Table 2). In contrast, no group differences

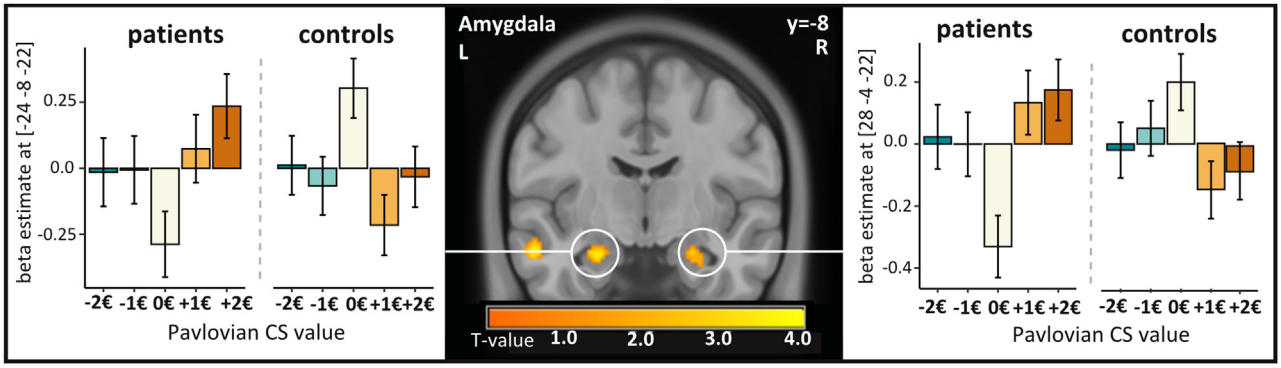

Figure 2. Stronger differential blood oxygen level–dependent responses in the bilateral amygdala during Pavlovian conditioning in patients with alcohol use disorder (AUD) compared with control participants (amygdala right: Z = 3.74, $p_{FWE\ ROI}$ = .012; amygdala left: Z = 3.35, $p_{FWE\ ROI}$ = .041). Group differences were driven by both increased blood oxygen level–dependent responses toward Pavlovian conditioned stimuli (CSs) in patients with AUD compared with healthy control participants ($p_{FWE\ ROI} < .001$), as well as increased blood oxygen level–dependent responses toward the neutral cue in healthy participants compared with patients with AUD ($p_{FWE\ ROI} \leq .012$) (see the Supplement). Visualization threshold of T-map at T ≥ 3. FWE, familywise error; L, left; R, right; ROI, region of interest.

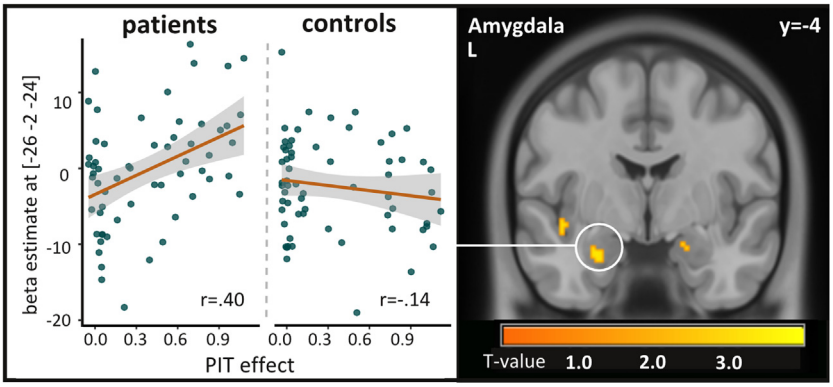

Figure 3. Blood oxygen level–dependent responses in the left amygdala during Pavlovian conditioning were positively associated with subsequent Pavlovian-to-instrumental transfer (PIT) behavior in patients with alcohol use disorder compared with control participants (amygdala left: $Z = 3.35$, $p_{FWE\ ROI} = .048$). Visualization threshold of T-map at $T \geq 3$. FWE, familywise error; L, left; ROI, region of interest.

emerged during aversive Pavlovian conditioning. Results remained significant when contingency-unaware participants were also included (see the Supplement).

