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DISSERTATION

Pavlovian-to-instrumental transfer in alcohol dependence: associations with OPRM1 polymorphism, alcohol approach bias, and cognitive bias modification

Pawlowsch-Instrumentelle Transfer bei Alkoholabhängigkeit: Verbindungen mit OPRM1-Polymorphismus, Alkohol-Annäherungsbias und „Cognitive bias modification"

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# List of abbreviations

aAAT: alcohol approach/avoidance task

AD: alcohol dependence

ADS: Alcohol Dependence Scale

ApB: approach bias

BIS-15: Barratt Impulsiveness Scale-15

CBM: cognitive bias modification

CS: conditioned stimulus

fMRI: functional magnetic resonance imaging

GLMM: generalized linear mixed-effect model

LMM: linear mixed-effect model

NAcc: nucleus accumbens

OPRM1: opioid receptor mu-1

PIT: Pavlovian-to-instrumental transfer

RT: reaction time

US: unconditioned stimulus

# Abstract

Background: Alcohol dependence (AD) is a prevalent problem characterized by a high relapse risk. The "incentive salience sensitization" theory of addiction proposes that alcohol exposure progressively sensitizes the brain circuitry related to attributing incentive salience to reward-predicting stimuli, which manifests in cue-triggered behavior. The Pavlovian-to-instrumental transfer (PIT) and the approach bias (ApB) to alcohol are two widely investigated cue-related effects in AD. Both effects have been linked to the development, maintenance, and relapse in AD. The studies in my dissertation investigated: (1) whether the opioid system, which has been associated with the alcohol ApB, interacts with the PIT effect; (2) whether the alcohol ApB is associated with PIT; and (3) whether the cognitive bias modification (CBM) training targeting on the alcohol ApB impacts on PIT effects.

Methods: Patients with AD (n = 186), young healthy subjects (n = 161), and middle-aged healthy subjects (n = 105) conducted a PIT task in study 1 of this dissertation. Genotyping was performed on whole blood samples to assess the A118G (rs1799971) polymorphism of the opioid receptor mu-1 (OPRM1) gene, which has been shown to influence the affinity of the mu-opioid receptor. In study 2 of this dissertation, 100 patients with AD who performed both the alcohol approach/avoidance task and the PIT were examined. In study 3, patients with AD (n = 95) completed the CBM or placebo training and performed PIT tasks (n = 81) as well as the alcohol approach/avoidance task (n = 88) before and after training.

Results: OPRM1 G-allele carriers compared to non-G-allele carriers showed a stronger PIT effect in all three groups. Interestingly, this gene-behavior association was present in prospectively relapsing but not in abstaining patients with AD. The alcohol ApB was associated with both behavioral and neural PIT effects in patients with AD. Moreover, this behavioral association was associated with the severity of AD and trait impulsivity. The CBM training did not significantly affect the PIT effects nor the alcohol ApB.

Conclusion: Findings of this dissertation highlight the role of the opioid system in Pavlovian mechanisms in humans that manifests in the PIT effect, which has implications for the treatment of AD. Furthermore, the results indicate an association between PIT, impulsive decision making, and a bias towards alcohol approach. However, the CBM intervention did not interact with mechanisms assessed by our PIT paradigms and may thus not be useful to target Pavlovian mechanisms in human alcohol addiction.


## Zusammenfassung

Hintergrund: Alkoholabhängigkeit ist durch ein hohes Rückfallrisiko gekennzeichnet. Die „Incentive Salience Sensitization Theorie" der Sucht geht davon aus, dass chronischer Alkoholkonsum jene Schaltkreise im Gehirn, die mit der Zuschreibung von Bedeutsamkeit belohnungsrelevanter Reize zusammenhängen, für Alkoholreize sensibilisiert. Der Pawlowsch-Instrumentelle Transfer (PIT) Effekt sowie ein Alkohol-Annäherungsbias sind zwei in der Literatur beschriebene Effekte, die Folgen einer solchen Sensibilisierung gegenüber Suchtreizen sein können. Beide Effekte wurden mit der Entwicklung, Aufrechterhaltung und dem Rückfall bei Alkoholabhängigkeit assoziiert. In dieser Dissertation untersuchte ich, (1) ob das Opioidsystem, das mit dem Alkohol-Annäherungsbias in Verbindung gebracht wurde, auch an PIT Effekten beteiligt ist, (2) ob der Alkohol-Annäherungsbias mit dem PIT-Effekt in Verbindung steht, und (3) ob ein Training zur Modifikation der kognitiven Verzerrung (CBM), welches auf eine Reduktion des Alkohol-Annäherungsbias, auch den PIT-Effekt beeinflusst.

Methoden: Patienten mit Alkohoholabhängigkeit (n = 186), junge gesunde Probanden (n = 161) und gesunde Probanden mittleren Alters (n = 105) führten in Studie 1 dieser Dissertation eine PIT-Aufgabe durch. Es wurde ein OPRM1-Polymorphismus genotypisiert, der die Affinität des mu-Opioidrezeptors beeinflusst. In Studie 2 wurden 100 Patienten mit Alkoholabhängigkeit untersucht, die sowohl die Alkohol-Annäherungs-/Vermeidungsaufgabe als auch die PIT-Aufgabe durchführten. In Studie

3 absolvierten Patienten mit Alkoholabhängigkeit (n = 95) ein CBM- oder Placebo-Training und führten vor und nach dem Training die PIT-Aufgabe (n = 81) sowie die Alkohol-Annäherungs-/Vermeidungs-Aufgabe (n = 88) durch.

Ergebnisse: Studie 1: OPRM1 G+ Träger zeigten im Vergleich zu G- Trägern einen stärkeren PIT-Effekt. Diese Gen-Verhaltens-Assoziation war bei Patienten, die prospektiv rückfällig wurden, nicht aber bei abstinenten alkoholabhängigen Patienten zu beobachten. Studie 2: Bei Patienten wurde der Alkohol-Annäherungsbias sowohl mit dem verhaltensbezogenen als auch mit dem neuronalen PIT-Effekt in Verbindung gebracht. Darüber hinaus war diese Verhaltensassoziation mit dem Schweregrad der Erkrankung und der Impulsivität assoziiert. Studie 3: Das CBM-Training wirkte sich weder auf den PIT-Effekt noch auf dem Alkohol-Annäherungsbias aus.

Schlussfolgerung: Die Ergebnisse dieser Dissertation unterstreichen die Rolle des Opioidsystems für den Einfluss Pawlowscher Reize auf das menschliche Verhalten, der sich im PIT-Effekt manifestiert. Darüber hinaus zeigen die Ergebnisse einen signifikanten Zusammenhang zwischen impulsiver Entscheidungsfindung, der Alkohol-Annäherungsbias und den Veränderungen der Reaktivität auf Pawlowsche Anreize hin. Die CBM-Intervention interagierte dagegen nicht mit den Mechanismen, die in dem von uns genutzten PIT-Paradigma untersucht wurden.

# 1. Introduction

Alcohol consumption is widespread in Germany with around 18% of adults reporting harmful use of alcohol [1, 2] and around 3.6% meeting the clinical criteria of alcohol dependence (AD) [3]. A critical characteristic of AD is the high incidence of relapse despite patients' desire to remain abstinent [4]. It is widely hypothesized that alcohol-related cues can elicit relapse [5]. According to the "incentive salience sensitization" theory of addiction, alcohol exposure has the ability to sensitize the brain circuitry related to attributing incentive salience to reward-predicting stimuli, as evidenced by cue-related behavior [6, 7]. In this dissertation, we focused on two tasks that have been used to investigate the cue-related behavior in research on AD: the Pavlovian-to-instrumental transfer (PIT) task, and the alcohol approach/avoidance task (aAAT). Studies provided evidence of these two tasks in association with the development, maintenance and relapse of AD, and their implications for treatment [e.g. 8, 9-12].

## 1.1 Pavlovian-to-instrumental transfer (PIT)

Pavlovian learning (or in other words, conditioned learning) describes a process in which a neutral stimulus (conditioned stimulus; CS) gains incentive salience when it repeatedly appears with a reward (unconditioned stimulus; US) [13]. PIT is a well-established paradigm to quantify the impact of Pavlovian cues on instrumental behavior. In PIT, Pavlovian CS-US associations are trained separately from instrumental response – outcome contingencies in subjects. Subsequently, instrumental behavior is assessed in the presence of Pavlovian CSs, usually under (nominal) extinction conditions (i.e., outcomes are not delivered during the test) [14].

To date, a variety of PIT paradigms have been utilized. Some studies focused on appetitive PIT: the effect of Pavlovian CS predicting an appetitive outcome on enhancing instrumental behavior that leads to the appetitive outcome [for a review, see 14], and other studies examined aversive PIT: the effect of aversive outcome-predictive Pavlovian CS on inhibiting approach to an appetitive outcome [e.g., 15, 16]

or on promoting response to cancel the aversive outcome [e.g., 17, 18]. Moreover, studies have disentangled outcome-specific PIT and general PIT that are underpinned by different neural substrates [19]. Outcome-specific PIT refers to the situation where the Pavlovian CS previously paired with a reward promotes instrumental behavior that leads to the same reward, whereas in general PIT a CS enhances instrumental behavior regardless of the identity of the reward [20, 21]. Research suggested that the nucleus accumbens (NAcc) shell mediates the outcome-specific PIT effect, whereas NAcc core mediates the general PIT effect [19].

## 1.1.1 Animal research on PIT

The interaction between Pavlovian and instrumental learning has been observed in animal studies a few decades ago. For instance, in a study conducted by Rescorla and Lolordo (22), dogs increased bar pressing to prevent shock when hearing a tone previously associated with shock (CS). Following that study, abundant animal studies have investigated PIT in addiction research [e.g., 23, 24-29]. Drug-related cues enhanced PIT effects in drug-exposed rats [23, 24]. Moreover, cues predicting ethanol delivery facilitated reward seeking for both ethanol and non-ethanol reward (i.e., a general PIT effect) [23].

In addition to drug-related cues, there are studies applying non-drug-related cues in PIT to study a general impact of drug use and addiction on cue-related behavior. Increased non-drug-related PIT effects were observed in rats repeatedly exposed to cocaine [25-28]. Comparably, mice chronically exposed to ethanol vapor showed enhanced non-ethanol-related (i.e., reward of food) PIT effects [29]. These findings suggest a general alteration in cue processes and motivational behavior caused by repeated exposure to drugs.

## 1.1.2 Human research on PIT

Following animal studies, PIT has been applied in human addition research, including alcohol use and dependence. Alcohol-related PIT effects were observed in nonclinical samples of social drinker [30-33]. This PIT effect was observed irrespective of alcohol

devaluation [32], but was not associated with craving [33] or alcohol consumption measured by the Alcohol Use Disorder Identification Test [30]. However, by applying a non-drug-related PIT paradigm that presents monetary Pavlovian CSs when participants performing an instrumental task to obtain monetary rewards or avoid monetary losses, our research group observed enhanced PIT effects in social drinkers with high-risk drinking compared to those with low-risk drinking [8, 34].

In addition to social drinkers, clinical samples of patients with AD have been investigated in several studies to examine the clinical relevance of PIT in AD. Our research group established and validated PIT tasks involving both drug-related and non-drug-related cues in patients with AD [35]. In the PIT tasks, participants completed an instrumental task to obtain monetary rewards or avoid monetary losses, while the Pavlovian cues that are associated with monetary outcomes or drink cues (i.e., alcohol or water pictures) were presented in the background. The non-drug-related PIT effect was more pronounced in patients with AD compared with non-dependent controls [9, 11, 35]. Moreover, the behavioral non-drug-related PIT was found to be associated with prospective relapse risk in patients with AD [36]. The alcohol cues, however, surprisingly inhibited instrumental button pressing for a monetary outcome, compared to water cues [11, 35], and this inhibitory drug-related PIT effect was observed in patients with AD but not in controls. Different findings were reported in another study using a PIT paradigm with outcome of snacks, in which differences were not found either between patients and controls, or between subsequent relapsers and abstainers, in either general or outcome-specific PIT [37]. The null findings may be explained, at least in part, by the limited sample size in that study [37], or might be associated with the type of reward used in PIT tasks.