Association of Pavlovian Conditioning With Instrumental PIT Behavior and Prospective Relapse

We further investigated whether neural responses during Pavlovian learning were related to the ability of Pavlovian cues to bias subsequent choice behavior (i.e., PIT effect) (see Figure S3 and Table S3) and to prospective relapse risk.

Across groups, this analysis revealed that increased conditioning-related BOLD activity in the right VS was associated with a stronger instrumental choice bias during the subsequent PIT phase ([x:4, y:−14, z:−8], $Z = 3.48$, $p_{FWE\ ROI} = .05$) (Figure S6). Group comparisons showed that BOLD activity in the left amygdala was predominantly predictive of

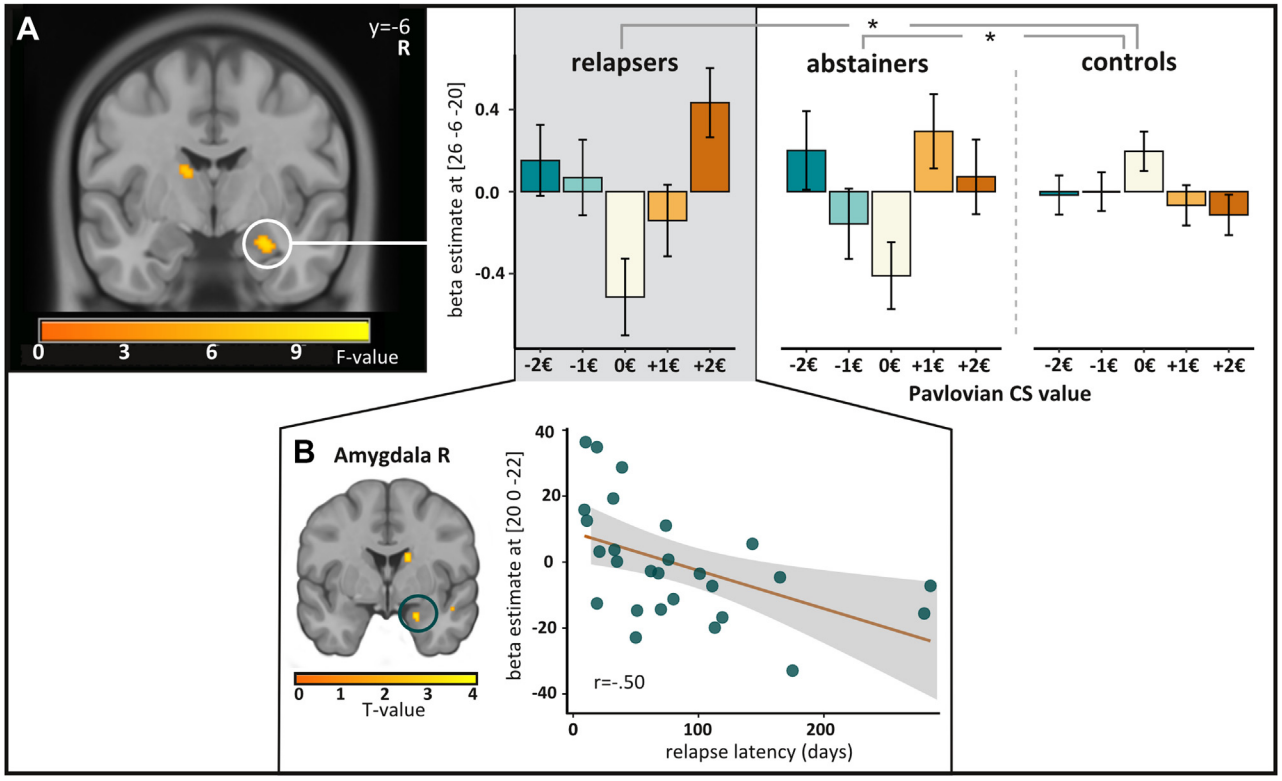

Figure 4. **(A)** Significant group difference between relapsers, abstainers, and control participants during Pavlovian conditioning in the right amygdala ($F_{2,416} = 8.58$, $p_{FWE\ ROI} = .033$). Both relapsers ($Z = 3.47$, $p_{FWE\ ROI} = .033$) and abstainers ($Z = 3.40$, $p_{FWE\ ROI} = .042$) showed increased blood oxygen level–dependent responses compared with control participants, while patient groups did not differ significantly. **(B)** Among patients who relapsed, differential amygdala responses during Pavlovian learning were inversely related to relapse latency ($Z = 2.94$, $p_{FWE\ ROI} = .047$). Visualization threshold of F-/T-map at $F \geq 6/T \geq 3$. *$p < .05$. CS, conditioned stimulus; FWE, familywise error; R, right; ROI, region of interest.

patients' subsequent choice bias, in contrast to HCs (left: [x:−26, y:−2, z:−24], $Z = 3.35$, $p_{FWE\ ROI} = .048$) (Figure 3).

Finally, we assessed prospectively whether neural signals during de novo conditioning were associated with relapse at 1-year follow-up. Contrasting relapsers with abstainers as well as HCs revealed a main effect of group in the right amygdala during Pavlovian learning ([x:26, y:−6, z:−20], $F_{2,416} = 8.58$, $p_{FWE\ ROI} = .033$) (Figure 4). Post hoc analyses confirmed that both patient groups showed increased amygdala activity relative to HCs (relapsers > HCs: [x:26, y:−6, z:−20], $Z = 3.47$, $p_{FWE\ ROI} = .033$; abstainers > HCs: [x:24, y:−4, z:−20], $Z = 3.40$, $p_{FWE\ ROI} = .042$). Although amygdala activation did not differ between prospective relapsers and abstainers ($p_{FWE\ ROI} = .22$), within patients who relapsed, increased right amygdala activation during Pavlovian learning was associated with reduced relapse latency ([x:20, y:0, z:−20], $Z = 2.94$, $p_{FWE\ ROI} = .047$) (Figure 4).

DISCUSSION

In this study, we investigated de novo Pavlovian conditioning of both appetitive and aversive associations in recently detoxified patients with AUD and healthy participants during fMRI. While both patients and healthy participants were equally likely to acquire the different CS-US associations in terms of explicit contingency knowledge, Pavlovian CSs elicited significantly stronger BOLD responses in bilateral amygdala in patients than in healthy controls. This difference was most pronounced for reward-predicting cues. We further related BOLD responses during Pavlovian conditioning to the behavioral choice bias induced by these cues in a subsequent PIT test, as well as to relapse during a 12-month follow-up period. In contrast to healthy participants, left amygdala activation during Pavlovian conditioning was positively associated with the subsequent behavioral PIT effect in patients with AUD, and among patients who relapsed, right amygdala activation was predictive of relapse latency in an exploratory analysis.

Patients With AUD Showed Elevated Amygdala Activation Toward Pavlovian Cues During De Novo Conditioning

We observed significant group differences in the amygdala during de novo Pavlovian conditioning, with stronger differential BOLD responses in patients than in healthy participants.

Converging lines of evidence have identified the amygdala as a core region subserving appetitive and aversive Pavlovian conditioning. The amygdala is critically involved in encoding the state value of motivationally salient stimuli, forming CS-US associations, and expression of conditioned responses (34,46–48). Amygdala responsivity has been shown to capture individual differences in human threat conditioning because BOLD signals in this region correlate with physiological conditioning indices (49–52). Furthermore, amygdala activation plays a vital role during Pavlovian relapse effects, i.e., the return of conditioned responses after extinction (53,54), highlighting the importance of this structure for the acquisition, recall, and expression of conditioned responses.

Therefore, our observation of elevated differential amygdala activation during Pavlovian conditioning in patients with AUD compared with healthy participants likely reflects enhanced neural encoding of Pavlovian associations—especially rewarding ones—and could reflect greater susceptibility to assigning incentive salience to novel, reward-related cues in AUD (1). To our knowledge, this is the first study to investigate appetitive Pavlovian de novo conditioning in patients with AUD. Additional evidence for enhanced drug-related Pavlovian learning in at-risk participants comes from Fleming *et al.* (25) who found that Pavlovian de novo conditioning using an alcoholic olfactory cue only induced subjective craving and conditioned event-related potentials in participants who were low in alcohol sensitivity but not in participants who were high in alcohol sensitivity, a phenotype associated with risk for developing AUD (24).