### 1.1.3 Neurobiological correlates of PIT in nucleus accumbens

Neurobiological correlates of PIT have been studied in both animal and human research. Applying functional magnetic resonance imaging (fMRI), areas of the ventromedial prefrontal cortex, putamen, amygdala, and NAcc were identified to be

associated with PIT [e.g., 9, 18, 38, 39-43]. We focused on the NAcc in this dissertation (study 2) concerning imaging PIT data, in line with previous studies of our research group [9, 43]. The NAcc plays an essential role in the human reward system [44], and was found to be correlated with both salience and valence during incentive anticipation [45]. In research on AD, heavy drinkers were found to have an enhanced activity in the NAcc in response to alcohol cues than light drinkers [46]. Furthermore, the activation in the NAcc induced by alcohol cues was associated with the severity of alcohol use disorder [47] and subjective craving in patients with AD [48]. In addition, it differentiated subsequent abstainers and relapsers [49]. Using the PIT task in clinical sample of patients with AD, our research group also observed activation in the left NAcc elicited by non-drug-related PIT among patients with AD as well as healthy controls (HCs), with the activation being greater in prospective relapsers than abstainers [9]. Activation in the NAcc induced by alcohol versus water cues in the drug-related PIT task was also observed, which was stronger in patients than in HCs and in prospective abstainers than in relapsers [43].

The neurotransmitter dopamine in NAcc functioning plays an important role in drug addiction [50]. It has long been known that drugs of abuse affect the dopamine transmission [51]. Less availability of dopamine D2-like receptors in the NAcc may represents a down regulation, and was displayed in patients with AD, and was associated with alcohol craving [52]. In one study using PIT, phasic dopamine release in the NAcc was observed in rats in response to reward-paired cues, and this was positively correlated with the general PIT effect [53]. In another study, rats that were administrated nonspecific dopamine antagonists showed reduced outcome-specific PIT effect [54].

### 1.1.4 Opioid system and incentive salience

In addition to the dopaminergic system, recent findings indicate that the opioid system also plays a role in the attribution of incentive salience [55], beyond its role in hedonic modulation that has long been postulated [56]. Animal studies revealed that the opioid

system is involved in motivation to different types of rewards guided by available cues [57], and choice decisions influenced by Pavlovian stimuli [58, 59]. In another study, researchers compared the ability of mu opioid stimulation to dopamine stimulation in the NAcc to amplify cue-triggered incentive salience measured by a PIT task, and observed similar amplification effects by both substances [60]. Research further suggests distinct functions of mu- and delta- opioid receptors across different brain regions such as the amygdala and the NAcc [58, 61].

In human research, evidence supports the involvement of the mu- opioid system in value-based decision making in healthy subjects who showed increased preference for the stimulus associated with a high monetary reward probability [62]. The involvement of the mu- opioid system was also implicated in social motivation, as pharmacological manipulation of the mu- opioid system affected both the "liking" of the opposite-sex faces and the motivation for viewing those faces [63]. In patients with AD, the unspecific opioid receptor antagonist naltrexone decreased activation in the ventral striatum induced by alcohol cues [64]. The function of the opioid system in PIT, however, has been less studied. In Weber et al. [65], an outcome-specific PIT task with the reward of chocolate was conducted by healthy subjects. Opioid receptor antagonist naltrexone suppressed the PIT effect, although the impact was less pronounced compared with a dopamine D2/D3 receptor antagonist [65].

## 1.2 Alcohol approach bias (ApB) and cognitive bias modification (CBM) training

Another area of research concerning the incentive salience of drug-related cues in addiction focuses on cue-driven behavioral biases, such as an automatic approach bias (ApB), for instance, a quick response to "approach" alcohol cues rather than to "avoid" them. This ApB to alcohol has been found to be greater in heavy drinkers than in light drinkers [e.g., 66, 67], and stronger in patients with AD compared with controls [e.g., 68]. One frequently used paradigm in laboratory research on AD is aAAT, the alcohol version of the approach/avoidance task [e.g., 69, 70]. Using aAAT, research found that the baseline alcohol ApB was predictive of future drinking [71]. Genetically,

the alcohol ApB was found to be related to the A118G (rs1799971) polymorphism of the opioid receptor mu-1 (OPRM1) gene [69], with OPRM1 G-allele carriers (G+ carriers) displaying a stronger alcohol ApB in male heavy drinkers than non-G-allele carriers (G- carriers) [69]. The OPRM1 A118G polymorphism influences the affinity of the mu-opioid receptor, such that the G variant causes a threefold increase in the binding affinity for beta-endorphin compared to the A variant [72].

In line with these findings, cognitive bias modification (CBM) training developed to retrain the automatic ApB to alcohol stimuli (hereafter the term "CBM training" in this dissertation specifically refers to alcohol ApB retraining) showed efficacy to reduce the relapse risk [10, 12, 73-76]. For example, in the first clinical study that applied CBM training targeted on alcohol ApB, patients with AD in the CBM training group were trained to avoid alcohol pictures, while other patients conducted the sham training (i.e., subjects had to approach and avoid alcohol and non-alcohol pictures equally) or received no training [10]. Patients in the CBM training condition shifted from an ApB to alcohol at baseline to an avoidance bias following training, and were less likely to relapse in a 1-year follow-up compared to the control groups [10].

Incongruent findings on the effect of CBM training, however, were reported in several studies, including a nonsignificant effect on the alcohol ApB in individuals with AD [77, 78], or heavy drinkers [79-83], and no effect on future alcohol drinking in heavy drinkers [79, 80] or high-risk young adults [84]. A recent systematic review suggests that CBM training exerts its effects mainly on individuals with more severe forms of AD [85]. Considering the mixed findings, the mechanisms for the efficacy of CBM training should be further investigated. Some studies suggest that the CBM effect on reducing relapse risk or alcohol drinking is modulated by the change of the alcohol ApB [e.g., 12, 86]. In other studies, CBM training effect generalized to other cue-related effects, such as reduced implicit alcohol-approach associations [10, 70], decreased behavioral arousal ratings to alcohol cues and cue reactivity in the amygdala [77]. These findings imply that CBM training might work on a more general effect on cue-guided behavior.

## 1.3 PIT and alcohol ApB

Although the relationship between PIT and the alcohol ApB has not been directly examined, findings suggest an association between them: both PIT and the alcohol ApB have been linked to AD, and have clinical relevance in treatment outcome of AD. Using fMRI, the NAcc was found to be correlated with both the PIT [e.g., 9, 39] and the alcohol ApB [68]. Furthermore, the opioid system is implicated in both effects, as the opioid receptor antagonist naltrexone suppressed the outcome-specific PIT effect [65], and male heavy drinkers who are OPRM1 G+ carriers exhibited a greater alcohol ApB than G- carriers [69]. From a theoretical perspective, both PIT and the alcohol ApB are cue-guided behavior and could be manifestations of incentive salience attribution to relevant stimuli [6]. According to "dual-process" accounts, the alcohol ApB manifests when an automatic system is activated and cognitive control is weakened [87], while the PIT effect in people with AD has been associated with impulsive choice measured by a delay-discounting task [11].

## 1.4 Questions and hypotheses

In this dissertation, three studies were conducted to investigate three questions.

1. Is a genotype affecting receptor affinity in the opioid system associated with the non-drug-related PIT effect in patients with AD and healthy control subjects? Does this association differ between patients with AD and controls, and between prospectively relapsing and abstaining patients?

   In study 1 [88] of this dissertation, three groups (detoxified patients with AD, middle-aged healthy control subjects, and young healthy subjects) were examined with a non-drug-related PIT task using monetary rewards. The interindividual difference in the opioid system in this study was quantified by A118G (or ASN40Asp) single nucleotide polymorphism of the OPRM1 gene. It was hypothesized that OPRM1 A118G polymorphism is associated with the non-drug-related PIT effect in all three groups, and this association is stronger in patients

with AD compared with age-matched healthy subjects, and stronger in prospective relapsers compared with abstainers.

2. Is alcohol ApB associated with the non-drug-related PIT effect in patients with AD?

In study 2 [89] of this dissertation, recently detoxified patients with AD conducted both the aAAT and the non-drug-related PIT task. We hypothesized that the strength of the alcohol ApB is associated with the behavioral non-drug-related PIT effect and neural PIT effect in the NAcc. Furthermore, we examined whether the behavioral association is further associated with the severity of AD and trait impulsivity, and whether it differs between prospective relapsers and abstainers.

3. Does the CBM training targeting on alcohol ApB impact the PIT effects in patients with AD?

In study 3 [90] of this dissertation, patients with AD received CBM or placebo training, and conducted the aAAT, drug-related and non-drug-related PIT tasks prior to and following the training. We hypothesized that CBM training would reduce patients' alcohol ApB, non-drug-related PIT effect, and increase the inhibition effect of alcohol cues in drug-related PIT. In addition, we explored whether CBM training reduces relapse risk in patients with AD.

## 2. Methods

### 2.1 Participants

Data were acquired from a bi-centric study that was conducted in Berlin and Dresden, Germany (ClinicalTrials.gov identifiers: NCT01744834, NCT01679145 and NCT02615977). All studies were approved by the ethic committee of Charité-Universitätsmedizin Berlin (EA1/267/14, EA/1/157/11 and EA1/268/14).

Three different groups (i.e., recently detoxified patients with AD, sex- and age-matched non-dependent healthy controls, and unmatched young healthy subjects) were recruited and assessed between 2012 and 2018. Patients with AD were followed up to until 2019. The inclusion criteria for participants were described in the publications of this dissertation [88-90]. Briefly, none of the participants had a history of substance dependence (expect for alcohol for patients with AD) or current substance use (except for tobacco); neurological disorders or DSM-IV axis 1 psychiatric disorders according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision [DSM-IV-TR; 91, 92]; medication that interacts with the central nervous system; and withdrawal symptoms. Patients with AD met the diagnosis criteria of AD according to DSM-IV-TR and were recently detoxified. The analyzed cohorts and sample size of the main analyses in the three studies of this dissertation are shown in Figure 1. Detailed demographic and clinical characteristics of participants can be found in the original publications [88, 90].

Patients with AD were followed up after study participation with the Time Line Follow Back procedure [93]. Relapse was defined as consuming ≥ four or five standard drinks on one drinking occasion for female and male respectively, according to the definition of high-risk consumption by World Health Organization [94]. In study 1 [88], patients' relapse status was determined by a 3-month follow-up, while study 2 [89] and 3 [90] used a 6-month follow-up .
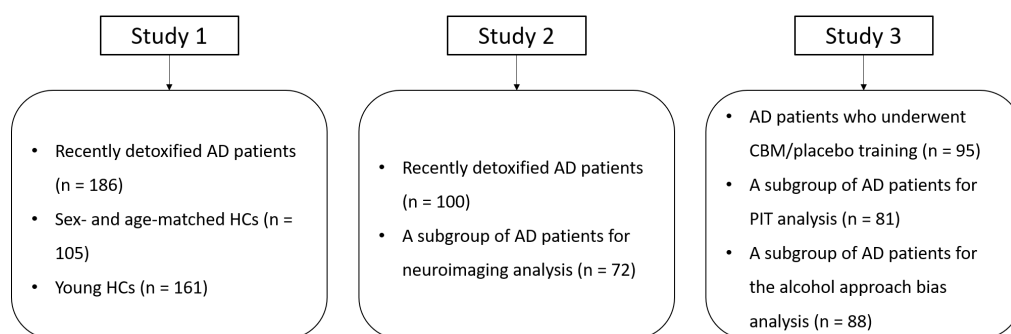


**Figure 1. Analyzed cohorts and sample sizes in the three studies of this dissertation.**

*Abbreviations: AD = alcohol dependence; CBM = cognitive bias modification; HC = healthy control. Originally created figure.*

## 2.2 Tasks and procedures

2.2.1 Pavlovian-to-instrumental transfer task

The PIT task has been introduced in all the three publications of this dissertation [88-90]. Briefly, there were four parts in the PIT task (see Figure 2). The first part was instrumental learning, in which participants learned to emit a go or a no-go response to six shells via probabilistic reward outcome. The second part was Pavlovian learning, in which participants passively viewed and remembered the presence of a conditioned stimulus (CS; a fractal image compound with an audible tone; five CSs were used) followed by an unconditioned stimulus (US; -2 vs. -1 vs. 0 vs. +1 vs. +2 euros). In the third part, participants performed the transfer test, in which the go/no-go instrumental task was conducted while the CSs from the Pavlovian learning tiled the background. The transfer test was performed in a fMRI scanner. The MRI acquisition parameters were reported in in Chen et al. [89]. In the fourth phase, participants conducted a forced-choice task, that is, they had to choose a CS from two simultaneously presented CSs.

In addition to the PIT trials with Pavlovian CSs presented in the background (non-drug-related PIT), participants completed trials in which alcohol and water pictures were used as background stimuli (drug-related PIT). The drug-related PIT was examined in study 3 [90] of this dissertation.
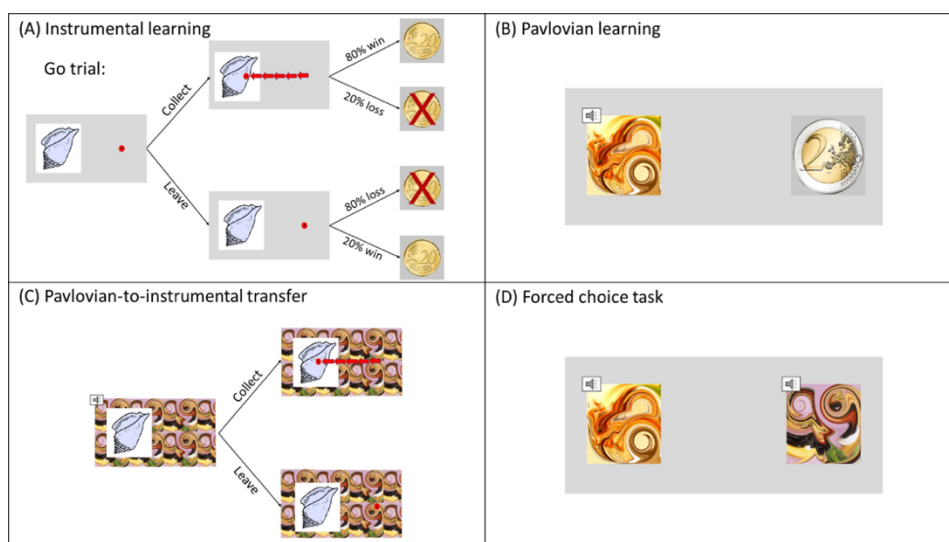
*Figure 2. The PIT paradigm.*

*(A) Instrumental learning. In a go-trial, collecting the shell by repeatedly pressing the button and moving the red dot towards the presented shell will result in a 20-cent reward with 80% probability or a 20-cent loss with 20% probability, while leaving the shell by no responses or less than five button presses will result in a 20-cent loss with 80% probability or a 20-cent reward with 20% probability. The outcome feedback of win or loss is presented following the response. The probability of win and loss for a no-go trial is reversed (not depicted here). Participants need to finish at least 60 trials and reach 80% accuracy over 16 trials or complete the whole 120 trials to end the instrumental learning. (B) Pavlovian learning. A conditioned stimulus (CS; compounded of a fractal image and an audio stimulus; five CSs in total) is presented paired with a monetary stimulus (unconditioned stimulus, US; +2€, +1€, 0€, -1€, -2€). Participants watch and memorize the Pavlovian CS – monetary US pairings. Eighty trials are conducted. (C) Pavlovian-to-instrumental transfer. The instrumental task (i.e., collect or leave the shell) is conducted with a Pavlovian CS tiling the background. There was no outcome feedback in this part to avoid further instrumental learning (nominal extinction). Participants performed 90 trials. In addition, there are 72 trials in which alcohol or water pictures are used as the background stimuli. (D) The forced choice task. The participant needs to choose one from two simultaneously presented Pavlovian CSs. Modified from Chen et al., 2022 [89].*

## 2.2.2 Alcohol approach/avoidance bias task

The aAAT applied in this dissertation was modified from Wiers et al. [10] to assess patients' alcohol ApB. Pictures of alcohol or soft drink were randomly presented inclined 3° to the left or right, in alignment with Cousijn et al. [95], and patients responded with a joystick according to the inclination of the picture. For example, one needed to push the joystick if the picture is inclined to the left and pull if inclined to the right (see Figure 3). The association between reaction (push/pull) and inclination (left/right) was randomized among participants.

***Figure 3. The alcohol approach/avoidance task.***

*In the depicted example, a push movement is required for a picture that is inclined to the left. A zooming-out effect shows upon pushing the joystick. Similarly, a right-inclined picture requires a pull movement, which leads to a zooming-in effect. Participants complete 168 trials. Modified from Chen et al., 2022 [89].*

2.2.3 Cognitive bias modification training

The CBM training used in this dissertation was adapted from the aAAT. In the CBM training condition, all alcohol pictures were presented with an inclination requiring a push movement and all soft drink pictures with an inclination requiring a pull movement [90]. In the placebo training condition, pictures of alcohol and soft drink were randomly presented inclined to the left or right, as in the original aAAT.

**2.3 Association between OPRM1 A118G polymorphism and non-drug-related PIT**

Study 1 [88] investigated the association between behavioral non-drug-related PIT and OPRM1 A118G Polymorphism in three groups (n = 186 patients with AD, n = 161 young controls, and n = 105 middle-aged controls). Participants conducted the PIT task introduced above. Whole blood samples were genotyped for the A118G polymorphism of the OPRM1 gene [88]. Based on the presence of the G allele, participants were categorized into G+ (AG and GG) and G- (AA) carriers.

## 2.4 Association between alcohol ApB and non-drug-related PIT

Study 2 [89] investigated the association between alcohol ApB and the non-drug-related PIT effect at both the behavioral and the neural level in patients with AD. N = 100 patients with AD who completed the PIT task and the aAAT were included in behavioral analysis, among which the fMRI PIT data were available for analysis in n = 72 patients. Participants fulfilled the Barratt Impulsiveness Scale-15 [BIS-15; 96] that measures the trait impulsivity, and the Alcohol Dependence Scale [ADS; 97] that measures the severity of AD.

## 2.5 CBM training on PIT

In study 3 [90], patients were randomly allocated to the CBM or placebo training group. The PIT task and the aAAT were conducted twice: once before the training to assess the baseline, and once after the training. Six training sessions were planned in total as suggested in previous literature [98]. After data cleaning, n = 95 (55/44 in the CBM/placebo training group respectively) patients with AD who finished the whole six sessions (no more than one missing session) were included into analyses. Among those patients, n = 88 completed the aAAT, and n = 81 completed the PIT tasks before and after the training.

## 2.6 Data analyses

Data analyses were conducted using the R programming language [99]. In all publications of this dissertation, the behavioral non-drug-related PIT data were analyzed using generalized linear mixed-effect models (GLMM; lme4 R package [100]), with the instrumental condition (go vs. no-go) and the valence [negative vs. neutral vs. positive; 88] or the value [+2, +1, 0, -1, -2; 89, 90] of Pavlovian CS as the basic predictors on trial-by-trial accuracy [88] or the number of button presses [89, 90] in the transfer test. In study 1 [88], in order to examine the association of the OPRM1 polymorphism with the PIT effect, information on the OPRM1 polymorphism (G− vs.

G+), as well as its interaction with the other two predictors of PIT (i.e., instrumental condition and Pavlovian CS valence), were included in the fixed effects of the model to regress the trial-by-trial accuracy (correct/incorrect). This analysis was conducted separately for the three groups (i.e., patients with AD, middle-aged HCs, young HCs). In addition, two analyses of the association between the OPRM1 polymorphism and PIT were conducted, comparing between patients and matched controls, and between prospective relapsers (n = 51) and abstainers (n = 94). For that, the group factor (AD patients vs. matched HCs, or realpsers vs. abstainers) and the interaction terms were included in the models.

To quantify participants' alcohol ApB in aAAT, the median reaction times (RT) to each drink category (alcohol or soft drink) under each movement condition (push or pull) were extracted for each participant. The alcohol ApB was reflected by a calculated D-diff score, which is the median RT difference between pushing and pulling alcohol pictures relative to soft drink pictures [90]. Therefore, a positive D-diff score reflects an ApB to alcohol relative to water cues, and a negative D-diff score reflects an avoidance bias to alcohol relative to water cues. To measure the association between alcohol ApB and behavioral PIT, study 2 of this dissertation added the predictor of the aAAT D-diff score as well as interaction terms in the GLMM to analyze participants' trial-by-trial number of button presses. In addition, it was further investigated if the severity of AD or the trait impulsivity is involved in the association between the alcohol ApB and behavioral PIT by adding the ADS score or the BIS-15 score as an additional predictor in separate GLMMs. For neural PIT analysis, a parametric modulator for non-drug-related PIT was established in the single-subject level analysis in Statistical Parametric Mapping 12 [101], which was calculated as the multiplication product of the Pavlovian CS value and the log-transformed number of button presses. Individual contrast images were subjected into a group analysis in which the aAAT D-diff score was added as a covariate. Small volume correction was used to restrict the search area in region of interest in bilateral NAcc (derived from Wake Forest University (WFU) PickAtlas software [102]) based on previous

observations [9, 39]. In addition, we explored the differences between prospective relapsers and abstainers in the alcohol ApB – PIT association.

In study 3 [90], in order to assess the impact of CBM training on the non-drug-related PIT, the GLMM included predictors of the training condition (CBM vs. placebo training), assessment time (pretest vs. posttest) and the interaction terms with Pavlovian CS value and instrumental condition to analyze trial-by-trial number of button presses. Similar analyses were conducted to investigate the impact of training on drug-related PIT, with beverage type (alcohol vs. water) instead of Pavlovian CS value included in the model. In addition, with regard to the training effect on the alcohol ApB, a linear mixed-effect model (LMM) with predictors of training condition and assessment time was established to predict the D-diff score. We further explored whether CBM training reduced the relapse risk in patients with AD by comparing the proportion of relapsers in two training groups using chi-squared test.

## 3. Results

### 3.1 Association between OPRM1 A118G polymorphism and non-drug-related PIT

GLMM results demonstrated a stronger PIT effect in OPRM1 G+ carrier than G-carriers in all three groups (instrumental condition × Pavlovian valence × OPRM1 polymorphism: $p = .002/.011/<.001$ and $\chi^2 = 12.72/9.03/20.69$ respectively for patients with AD, middle-aged controls, and young controls; see Figure 4) [88]. This OPRM1 polymorphism – PIT association did not differ between patients and age-matched controls (instrumental condition × Pavlovian valence × OPRM1 polymorphism × group: $p = .85$). When comparing subsequent relapsers (n = 51) and abstainers (n = 94), a significant group difference was present in the association between OPRM1 polymorphism and PIT (instrumental condition × Pavlovian valence × OPRM1

polymorphism × relapse: $\chi^2 = 30.35$, p < .001). Post-hoc tests indicated that this gene-behavior association was significant only in relapsers (p < .001) but not in abstainers (p = .33), see Figure 5. In G+ carriers, prospective relapsers exhibited a stronger non-drug-related PIT effect than abstainers (p < .001), while no significant difference in PIT was shown between relapsers and abstainers who are G- carriers (p = .09). Further detailed results can be found in the original publication [88].
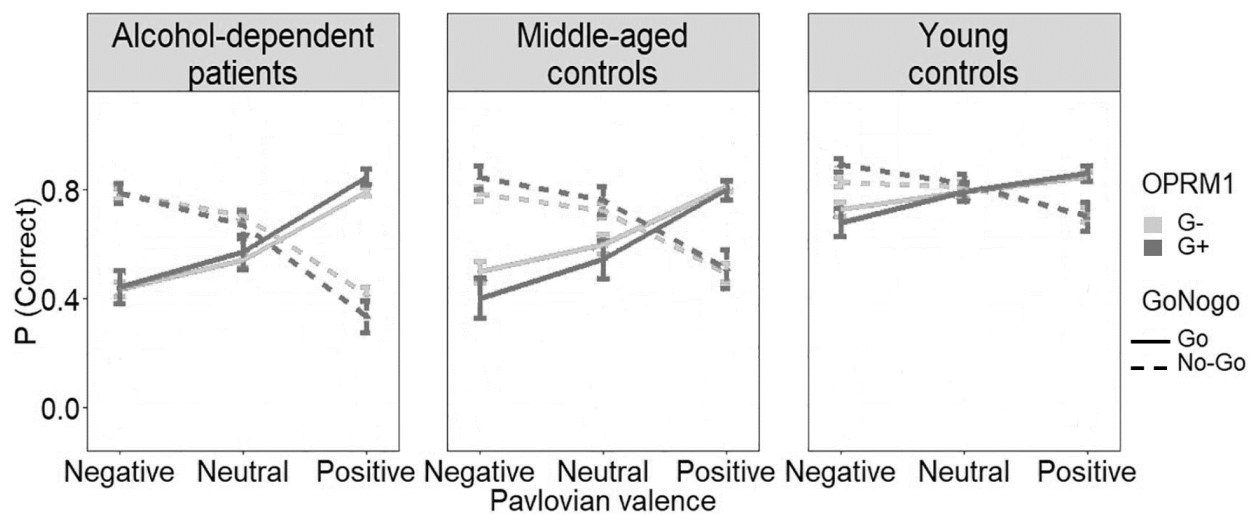


***Figure 4. Non-drug-related PIT effect as a function of OPRM1 polymorphism in three groups: alcohol-dependent patients, middle-aged controls, and young controls.***