Regarding aversive Pavlovian conditioning, Muench *et al.* (13) observed overall attenuated differential amygdala activation during de novo Pavlovian threat conditioning in patients with AUD compared with healthy participants. Interestingly, however, differential amygdala responses scaled positively with AUD symptom severity (13). During instructed threat conditioning, where CS-US contingencies are known in advance, men with alcohol dependence showed attenuated differential BOLD responses toward a high- versus a low-heat-predicting cue in cortical regions associated with negative affect regulation, including the pregenual anterior cingulate cortex and medial prefrontal cortex, together with increased posterior insula activation toward the high-intensity versus the low-intensity US itself (12). Further evidence for altered threat conditioning in AUD comes from 2 studies in high-risk populations (14,15). Finn *et al.* (15) found that men with a high family history of AUD compared with men without such a family history failed to acquire differential skin conductance responses toward threat compared with neutral cues due to reduced responsiveness toward the threat-associated CS. Attenuated differential skin conductance responses and startle responses toward aversive versus neutral CSs were also observed in young binge drinkers compared with nonbinge drinkers (13), also corroborating rat studies showing that multiple episodes of ethanol withdrawal can impair fear conditioning due to lower responsiveness toward fear-associated CSs (55,56).

In our study, investigating aversive and appetitive Pavlovian conditioning separately showed that the group difference in amygdala activation was primarily driven by enhanced BOLD responses toward reward-predicting but not loss-predicting cues. However, we refrain from drawing specific conclusions about aversive conditioning in AUD because the aversive contrast in our paradigm did not elicit significant differential BOLD activation across participants [see also (35)]. Therefore, cues signaling threat, like electric shock or loud noise, may be better suited to studying aversive associative learning in future studies.

Conditioned Amygdala Responses Are Related to PIT and Relapse Latency in Patients With AUD

The PIT paradigm enables investigation of the influence of Pavlovian cues on instrumental behavior—an effect called PIT (27,28,57). PIT effects are mediated by distinct regions within the NAcc and amygdala (44,58,59) and have been discussed as a potential mechanism contributing to habit formation and habitual drug use in AUD (3,60,61).

By relating neural activity during Pavlovian conditioning to the subsequent behavioral PIT effect, we showed that VS BOLD responses were positively correlated with the strength of the PIT effect in both patients and control participants. Amygdala activation during Pavlovian conditioning was significantly correlated with instrumental choices during PIT in patients with AUD compared with HCs. This observation suggests that amygdala engagement during Pavlovian conditioning contributes to instrumental choices toward these Pavlovian cues and that this association is pronounced in patients with AUD, underlining the behavioral relevance of our neural finding.

Previous research showed that BOLD responses in the NAcc during the PIT phase were predictive of subsequent relapse during follow-up in patients with AUD (26,28). Therefore, we assessed whether the neural signatures of Pavlovian conditioning represent a potential marker for prospective relapse within a 12-month follow-up period. Both prospective relapsers and abstainers showed elevated amygdala responses during Pavlovian conditioning compared with healthy participants, and patient groups did not differ significantly in overall amygdala reactivity. However, we found a significant inverse correlation between right amygdala activation in response to Pavlovian CSs and relapse latency in prospectively relapsing patients. The results of this study, if replicated, may suggest that increased amygdala reactivity toward Pavlovian cues is not a general risk factor of AUD but could decrease relapse latency in vulnerable persons. Patients who abstained might have had additional protective factors helping them to stay abstinent despite increased amygdala reactivity during Pavlovian learning. Cue-reactivity research has revealed that abstinent compared with nonabstinent patients with AUD showed increased functional connectivity between limbic regions and prefrontal areas in a cue-reactivity paradigm, potentially helping them to stay abstinent in the presence of craving-inducing alcohol cues (62). However, increased cue-induced limbic brain activation may not simply promote relapse, but could contribute to salience attribution, which is also required for inhibitory control (20,63). Complex top-down and bottom-up mechanisms may constitut important moderating factors also shown to critically interact with cue reactivity in AUD (64,65).