*The PIT effect in study 1 of this dissertation was reflected as the influence of Pavlovian CS valence (negative, neutral, or positive) on the accuracy of the instrumental response in the transfer test. Positive CSs enhanced accuracy in instrumental go trials, and decreased accuracy in no-go trials, vice versa for the effect of negative CSs, as shown by the slope of the lines. In all three groups, the non-drug-related PIT effect was associated with the OPRM1 polymorphism [88]. That is, G+ carriers showed a stronger PIT effect than G− carriers. Modified from Sebold et al., 2021 [88].*
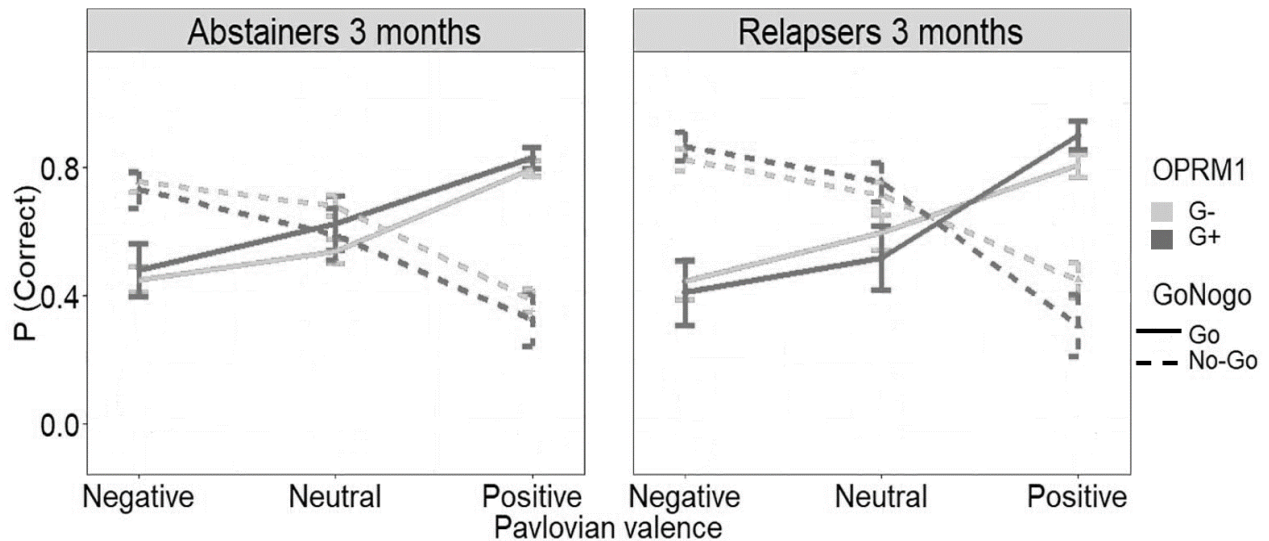
***Figure 5. Non-drug-related PIT effect in prospectively abstaining and relapsing alcohol-dependent patients with a follow-up of three months.***

*The OPRM1 polymorphism – PIT association was stronger in future relapsers compared to abstainers. Modified from Sebold et al., 2021 [88].*

## 3.2 Association between alcohol ApB and non-drug-related PIT

There was a significant association of the alcohol ApB with the behavioral non-drug-related PIT effect in patients with AD, as suggested by the interaction of aAAT D-diff score with Pavlovian CS value (Pavlovian CS value × aAAT D-diff score: estimate = 0.14, z = 11.34, p < .001; Figure 6) [89]. Moreover, this association was associated with the severity of AD in patients (Pavlovian CS value × aAAT D-diff score × ADS score: estimate = 0.02, z = 12.51, p < .001) and the degree of trait impulsivity (Pavlovian CS value × aAAT D-diff score × BIS-15 score: estimate = 0.04, z = 14.58, p < .001) [89]. Exploratory analyses showed a stronger association of the alcohol ApB with the non-drug-related PIT in relapsers compared with abstainers with intention-to-treat analysis, when patients with missing relapse information were categorized as relapsers (estimate = 0.08, z = 2.34, p = .020) [89], while this finding was not present

with per-protocol analysis when only patients with clear relapse information were included (estimate = -0.03, z = -0.81, p = .42) [89].
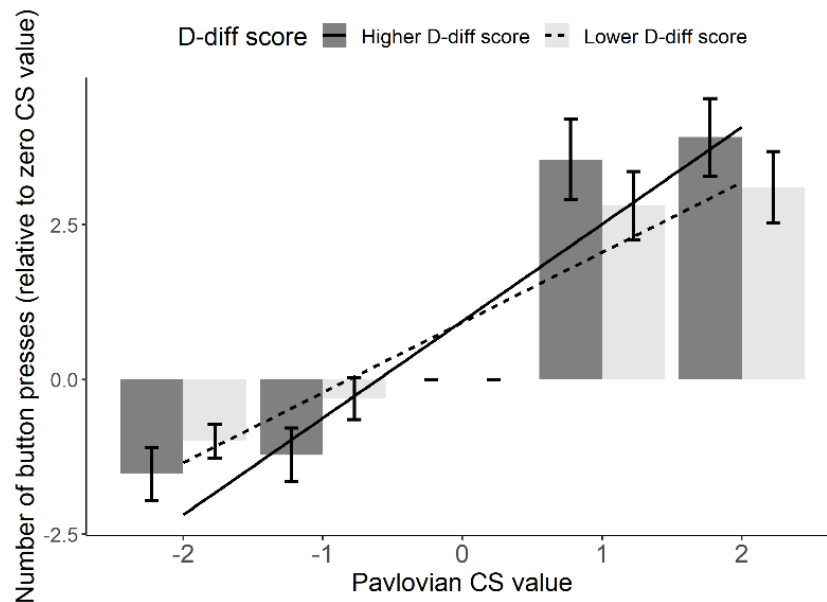


**Figure 6. The behavioral non-drug-related PIT effect as a function of the alcohol approach bias (i.e., D-diff score).**

*The PIT effect in study 2 [89] of this dissertation was reflected as the influence of Pavlovian CS value on the number of button presses in the transfer test (visualized as the slopes of lines). In this figure, the D-diff score was transformed into a two-level factor using median split for illustrative purposes. Patients who showed a greater alcohol approach bias (ApB) exhibited a stronger PIT effect than patients with a lower alcohol ApB. Modified from Chen et al. 2022 [89].*

At the neural level, the alcohol ApB was associated with the PIT-related activity in the right NAcc (x = 16, y = 14, z = −12, $t_{(67)}$ = 3.40, $p_{svc-FWE}$ = .010) [89]. Further detailed results can be found in the original publication [89]. The association between the alcohol ApB and NAcc PIT effect did not differ between relapsers and abstainers, either using per-protocol or intention-to-treat analysis ($p_{svc-FWE}$ ≥ .156) [89].

### 3.3 CBM training effects

Patients who underwent the CBM training showed a distinct change of the alcohol ApB in comparison to those who underwent the placebo training, as indicated by an interaction between training condition and assessment time on aAAT D-diff score (estimate = -0.21, t = -2.20, p = .03) in the expected direction, i.e., bias decreased after the CBM training and increased after the placebo training (Figure 7) [90]. However, post-hoc analyses suggest that the ApB did not change significantly in either training group (CBM training group: estimate = -0.08, t = -1.30, p = .20; placebo group: estimate = 0.13, t = 1.78, p = .08) [90], which did not support an impact of CBM training on reducing alcohol ApB in a strict interpretation manner.
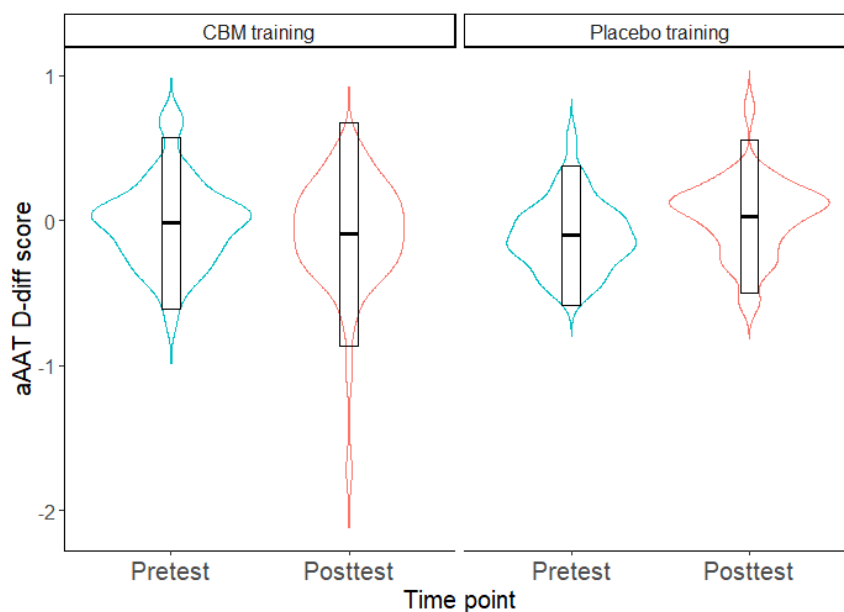


***Figure 7. Alcohol approach bias (ApB) (i.e., D-diff score) in alcohol-dependent patients as a function of training condition and assessment time.***

*There was no significant change in ApB following CBM training or placebo training. From Chen et al., 2022 [90].*

With respect to the impact of training on the non-drug-related PIT, the nonsignificant interaction (Pavlovian CS value * training condition * assessment time: estimate =

0.006, z = 0.55, p = .58) indicates no difference between the two training conditions in PIT change [90]. Exploratory analyses indicated that patients' non-drug-related PIT effect decreased following both the CBM training (Pavlovian CS value × assessment time: estimate = -0.04, z = -5.41, p < .001) and the placebo training (Pavlovian CS value × assessment time: estimate = -0.04, z = -5.01, p < .001) [90]. A null finding was also observed for the training on drug-related PIT (beverage type × training condition × assessment time: estimate = 0.02, z = 0.58, p = .56) [90]. The drug-related PIT effect did not change significantly in either training group.

The exploratory analysis regarding the relapse status in the six-month follow-up showed nonsignificant difference in relapse risk between the two training groups with either per-protocol or intention-to-treat analysis (p ≥ .17) [90]. Further exploratory analyses examined if prospective relapsers and abstainers differed in the alcohol ApB or PIT effects. Result indicated that prospective relapsers showed a greater alcohol ApB than abstainers, especially at the posttest aAAT (estimate = 0.19, t = 2.41, p = .02) [90]. Similarly, relapsers showed a stronger non-drug-related PIT effect than abstainers across two assessment times (estimate = -0.05, z = -3.92, p < .001) [90]. The two groups did not differ significantly regarding drug-related PIT. Further detailed results can be found in the original publication [90].

Internal consistency analyses were additionally conducted for both the aAAT and PIT tasks. Followed Cousijn et al. [103], Cronbach's α was calculated using each approach bias score per picture stimulus in the aAAT, resulting in Cronbach's α values ranging from 0.51 to 0.71 (alcohol stimuli at pretest aAAT: 7 items, Cronbach's α = 0.51; soft drink stimuli at pretest aAAT: 7 items, Cronbach's α = 0.61; alcohol stimuli at posttest aAAT: 7 items, Cronbach's α = 0.52; soft drink stimuli at posttest aAAT: 7 items, Cronbach's α = 0.71), which are comparable to similar studies assessing implicit approach bias [95, 103, 104]. Regarding PIT tasks, split-half reliability analyses showed high correlations between individual non-drug-related PIT effects calculated separately for odd and even trials (r = 0.93 and 0.94 at pretest and

posttest, respectively). These correlations remained consistent across both the CBM training group (r = 0.93 and 0.94 at pretest and posttest respectively) and the placebo training group (r = 0.92 and 0.95 at pretest and posttest respectively). Likewise, high internal consistency was observed for drug-related PIT (r = 0.91 and 0.94 at pretest and posttest, respectively). These results align with the previously demonstrated moderate to high reliability of our PIT tasks [105].

# 4. Discussion

In conclusion, study 1 [88] of this dissertation found an association of the OPRM1 A118G polymorphism with the non-drug-related PIT in patients with AD and healthy subjects. That is, OPRM1 G+ carriers exhibited a stronger behavioral non-drug-related PIT effect than G- carriers. This OPRM1 polymorphism and PIT association was not significantly different between patients and age-matched controls, but was different between prospectively relapsing and abstaining patients, with the significant interaction showed only in relapsing but not in abstaining patients [88]. Study 2 [89] observed an association of the alcohol ApB with behavioral and neural non-drug-related PIT effects in patients with AD, and the association with the behavioral PIT was associated with the severity of AD and trait impulsivity of patients. Study 3 [90] of this dissertation did not observe a significant impact of CBM training on either drug-related or non-drug-related PIT in patients with AD. Findings from the three studies are discussed below.

## 4.1 Association between OPRM1 A118G polymorphism and non-drug-related PIT

Study 1 [88] shows for the first time that a OPRM1 polymorphism that affects receptor affinity [72] is also a modulator of the magnitude of PIT effect in human, and indicates

a difference between prospective relapsers and abstainers in this gene–behavior interaction.

The finding that OPRM1 polymorphism is associated with PIT is in line with Weber et al. [65], in which decreased PIT effects were observed in healthy subjects administrated with opioid receptor antagonist. Compared to Weber et al. [65], study 1 used monetary rewards rather than food, and applied a PIT task that consists of both excitatory and inhibitory Pavlovian CSs. The converging evidence implies an effect of the OPRM1 gene on Pavlovian mechanisms, in both people with AD and non-dependent healthy subjects.

There was no significant difference in this gene-behavior association between patients and age-matched controls in this study. Past research showed contradictory findings with respect to the effect this polymorphism on alcohol-related behavior. It was reported that OPRM1 G+ carriers have a higher subjective feeling of intoxication [106], alcohol craving [107] and an increased risk of a family history of AD [106]. On the other hand, a meta-analysis with 17 studies suggests no association between the OPRM1 genotype with AD [108]. In study 1 of this dissertation, instead of being a marker of AD, the OPRM1 gene-PIT association may have more importance in predicting mechanisms related to relapse in patients with AD. As found in study 1, the OPRM1 gene-PIT association was significant only in prospective relapsers but not abstainers, and a difference in PIT effect between relapsers and abstainers was found only in G+ carriers, not in G- carriers, indicating that OPRM1 may modulate PIT and interact with treatment outcome in some persons with AD [88].

## 4.2 Association between alcohol ApB and non-drug-related PIT

Converging evidence from study 1 [88] and previous findings [69] suggest that both the alcohol ApB and the non-drug-related PIT are modulated by OPRM1 gene. Study 2 [89] provides further direct evidence of a significant association between the alcohol

ApB and behavioral as well as neural non-drug-related PIT in patients with AD. This finding links two well-developed paradigms in AD research, and indicates that the mechanisms of the two paradigms overlap at least partially. The magnitude of the alcohol ApB was positively associated with the right NAcc PIT effect in our study. Previous research suggests the involvement of the right NAcc in alcohol-related CS (i.e., beer flavor) [109], finding in study 2 of this dissertation indicates a role of the right NAcc in Pavlovian conditioning assessed by both paradigms.

The behavioral association was modulated by trait impulsivity, which is in line with the previous findings that impulsive processes may play a role in both the alcohol ApB [87] and the PIT effect [11], hypothetically reflecting shared mechanisms of the alcohol ApB and the PIT effect that are also implicated in impulsive processes. Alcohol cues were used in aAAT, while non-drug-related cues were used in PIT in our study, reflecting that impulsive decision making in individuals with AD can be triggered by both alcohol cues as well as non-alcohol cues [89].

The aAAT-PIT association was associated with the severity of AD in patients (note that the trait impulsivity was positively correlated with the severity of AD in this study; see the original publication [89]). We speculate that more severe form of alcohol intake and dependence could contribute to increasingly fast and hence "impulsive" decision making elicited by conditioned cues, which contributes to the association of the alcohol ApB with the non-drug-related PIT observed in this study. However, impulsive decision making, on the other hand, can also be the cause and not only the consequence of excessive alcohol intake. Longitudinal studies are needed to further elucidate the associations between impulsive decision making, development of AD, and cue reactivity.

### 4.3 CBM training effects

Although previous findings suggest that CBM training can affect other alcohol cue-related behavior [10, 70], and the study 2 of this dissertation provides evidence of an

association between the alcohol ApB targeted by CBM training and the non-drug-related PIT, study 3 of this dissertation did not observe an impact of CBM training on drug-related or non-drug-related PIT effects in people with AD. These findings imply that CBM may not interact directly with the processes evaluated by our PIT paradigms.

The nonsignificant impact of training on drug-related PIT may be due in part to an already existing inhibition effect of the alcohol cues compared to the water cues on instrumental behavior shown in the PIT alcohol versus water cue task prior to training, which may lead to a ceiling effect to further increase the inhibition of alcohol cues. The non-drug-related PIT, although found to be associated with the alcohol ApB in study 2, was not affected by CBM training. Previous research indicates that changes in the alcohol ApB may be related to the CBM training efficacy [12, 85, 86, 110]. Several previous studies that did not find a generalized impact of the CBM training on implicit alcohol association also did not observe a significant change of the alcohol ApB [80, 81, 83]. Study 3 of this dissertation did not support an effect of CBM on decreasing the alcohol ApB in patients with AD, which may be associated with our null findings regarding the PIT tasks. The nonsignificant decrease in the alcohol ApB may be due to the absence of an alcohol ApB prior to the training. Similar to the inhibition effect of alcohol cues in drug-related PIT, this hypothetically "aversive" impact of alcohol cues in our study could be due to the motivation to remain abstinent after detoxification [43]. Together, CBM training in study 3 did not significantly affect the non-drug-related or the drug-related PIT effects nor the alcohol ApB [90]. Given the mixed findings on the efficacy of training, further studies are needed to elucidate the mechanisms of CBM interventions in AD.

## 4.4 Limitations

Several limitations of this dissertation should be stated. First, the non-drug-related PIT task applied in all three studies of this dissertation cannot be categorized as an

outcome-specific or a general PIT task, because monetary outcomes were used as both the instrumental outcomes and the Pavlovian USs, albeit with different values. Future studies applying a PIT task that can disentangle out-specific and general PIT are needed. Second, in all three studies, there are a larger number of patients who had missing relapse information (n = 41/49/38 in study 1/2/3 respectively), which leads to a relatively small sample size for the analyses involving future relapse. In study 1, the sample size of alcohol-dependent G+ carriers who relapsed versus who abstained in follow-up was 16 versus 14 respectively, requiring replication studies with larger sample sizes. In study 2, group differences between relapsers and abstainers did not reach significance when excluding those with missing relapse information, which was probably due to insufficient statistical power. Similarly, the null effect of CBM training in study 3 on reducing the relapse risk may be explained by a lack of statistical power, as the sample sizes in past studies were much larger [10, 12, 74, 76]. Third, the aAAT used in this dissertation, as well as in prior research using similar implicit measures [95, 103, 104], displayed relatively low internal reliability. The limited internal reliability may contribute to the inconsistent results observed across studies using the aAAT. It is critical to develop measures of approach bias with improved internal reliability in future research [103]. Fourth, causal interpretations cannot be derived from association findings (e.g., the alcohol ApB - PIT association observed in study 2), longitudinal research is warranted to further illustrate potentially causal relationships and their underlying mechanisms.

## 5. Conclusion

In conclusion, this dissertation presents evidence that non-drug-related PIT effect is modulated by the OPRM1 polymorphism in patients with AD and healthy subjects, and this gene-behavior association differed between subsequent relapsers and abstainers [88]. This finding suggests that Pavlovian mechanisms could be a target for therapeutic interventions, and such interventions could be particularly effective in a genetically defined subgroup. Moreover, alcohol approach bias is associated with both the behavioral and neural non-drug-related PIT in patients with AD, and the association with behavioral PIT was related to the severity of AD and trait impulsivity [89]. The cognitive bias modification training targeting on the alcohol approach bias, however, did not significantly influence the non-drug-related or the drug-related PIT effects nor the alcohol approach bias [90]. Therefore, modifying Pavlovian mechanisms should not rely on the modification of the approach bias but explore other interventions, e.g., increasing cognitive inhibition rather than motor approach.

Findings of this dissertation underscore the role of the opioid system in Pavlovian mechanisms in humans that manifests in the PIT effect and may have clinical relevance for the treatment of AD. Specifically, pharmacological modification of opiate receptors (e.g., by antagonists) may be a promising approach. Furthermore, our results indicate an association between impulsive decision making, an alcohol approach bias and alterations in PIT. Future studies should further elucidate the underlying mechanisms of how the neurobiological correlates of impulsivity and their interaction with endorphinergic neurotransmission can affect PIT, which will contribute to a better understanding of the mechanism, mediators, and moderators of cognitive bias modification interventions, and thus may enhance the effectiveness of cognitive bias modification interventions for AD.

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## Statutory Declaration

"I, Ke Chen, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic "Pavlovian-to-instrumental transfer in alcohol dependence: associations with OPRM1 polymorphism, alcohol approach bias, and cognitive bias modification (German translation: Pawlowsch-Instrumentelle Transfer bei Alkoholabhängigkeit: Verbindungen mit OPRM1-Polymorphismus, Alkohol-Annäherungsbias und „Cognitive bias modification")" independently and without the support of third parties, and that I used no other sources and aids than those stated.

All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; http://www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me."

Date: Signature:

## Declaration of Contribution to the Publications

Ke Chen contributed the following to the below listed publications:

Publication 1: Sebold M, Garbusow M, Cerci D, **Chen K**, Sommer C, Huys QJ, Nebe S, Rapp M, Veer IM, Zimmermann US, Smolka MN. Walter H, Heinz A, Friedel E. Association of the OPRM1 A118G polymorphism and Pavlovian-to-instrumental transfer: Clinical relevance for alcohol dependence. Journal of Psychopharmacology. 2021 May; 35(5):566-78.

Contribution (in detail): Ke Chen performed the analysis regarding relapse in the sample of alcohol-dependent patients, and provided suggestions for the interpretation of the result. She also contributed to the revision of the manuscript by providing comments.

Publication 2: **Chen K***, Garbusow M*, Sebold M, Kuitunen-Paul S, Smolka MN, Huys QJ, Zimmermann US, Schlagenhauf F, Heinz A. Alcohol approach bias is associated with both behavioral and neural Pavlovian-to-instrumental transfer effects in alcohol-dependent patients. Biological Psychiatry Global Open Science. 2022 Apr 14.

*Equal contribution.

Contribution (in detail): Prof. Dr. phil. Dr. med. Andreas Heinz, Dr. rer. nat. Maria Garbusow, Dr. rer. nat. Miriam Sebold and Ke Chen were responsible for the conceptualization and design of this publication. Ke Chen performed data evaluation, the behavioral data analyses, the fMRI data first-level and second-level analyses under the guidance of Dr. rer. nat. Maria Garbusow (co-first author of this publication) and Prof. Dr. med. Florian Schlagenhauf. Ke Chen worked on the interpretation of the results with the help from Dr. rer. nat. Maria Garbusow and Prof. Dr. phil. Dr. med. Andreas Heinz. Ke Chen produced all the figures and tables for this publication. In addition, she wrote the initial manuscript and prepared the revision prior to publication with crucial help from Dr. rer. nat. Maria Garbusow. All co-authors gave important comments on the initial manuscript and the revision.

Publication 3: **Chen K**, Garbusow M, Sebold M, Zech HG, Zimmermann U, Heinz A. Automatic Approach Behaviors in Alcohol Dependence: Does a Cognitive Bias Modification Training Affect Pavlovian-to-Instrumental-Transfer Effects? Neuropsychobiology. 2022;81(5):387-402

Contribution (in detail): Prof. Dr. phil. Dr. med. Andreas Heinz was responsible for the study concept and design. Ke Chen performed all the statistical analyses. She also worked on the interpretation of the results with the help from Prof. Dr. phil. Dr. med. Andreas Heinz. She produced all the figures and tables for this publication. In addition, she wrote the initial manuscript and prepared the revisions prior to publication with the help from Prof. Dr. phil. Dr. med. Andreas Heinz. All co-authors provided critical comments on the initial manuscript and the revisions.

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Signature, date, and stamp of the supervising university professor (Herr Prof. Dr. phil. Dr. med. Andreas Heinz)

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Signature of doctoral candidate (Ke Chen)

## Declaration of the contribution of the co-first author

Publication 2: Chen K*, **Garbusow M\***, Sebold M, Kuitunen-Paul S, Smolka MN, Huys QJ, Zimmermann US, Schlagenhauf F, Heinz A. Alcohol approach bias is associated with both behavioral and neural Pavlovian-to-instrumental transfer effects in alcohol-dependent patients. Biological Psychiatry Global Open Science. 2022 Apr 14.

*Equal contribution.

Contribution of Dr. rer. nat. Maria Garbusow (in detail): Prof. Dr. phil. Dr. med. Andreas Heinz, Dr. rer. nat. Maria Garbusow, Dr. rer. nat. Miriam Sebold and Ke Chen were responsible for the conceptualization and design of this publication. Dr. rer. nat. Maria Garbusow guided data evaluation, the behavioral data analyses, the fMRI data first-level and second-level analyses conducted by Ke Chen. Dr. rer. nat. Maria Garbusow worked in cooperation with Ke Chen on the interpretations of the results. In addition, Dr. rer. nat. Maria Garbusow provided crucial intellectual inputs to the initial manuscript and the revision prior to publication.