Limitations

Several limitations of the current study need to be considered. First, prospective studies in participants at risk are needed to elucidate whether the observed alterations in Pavlovian conditioning represent a predisposing factor for AUD or rather develop throughout the course of the disease. Second, our paradigm may not be optimal for disentangling appetitive and aversive conditioning because the aversive contrast failed to significantly engage relevant brain structures. Third, we acquired no additional psychophysiological measure of conditioned responding, e.g., skin conductance, which limits comparability between studies. Fourth, the observed group difference in amygdala activation during Pavlovian conditioning was due to both increased BOLD responses toward Pavlovian cues in AUD as well as toward the neutral cue in HCs. Our control condition may be affectively more ambiguous than a neutral cue not paired with any outcome, as is often used in (fear) conditioning paradigms, highlighting the need for careful consideration of adequate baseline conditions in future studies. Moreover, we did not investigate conditioning of drug-related cues, which may tap into more disease-specific mechanisms within largely overlapping neural circuits (66,67).

Conclusions and Future Directions

In conclusion, we have provided evidence for altered Pavlovian learning processes in patients with AUD, which was reflected in increased amygdala recruitment that was especially pronounced during reward-associative learning. Increased amygdala reactivity was related to subsequent PIT behavior as well as to relapse latency during a 12-month follow-up period. These findings may reflect greater susceptibility to assigning incentive salience to novel, reward-related cues in patients with AUD (1), a process that may contribute to biasing patients' behavioral choices in the presence of these Pavlovian stimuli.

Our findings extend evidence on Pavlovian conditioning in patients with AUD and related high-risk populations and point toward patterns of associative learning alterations, whereby conditioned responses toward reward- or drug-associated Pavlovian cues are increased (25), while learning from threat-signaling cues is abolished (12–15). This may promote elevated reactivity toward reward-associated cues including drug cues while people also engage in conditioned behaviors despite severe negative consequences.

Interestingly, the reported conditioning alterations in AUD are different from those seen in other patient populations including patients with posttraumatic stress disorder and anxiety, wherein both threat and safety cues elicit increased physiological responses and neural activation of the amygdala, suggesting abnormal fear generalization (68,69). Given the evidence here, investigating both reward and threat conditioning processes in mental disorders could represent a fruitful avenue for future research because it enables the dissociation of learning alterations in different value domains (70,71). Moreover, investigating individual differences in these learning mechanisms may provide valuable insights into the role of Pavlovian conditioning in addiction maintenance [e.g., (72)].

Ultimately, characterizing alterations in neural structures subserving Pavlovian learning processes, that is, mechanisms at the center of influential theories of addiction (1–4), could help develop our understanding of AUD as a disorder driven by maladaptive learning and provide targets for future therapeutic interventions aimed at counteracting the motivational power of alcohol-related cues (73).

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ARTICLE INFORMATION

From the Charité Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Psychiatry and Neurosciences, CCM, NeuroCure Clinical Research Center, Berlin, Germany (CE, MG, MS, KC, FS, AH); Department of Psychiatry and Psychotherapy, Technische Universität Dresden, Dresden, Germany (CE, MNS, USZ); Technische Hochschule Aschaffenburg, University of Applied Sciences, Aschaffenburg, Germany (MS); Neuroimaging Center, Technische Universität Dresden, Dresden, Germany (MNS); Applied Computational Psychiatry Laboratory, Division of Psychiatry, Mental Health Neuroscience Department, University College London, London, England, United Kingdom (QJMH); Applied Computational Psychiatry Laboratory, Max Planck UCL Centre for Computational Psychiatry and Ageing Research, Queen Square Institute of Neurology, University College London, London, England, United Kingdom (QJMH); Camden and Islington NHS Foundation Trust, London, England, United Kingdom (QJMH); Department of Addiction Medicine and Psychotherapy, kbo Isar-Amper Klinikum Region München, Haar, Germany (USZ); and Charité Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, NeuroCure Cluster of Excellence, Berlin, Germany (AH).

FS and AH contributed equally to this work.

Address correspondence to Claudia Ebrahimi, Ph.D., at claudia.ebrahimi@charite.de.

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