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Signature of the co-first author (Dr. rer. nat. Maria Garbusow)

# Printing Copies of the Publications

**Association of the OPRM1 A118G polymorphism and Pavlovian-to-instrumental transfer: Clinical relevance for alcohol dependence.**

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| 3 | JAMA Neurology | 21,007 | 29.907 | 0.05043 |
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| 17 | Brain Stimulation | 10,760 | 9.184 | 0.01497 |
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| 38 | Current Neurology and Neuroscience Reports | 5,335 | 6.030 | 0.00686 |
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| 50 | JOURNAL OF PAIN | 13,915 | 5.383 | 0.01264 |
| 51 | BIPOLAR DISORDERS | 6,472 | 5.345 | 0.00541 |
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| 56 | JOURNAL OF THE PERIPHERAL NERVOUS SYSTEM | 2,488 | 5.188 | 0.00249 |
| 57 | JPAD-Journal of Prevention of Alzheimers Disease | 873 | 5.020 | 0.00220 |
| 58 | AMERICAN JOURNAL OF NEURORADIOLOGY | 29,851 | 4.966 | 0.02313 |
| 59 | NEUROREHABILITATION AND NEURAL REPAIR | 7,555 | 4.895 | 0.00615 |
| 60 | JOURNAL OF NEUROTRAUMA | 19,581 | 4.869 | 0.01717 |
| 61 | DEVELOPMENTAL MEDICINE AND CHILD NEUROLOGY | 17,461 | 4.864 | 0.01202 |
| 62 | CLINICAL NEUROPHYSIOLOGY | 25,162 | 4.861 | 0.01585 |
| 63 | SLEEP MEDICINE | 17,340 | 4.842 | 0.01628 |
| 64 | Multiple Sclerosis and Related Disorders | 7,487 | 4.808 | 0.01284 |

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|------|-------------------|-------------|----------------------|-------------|
| 65 | Journal of Neurogastroenterology and Motility | 2,606 | 4.725 | 0.00349 |
| 66 | Journal of Neuromuscular Diseases | 1,350 | 4.693 | 0.00275 |
| 67 | Journal of Neurologic Physical Therapy | 1,571 | 4.655 | 0.00153 |
| 68 | CNS SPECTRUMS | 3,558 | 4.604 | 0.00290 |
| 69 | JOURNAL OF NEURORADIOLOGY | 1,789 | 4.600 | 0.00198 |
| 70 | JOURNAL OF PSYCHOPHARMACOLOGY | 9,324 | 4.562 | 0.00949 |
| 71 | JOURNAL OF THE NEUROLOGICAL SCIENCES | 23,403 | 4.553 | 0.01804 |
| 72 | Movement Disorders Clinical Practice | 2,088 | 4.514 | 0.00399 |
| 73 | JOURNAL OF NEURO-ONCOLOGY | 16,733 | 4.506 | 0.01490 |
| 74 | Neurology and Therapy | 917 | 4.446 | 0.00196 |
| 75 | JOURNAL OF NEURO-OPHTHALMOLOGY | 2,897 | 4.415 | 0.00286 |
| 76 | PARKINSONISM & RELATED DISORDERS | 13,677 | 4.402 | 0.01510 |
| 77 | Pain Physician | 6,200 | 4.396 | 0.00630 |
| 78 | CLINICAL NEUROPSYCHOLOGIST | 6,059 | 4.373 | 0.00451 |
| 79 | Neurosurgical Focus | 10,856 | 4.332 | 0.01139 |
| 80 | Journal of Clinical Sleep Medicine | 11,389 | 4.324 | 0.01245 |
| 81 | REVUE NEUROLOGIQUE | 3,295 | 4.313 | 0.00325 |
| 82 | Spine Journal | 13,705 | 4.297 | 0.01567 |
| 83 | Expert Review of Neurotherapeutics | 5,795 | 4.287 | 0.00476 |
| 83 | BRAIN TOPOGRAPHY | 3,529 | 4.275 | 0.00351 |
| 85 | Journal of Movement Disorders | 709 | 4.229 | 0.00126 |
| 86 | PEDIATRIC NEUROLOGY | 7,875 | 4.210 | 0.00711 |
| 87 | Sleep Health | 2,865 | 4.207 | 0.00513 |

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*Original Paper*

# Association of the *OPRM1* A118G polymorphism and Pavlovian-to-instrumental transfer: Clinical relevance for alcohol dependence

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## Abstract

**Background:** Pavlovian-to-instrumental transfer (PIT) quantifies the extent to which a stimulus that has been associated with reward or punishment alters operant behaviour. In alcohol dependence (AD), the PIT effect serves as a paradigmatic model of cue-induced relapse. Preclinical studies have suggested a critical role of the opioid system in modulating Pavlovian–instrumental interactions. The A118G polymorphism of the *OPRM1* gene affects opioid receptor availability and function. Furthermore, this polymorphism interacts with cue-induced approach behaviour and is a potential biomarker for pharmacological treatment response in AD. In this study, we tested whether the *OPRM1* polymorphism is associated with the PIT effect and relapse in AD.
**Methods:** Using a PIT task, we examined three independent samples: young healthy subjects ($N=161$), detoxified alcohol-dependent patients ($N=186$) and age-matched healthy controls ($N=105$). We used data from a larger study designed to assess the role of learning mechanisms in the development and maintenance of AD. Subjects were genotyped for the A118G (rs1799971) polymorphism of the *OPRM1* gene. Relapse was assessed after three months.
**Results:** In all three samples, participants with the minor *OPRM1* G-Allele (G+ carriers) showed increased expression of the PIT effect in the absence of learning differences. Relapse was not associated with the *OPRM1* polymorphism. Instead, G+ carriers displaying increased PIT effects were particularly prone to relapse.
**Conclusion:** These results support a role for the opioid system in incentive salience motivation. Furthermore, they inform a mechanistic model of aberrant salience processing and are in line with the pharmacological potential of opioid receptor targets in the treatment of AD.

## Keywords
Alcohol dependence, learning, decision making, *OPRM1* A118G, opioid system

## Introduction

Contextual stimuli are important modulators in the way we learn and can promote specific behaviours. One mechanism underlying contextual learning is the so-called Pavlovian-to-instrumental transfer (PIT). The PIT effect capture the influence of Pavlovian conditioned stimuli (CSs) on instrumental behaviour, with appetitive Pavlovian stimuli specifically promoting approach and reducing withdrawal, and aversive Pavlovian stimuli promoting withdrawal and reducing approach (Huys et al., 2011), thus reflecting a powerful mechanism affecting behavioural choices across humans (Talmi et al., 2008) and animals (Dickinson et al., 2000; O'Connor et al., 2010). Moreover, the PIT effect has been used as a quantification of incentive salience attribution, that is, the extent to which formerly neutral cues become attractive, themselves desired, and therefore 'wanted' (Huys et al., 2014; Meyer et al., 2012).

Crucially, incentive salience attribution is one prominent mechanism underlying several disorders of compulsivity, such as alcohol dependence (AD; Corbit and Janak, 2007) and other addictive disorders (LeBlanc et al., 2012). Also, interindividual differences in PIT have been associated with addiction vulnerability and maintenance. For instance, preclinical work suggests an association between the magnitude of PIT and addictive behaviour, such as

compulsive alcohol drinking (Barker et al., 2012; Corbit and Janak, 2007). Preclinical studies have also consistently reported that non-drug-related (e.g. food or sucrose reward) CSs lead to increased

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responding during PIT in addicted animals (LeBlanc et al., 2013; Ostlund et al., 2014; Saddoris et al., 2011). Moreover, we have recently shown that the PIT effect in humans serves as a vulnerability marker for the development and maintenance of AD (Garbusow et al., 2014, 2019; Schad et al., 2019a; but see van Timmeren et al., 2020). The behavioural and neural correlates of PIT have been associated with relapse in AD (Garbusow et al., 2016; Sekutowicz et al., 2019; Sommer et al., 2020) and were predictive of future drinking behaviour in adolescents (Sekutowicz et al., 2019).

Although contemporary theories emphasise the involvement of the dopaminergic system in incentive salience, recent findings suggest the opioid system as another important player (Pecina and Berridge, 2013; van Steenbergen et al., 2019). The opioid system has been primarily linked to hedonic features of a reward, also termed 'liking' as opposed to 'wanting', which reflects the motivational properties to promote a certain behaviour rather than its hedonic value. However, preclinical studies have shown that stimulation of the μ-opioid (MOP) system in the nucleus accumbens directly enhances incentive motivation (or 'wanting') for reward (Pecina and Berridge, 2013). In animals, experimental manipulation of the opioid system can mediate the influence of reward-guided and stimulus-guided decisions on choice (Laurent et al., 2012), increase motivation for different reward types (Mahler and Berridge, 2012) and mediate the motivating influence of cue-triggered reward expectations (Lichtenberg and Wassum, 2017). In humans, evidence for a functional role of the opioid system in mediating 'wanting' mainly stems from pharmacological challenges. For instance, MOP agonists and antagonists selectively enhance and decrease processing efficiency in a reward task (Eikemo et al., 2017) and increase and decrease the motivation to view positive valenced stimuli, respectively (Chelnokova et al., 2014). Likewise, opioid receptor antagonists reduced physical effort produced to obtain reward and increased negative facial reactions during reward anticipation (Korb et al., 2019).

In humans, the role of the opioid system in mediating the PIT effect as one further quantification of incentive salience (or 'wanting') is less clear. The opioid receptor antagonist naltrexone could decrease alcohol cue-induced activation of the ventral striatum (Myrick et al., 2008) and cue-induced impulsive responding (Mitchell et al., 2007). However, to date, there are only two studies investigating the role of the opioid system in mediating human PIT-like effects (Weber et al., 2016; Wiers et al., 2009), reporting reduced PIT after blockade of the MOP receptor (naltrexone) in healthy humans (Weber et al., 2016) and increased automatic approach tendencies in G+ carriers of the OPRM1 polymorphism to alcohol-associated stimuli (Wiers et al., 2009). The overarching aim of our study was to further elucidate the role of the human opioid system in mediating the PIT effect in both healthy subjects and those with AD.

A common mechanism of quantifying interindividual differences in the human opioid system is the determination of the MOP receptor single nucleotide polymorphism (*OPRM1*). The *OPRM1* gene codes for the MOP receptor, an inhibitory G-protein coupled receptor that binds endogenous opioid peptides such as β-endorphin and enkephalins as well as exogenous opioids such as morphine and heroin (Burns et al., 2019; Kieffer and Gaveriaux-Ruff, 2002). Opioid receptors are distributed widely in the human brain and modulate brain function at all levels of neural integration, including the mesolimbic system as part of the brain's reward pathway.

Human studies investigating the *OPRM1* polymorphism have suggested a crucial role of this single nucleotide polymorphism (SNP) in AD, treatment response and automatic approach biases to conditioned cues (Chamorro et al., 2012; Filbey et al., 2008; Ray and Hutchison, 2004; Wiers et al., 2009). The A118G (rs1799971) polymorphism of the *OPRM1* gene alters the function of MOP receptors, such that the G variant binds beta-endorphin three times more strongly than the A variant, potentially also affecting receptor availability (Heinz et al., 2005). We henceforth refer to the minor *OPRM1* G-allele carriers as G+ carriers. G+ carriers were shown to report higher subjective alcohol-associated feelings of intoxication (Ray and Hutchison, 2004) and craving (Van Den Wildenberg et al., 2007) and have a higher risk for positive family history (Ray and Hutchison, 2004). However, conflicting results stem from large genome-wide association studies (GWAS) and candidate gene studies (Kong et al., 2017), which could not replicate an association between AD and *OPRM1* genotype, corresponding with a recent report on converging evidence against an association between the *OPRM1* A118G polymorphism and alcohol consumption and sedation (Sloan et al., 2018).

The analyses presented here aimed to answer three questions. (1) Is the *OPRM1* polymorphism associated with the PIT effect across three independent cohorts? (2) Is the association between the PIT effect and the *OPRM1* polymorphism different in patients with AD compared to healthy controls (HCs)? (3) Is the association between the PIT effect and the *OPRM1* polymorphism relevant for treatment outcome in the way that it is different in prospectively relapsing and abstinent patients with AD?

## Methods

### Subjects

All subjects were recruited between 2012 and 2018 as part of a larger study (LeAD study, ClinicalTrials.gov identifiers: NCT01744834, NCT01679145 and NCT02615977) investigating behavioural, genetic and neuroimaging alterations associated with reward-based learning as (a) predictors for the development of AD in a sample of young 18-year-old male subjects recruited from the national registry and (b) the maintenance of AD with respect to relapse and drinking behaviour in a sample of patients suffering from AD and an age, education and sex-matched HC sample (for previously published results of our sample, see, amongst others, Garbusow et al., 2014, 2016, 2019). Thus, this study comprised two independent HC samples that significantly differed with regards to several sociodemographic variables (see Supplemental Table S2 for between-group differences). As previous analyses (Sebold et al., 2016) indicated substantial differences in PIT effects between these cohorts, we did not merge both control samples but instead analysed the influence of the *OPRM1* polymorphism on the PIT effect separately in these two control cohorts (analysis 1).

The assessed samples were a subsample of the three cohorts mentioned above for which genetic data were available: 18-year-old male subjects (*N* = 161, henceforth referred to as young controls), recently detoxified patients with AD (*N* = 186) and age-matched HCs (*N* = 105, henceforth referred to as middle-aged controls). For a precise overview of the selection procedures, see Supplemental Information 1 and Supplemental Figure S1.

For a complete description of exclusion criteria, see Garbusow et al. (2016). Briefly, all subjects were free from psychotropic medication, had no history of substance dependence (DSM-IV, except from AD in the AD group) or current substance use (DSM-IV, except for nicotine use), no other current DSM-IV axis 1 psychiatric or neurological disorders and no borderline personality disorder as assessed by the computer-based Composite International Diagnostic Interview (Jacobi et al., 2013; Wittchen, 1997). Participants' demographic and clinical characteristics are outlined in Table 1. Participants gave written informed consent before study inclusion. The study was approved by the local ethics committees of the Technical University of Dresden and Charité Universitätsmedizin Berlin.

To define relapse across patients with AD, a three-month follow-up was performed using the Time Line Follow Back procedure (Sobell and Sobell, 1992). Relapse was defined as at least five standard drinks (e.g. one standard drink = 0.33 L beer) on one occasion for male participants and at least four standard drinks for female participants according to the World Health Organization (WHO; 2014) definition of high-risk consumption. A total of 51 patients were classified as relapsers (of whom 37 were G− and 14 were G+ carriers), whereas 94 patients were classified as abstainers (of whom 78 were G− and 16 were G+ carriers). The remaining 41 patients could not be contacted during the follow-up period.

## Task

We used a PIT task as previously described (Garbusow et al., 2014, 2016; Sommer et al., 2017). The task consisted of four phases (of which the first three phases are depicted in Figure 1): (a) instrumental learning, (b) Pavlovian learning, (c) PIT and (d) forced choice task followed by a rating scale of the stimuli.

*Instrumental learning.* Subjects had to learn to collect 'Go' shells and leave 'No-Go' shells by repeatedly pressing a button while receiving probabilistic feedback. In order to collect a shell, subjects had to move a red dot onto the selected shell by repeated button presses within two seconds. We instructed the subjects to maximise their profit. For this, they should use the probabilistic feedback to find out via trial and error what is a 'good shell', which in 'most cases' lead to wins when collected, and leave 'bad shells', which in 'most cases' lead to wins when not collected. Each button press moved the red dot a fraction of the way towards the shell. To collect a 'Go' shell correctly, subjects had to press the button five or more times, and to leave a 'No-Go' shell, subjects had to perform between zero and four button presses. The subjects did not know about the number of button presses, but we instructed them to press the button as often as possible to collect a shell to maximise instrumental performance. Correct responses were rewarded with 20 cents in 80% of trials and punished with a loss of 20 cents in 20% of trials, and for wrong responses it was vice versa (see Figure 1.1 for 'Go' and 'No-Go' trials). The shell set consisted of six different shells (three 'Go' shells and three 'No-Go' shells).

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 after a minimum of 60 trials).

*Pavlovian learning.* Pavlovian learning consisted of 80 trials in which compound visual and auditory stimuli (CS) were predictive of distinct monetary rewards or punishments (unconditioned stimulus (US); Figure 1.2). Each trial began with a three-second presentation of a compound CS (fractal picture and tone) which was then followed by a three-second presentation of two fixation crosses (on the left and right side of the screen). Then, the US (monetary reward or punishment) was presented for threeseconds on the side where the CS had not been presented. Subjects were instructed to view the CS–US pairings passively and to memorise these associations. The set of CS consisted of six stimuli of which each was paired with positive (+2€/+1€), neutral (0€) or negative (−1€/−2€) outcomes, henceforth referred to as 'money CS'.

*PIT phase.* Subjects performed 162 trials of the instrumental task again, this time without outcome feedback. Subjects were instructed that their choices still counted towards the final monetary outcome (so-called nominal extinction). The instrumental stimuli superimposed one of the money CSs presented during Pavlovian training (Figure 1.3), or one of four beverage stimuli (results not presented here, but see (Schad et al., 2019a; Sekutowicz et al., 2019; Sommer et al., 2017, 2020). Each instrumental stimulus (three 'Go' shells and three 'No-Go' shells) was combined with each money CS (fractal stimulus previously associated with either of −2€, −1€, 0€, +1€, +2€) for three times, resulting in 90 trials, which were of primary interest for this study. Each trial lasted 3.6 seconds.

*Forced-choice task.* This part of the task aimed to verify the acquisition of Pavlovian learning. In each trial, subjects had to choose between two sequentially presented compound money CSs from the Pavlovian training, each presented for two seconds. All possible compound CS pairings were presented three times in an interleaved randomised order.

*Pleasantness ratings.* After the task, subjects were asked to rate the pleasantness of the CSs (fractals and shells) from the Pavlovian learning phase and the instrumental learning phase on a Likert scale from 1 to 7 on the screen.

## Genotyping

To genotype our sample, DNA was extracted semi-automatically with a Chemagen Magnetic Separation Module (PerkinElmer, Waltham, MA) from whole blood. All samples were genotyped with the Illumina Infinium Psych Array Bead Chip (Illumina, San Diego, CA). We assessed rs1799971, a SNP that is an A-to-G substitution (A118G), resulting in a functional amino acid substitution (Asn40Asp; Hartwell et al., 2020).

Because of the limited sample size, G-allele carriers (AG and GG) were grouped together. This approach is in keeping with precedent in the field (Persson et al., 2019; Way et al., 2009).

## Behavioural analyses

Data were analysed using the R programming language (R Foundation for Statistical Computing, Vienna, Austria). Demographic, clinical and neuropsychological comparisons between G+ and G− *OPRM1* carriers were examined using chi-square and *t*-tests (Table 1).

Table 1. Demographic, clinical and neuropsychological characteristics for all cohorts; young controls, middle-aged controls and patients with AD, split by OPRM1 polymorphism.

| Cohort | Alcohol-dependent patients (N = 186) | | | Middle-aged controls (N=105) | | | Young controls (N=161) | | |
|---|---|---|---|---|---|---|---|---|---|
| OPRM1 | G− (N=154) | G+ (N=32) | Test statistics | G− (N=79) | G+ (N=26) | Test statistics | G− (N=120) | G+ (N=41) | Test statistics |
| | M (SD) | M (SD) | | M (SD) | M (SD) | | M (SD) | M (SD) | |
| *Demographic variables* | | | | | | | | | |
| Age | 46.17 (10.49) | 47.09 (11.03) | $t=-0.42, p=0.67$ | 43.64 (11.1) | 46.04 (10.5) | $t=-0.99, p=0.33$ | 18.36 (0.2) | 18.37 (0.2) | $t=-0.33, p=0.74$ |
| Sex (% male) | 84% | 81% | $\chi^2=0.2, p=0.67$ | 83% | 81% | $\chi^2=0.11, p=0.75$ | 100% | 100% | NA |
| Years of education | 14.97 (4.07) | 14.29 (2.52) | $t=-1.3, p=0.22$ | 15.98 (3.22) | 15.37 (3.32) | $t=-0.79, p=0.43$ | 11.7 (0.75) | 11.51 (1.34) | $t=0.85, p=0.4$ |
| *Clinical characteristics* | | | | | | | | | |
| Anxiety[a] | 4.37 (3.41) | 4.8 (3.37) | $t=-0.63, p=0.53$ | 2.32 (2.04) | 1.88 (2.21) | $t=0.89, p=0.38$ | 2.31 (2.19) | 2.92 (2.89) | $t=-1.2, p=0.23$ |
| Depression[b] | 3.5 (3.7) | 4.33 (3.33) | $t=-1.23, p=0.23$ | 1.48 (1.98) | 1.85 (2.62) | $t=-0.65, p=0.52$ | 1.67 (1.75) | 1.8 (2) | $t=-0.39, p=0.7$ |
| Craving[c] | 12.76 (7.94) | 12.52 (8.57) | $t=0.14, p=0.88$ | 2.4 (2.41) | 3.68 (4.03) | $t=-1.31, p=0.2$ | 3.47 (3.01) | 4.65 (3.48) | $t=-1.91, p=0.7$ |
| Impulsivity[d] | 31.63 (6.67) | 31.84 (5.57) | $t=-0.19, p=0.85$ | 29.32 (5.4) | 28.84 (5.3) | $t=0.39, p=0.69$ | 29.99 (5.15) | 31.82 (4.56) | $t=-2.13, p=0.04$ |
| *Neuropsychological testing* | | | | | | | | | |
| Cognitive speed[e] | 9.27 (2.76) | 9.48 (2.78) | $t=-0.39, p=0.7$ | 10.58 (2.82) | 10.92 (3.78) | $t=-0.42, p=0.68$ | 11.5 (2.2) | 11 (2.59) | $t=1.11, p=0.27$ |
| Working memory[f] | 6.5 (1.93) | 6.77 (1.61) | $t=-0.82, p=0.41$ | 7.41 (1.95) | 7.62 (2.43) | $t=-0.40, p=0.69$ | 8.04 (1.95) | 8.02 (2.21) | $t=0.04, p=0.96$ |

The variables were assessed by means of [a]the anxiety and [b]depression subscale of the Hospital Depression and Anxiety Questionnaire; [c]the Obsessive Compulsive Drinking Scale, [d]the Barratt Impulsiveness Scale and the following subtest of the Wechsler Intelligence Test: [e]the Digit Symbol Substitution Test and [f]the Digit Span Backwards Test.
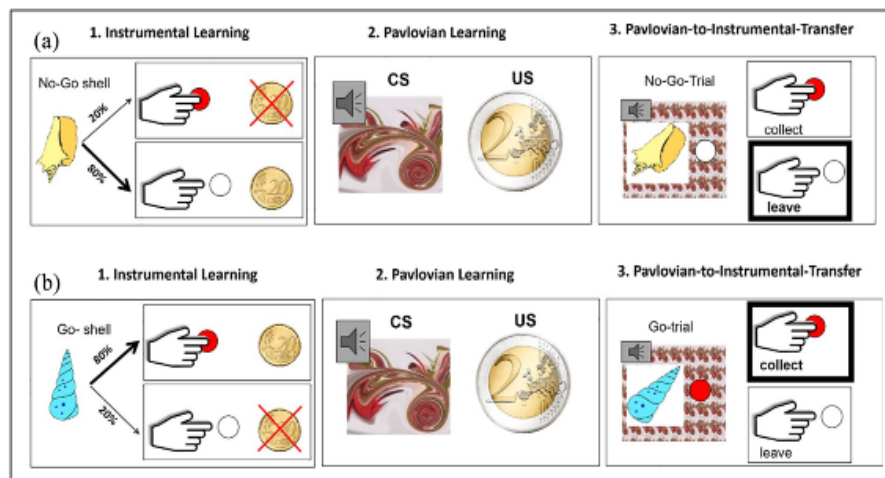AD: alcohol dependence.

**Figure 1.** Phases 1–3 of the paradigm for (a) the 'No-Go' trial and (b) the 'Go' trial. 1. Instrumental learning: The subject's task was to move a dot towards the stimulus by repeated button presses in order to collect it or to do nothing within two seconds. These two instrumental choices resulted in monetary wins or losses, presented immediately after each trial via a picture of a 20€ coin for 1.5 seconds. Feedback was probabilistic. A 'Go' shell was rewarded in 80% and punished in 20% of trials if collected and vice versa if not collected. A 'No-Go' shell was rewarded in 80% and punished in 20% of the trials if not collected and vice versa if collected. 2. Pavlovian learning: Neutral fractal and audio stimulus compounds (CS) are repeatedly paired with monetary outcomes (US: e.g. here a 2€ coin). 3. Pavlovian-to-instrumental transfer (PIT) phase: Subjects performed the instrumental task in nominal extinction, that is, no explicit monetary outcomes were presented (A. leave button to not collect a 'No-Go' shell and B. press button to collect 'Go' shell superimposed on the audiovisual Pavlovian stimulus; here: the Pavlovian stimulus previously paired with 2€ and the respective tone pitch).

Analysis of the PIT phase was of primary interest, but we analysed all other phases as well (see Supplemental Information 6, Supplemental Information 7, Supplemental Information 8 and Supplemental Information 10). In the PIT phase, the PIT effect reflects the interaction between the valence of the background stimulus and the accuracy of the foreground instrumental action. We were specifically interested whether the *OPRM1* genotype covaried with PIT effect, that is, the way that positive and negative stimuli influence 'Go' and 'No-Go' actions. More precisely, we asked whether the genetic phenotype would interact with the extent to which a positive stimulus facilitates 'Go' responses but impairs 'No-Go' responses and, vice versa, a negative stimulus facilitates 'No-Go' responses but impairs 'Go' responses.

As outlined in the introduction, the analyses presented here aimed to elucidate: (a) the association between the *OPRM1* polymorphism and the PIT effect, (b) the clinical relevance of this association for AD and (c) the relevance of this association for treatment outcome. Across these different analyses, we coded a participant's accuracy of the PIT phase as correct (1) if a 'Go' shell was collected or a 'No-Go' shell was left, and as false (0) if a 'No-Go' shell was collected or a 'Go' shell was left. We used a binomial mixed effect regression as implemented in the lme4 package (Bates et al., 2015). We regressed the participant's accuracy (correct or incorrect) on Pavlovian valence (negative, neutral or positive, dummy coded with neutral as the reference), instrumental action ('Go' or 'No-Go', coded as 0.5 and −0.5) and *OPRM1* polymorphism (G− or G+, coded as −0.5 and +0.5) and tested for interaction between these factors. Subjects were added as random effects (random intercept model). We performed model

comparisons to ensure that this model was the best-fitting model across subjects (see Supplmental Information 2).

*Analysis 1: Association between the PIT effect and the* OPRM1 *polymorphism across cohorts.* To test whether the *OPRM1* polymorphism was associated with the PIT effect in all three cohorts, we performed the above-described analysis for all three cohorts separately (Supplemental Figure S1).

*Analysis 2: Alcohol-related group differences for the association between the PIT effect and the* OPRM1 *polymorphism.* To test whether the interaction between the PIT effect and the *OPRM1* polymorphism was significantly different between HCs and patients with AD, we performed the above-described regression model (see analysis 1) but additionally added group (HC or AD, coded as 0.5 and −0.5) as an additional fixed effect and allowed interaction with all predictors (Supplemental Figure S1). For this analysis, we only included patients with AD and middle-aged control subjects (who were initially sampled as a comparison group of patients with AD). Both groups profoundly differed across several socio-economic and clinical variables (Supplemental Table S2). Increased depression, anxiety, craving and impulsivity as well as reduced cognitive speed and working memory are features instead of confounders of AD. Thus, as suggested by Miller and Chapman (2001), we did not control for these variables. Years of education was the only variable we added as a covariate because groups significantly differed in these variables despite our efforts of matching.

**Table 2.** Results of analysis 1. Effects of the regression analysis from the PIT part for all three cohorts.

| Group | Alcohol-dependent patients (*N*=186) | | Middle-aged controls (*N*=105) | | Young controls (*N*=161) | |
|---|---|---|---|---|---|---|
| | $\chi^2$ | *p*-Value | $\chi^2$ | *p*-Value | $\chi^2$ | *p*-Value |
| Pavlovian CS valence | 11.723 | **0.003** | 5.599 | 0.061 | 15.105 | **0.001** |
| Instrumental behavior | 7.057 | **0.008** | 13.108 | **0.0003** | 0.159 | 0.690 |
| *OPRM1* polymorphism | 0.002 | 0.963 | 0.046 | 0.831 | 0 | 0.994 |
| Pavlovian valence × instrumental behavior | 2074.63 | **<0.0001** | 912.67 | **<0.0001** | 365.68 | **<0.0001** |
| Pavlovian valence × *OPRM1* polymorphism | 0.224 | 0.894 | 0.074 | 0.964 | 0.629 | 0.730 |
| Instrumental behavior × *OPRM1* polymorphism | 13.917 | **0.0002** | 18.930 | **<0.0001** | 7.757 | **0.005** |
| Pavlovian valence × instrumental behavior × *OPRM1* polymorphism | 12.723 | **0.002** | 9.027 | **0.011** | 20.691 | **<0.0001** |

All interaction effects with the *OPRM1* polymorphism in the young control cohort remained significant after controlling for self-reports of impulsivity, which was significantly different between G+ and G− carriers in this cohort (see Table 1). Statistically significant values are shown in bold.
PIT: Pavlovian-to-instrumental transfer; CS: conditioned stimulus.

*Analysis 3: Relapse-related group differences for the association between the PIT effect and the* OPRM1 *polymorphism.* To test whether the interaction between the *OPRM1* polymorphism and the PIT effect was significantly different between patients with AD who relapsed and those who remained abstinent, we performed the above described regression analysis (see analysis 1) but added relapse (relapsers or abstainers, coded as 0.5 and −0.5) as an additional fixed factor and allowed interaction with all predictors. For this analysis, we only included patients with AD for whom relapse data were available (*n*=145; Supplemental Figure S1). Relapsing patients did not differ from abstaining patients in any demographic or clinical variables, except for craving (where relapsing patients had significantly higher OCDS scores (Anton et al., 1995; Mann and Ackermann, 2000) than abstaining patients (*t*=−2.66, *p*=0.01). Thus, we added craving as a covariate of no interest in this analysis.

*Post hoc analyses*

For analyses 2 and 3, we were particularly interested in how the PIT effect was modulated by the *OPRM1* polymorphism and whether this depended on group, respectively. Thus, in our post hoc analyses, we focused on these contrasts (analysis 2: G+ vs. G− carriers/HCs vs. ADs; analysis 3: G+ vs. G− carriers/relapsers vs. abstainers) and considered effects as significant when they survived Bonferroni correction for four comparisons (*p*<0.01).

**Results**

*Genotyping*

Genotyping resulted in 353 participants homozygous for the major A allele, 89 participants with the AG combination and 10 participants homozygous for the G allele. *OPRM1* genotype distribution did not significantly differ from Hardy–Weinberg equilibrium ($\chi^2_{(df=1)}$=2.31, *p*=0.13).

Demographic, clinical and neuropsychological comparisons between G+ and G− carriers in all three cohorts indicated no group differences (Table 1), except from increased self-reports of impulsivity assessed via BIS-15 (Meule, 2011) in G+ carriers compared to G− carriers in young healthy adults. Moreover, we

found no evidence for a functional association between the *OPRM1* polymorphism and AD. Descriptively, there were proportionally more G+ carriers among the HCs compared to the AD group – from the literature we would have expected the reverse results – although this difference was formally not statistical significant ($\chi^2_{(df=1)}$=3.62, *p*=0.06). Also, we found no evidence for a functional association between the *OPRM1* polymorphism and relapse ($\chi^2_{(df=1)}$=1.60, *p*=0.21).

*Behavioural data*

*Analysis 1: Association between the PIT effect and the* OPRM1 *polymorphism across cohorts.* The first aim of this study was to test whether the *OPRM1* polymorphism influences the PIT effect across three independent cohorts. In all three cohorts we found a significant PIT effect, that is, the interaction between Pavlovian valence (negative, neutral or positive) and instrumental action ('Go' or 'No-Go'; Table 2), indicating that positive stimuli facilitated 'Go' responses but impaired 'No-Go' responses, whereas negative stimuli facilitated 'No-Go' responses but impaired 'Go' responses.

In all groups, respectively, we found no interaction between Pavlovian valence and *OPRM1* polymorphism. However, the *OPRM1* polymorphism interacted with instrumental action (Table 2). Crucially, we found a three-way interaction between Pavlovian valence, instrumental action and *OPRM1* polymorphism in all cohorts. This result suggests that the *OPRM1* polymorphism strongly interacts with the PIT effect in all three independent cohorts. In fact the PIT effect was significantly higher in G+ carriers compared to G− carriers (Figure 2 and Table 2).

To rule out that our PIT-related *OPRM1* effect was simply due to the fact that G+ carriers showed stronger cue-induced modulation of liking, we further performed analyses of the rating data of the Pavlovian stimuli (pleasantness ratings; Supplemental Information 10). To this end, we first tested whether the *OPRM1* polymorphism was associated with ratings of the stimuli, depending on the Pavlovian valence. In all cohorts, the *OPRM1* polymorphism did not interact with Pavlovian valence (Supplemental Information 10). Moreover, adding the rating data as an additional covariate in our PIT analyses, all interaction between the
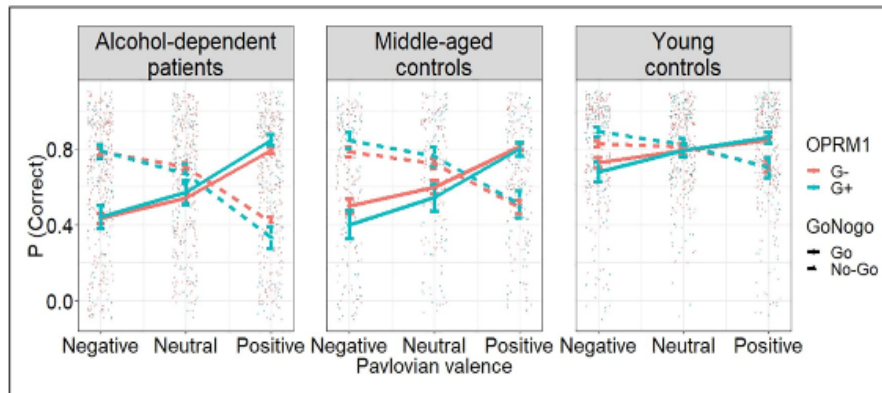
**Figure 2.** Results of the PIT phase as a function of group (patients with alcohol dependence (AD), middle-aged controls and young controls) and *OPRM1* polymorphism. Each panel shows the PIT effect in the respective group, that is, there was a significant influence of Pavlovian background valence on instrumental action (accuracy: percent correct choices), here visualised by the slope of the lines. Crucially, in each of the three cohorts, this was steeper in G+ carriers compared to G– carriers, as indicated by the three-way interaction between *OPRM1* polymorphism, Pavlovian valence and instrumental action (analysis 1), that is, in each of the three independent cohorts, the PIT effect was modulated by the *OPRM1* polymorphism. However, this was not different between alcohol-dependent patients and matched middle-aged controls (analysis 2).

**Table 3.** Results of analysis 2. Effects of the regression analysis from the PIT part where we tested whether the interaction between the PIT effect and the *OPRM1* polymorphism was significantly different between patients with AD and HCs.

|  | $\chi^2$ | *p*-Value |
| --- | --- | --- |
| Pavlovian valence | 13.183 | 0.001 |
| Instrumental action | 18.391 | **<0.0001** |
| *OPRM1* polymorphism | 0.007 | 0.933 |
| Group | 2.316 | 0.128 |
| Years of education | 7.651 | 0.006 |
| Pavlovian valence × instrumental action | 2888.726 | **<0.0001** |
| Pavlovian valence × *OPRM1* polymorphism | 0.031 | 0.984 |
| Instrumental action × *OPRM1* polymorphism | 0.374 | 0.540 |
| Pavlovian valence × group | 3.661 | 0.160 |
| Instrumental action × group | 4.187 | 0.041 |
| *OPRM1* polymorphism × group | 0.015 | 0.901 |
| Pavlovian valence × instrumental action × *OPRM1* polymorphism | 16.909 | **<0.0001** |
| Pavlovian valence × instrumental action × group | 22.695 | **<0.0001** |
| Pavlovian valence × *OPRM1* polymorphism × group | 0.257 | 0.880 |
| Instrumental action × *OPRM1* polymorphism × group | 30.727 | **<0.0001** |
| Pavlovian valence × instrumental action × *OPRM1* polymorphism × group | 0.318 | 0.853 |

HC: healthy control.

*OPRM1* polymorphism, Pavlovian valence and instrumental action remained significant (patients with AD: $p=0.0004$; middle-aged controls: $p=0.006$; young controls: $p<0.0001$).

*Analysis 2: Alcohol-related group differences for the association between the PIT effect and the* OPRM1 *polymorphism.* The second aim of this study was to test whether the interaction between the PIT effect and *OPRM1* polymorphism was significantly different between patients with AD and HCs. This analysis indicated a three-way interaction between Pavlovian valence, instrumental action and group and also a three-way

interaction between Pavlovian valence, instrumental action and *OPRM1* polymorphism. Thus, AD and the *OPRM1* polymorphism were significantly and independently associated with the strength of the PIT effect per se (see Figure 2). Moreover, we found a three-way interaction between instrumental action, group and *OPRM1* polymorphism. However, the four-way interaction between Pavlovian valence, instrumental action, group and *OPRM1* polymorphism was not statistically significant (Table 3). Thus, the interaction between the PIT effect and the *OPRM1* polymorphism was not statistically different between patients with AD and matched control subjects (Figure 2).

**Table 4.** Results of analysis 3. Effects of the regression analysis from the PIT part where we tested whether the interaction between the PIT effect and the *OPRM1* polymorphism was significantly different between relapsers and abstainers.

|  | $\chi^2$ | *p*-Value |
|---|---|---|
| Pavlovian valence | 10.27 | **0.006** |
| Instrumental action | 0.002 | 0.965 |
| *OPRM1* polymorphism | 0.324 | 0.569 |
| Relapse | 0.706 | 0.401 |
| Craving | 0.053 | 0.817 |
| Pavlovian valence × instrumental action | 1535.13 | **<0.0001** |
| Pavlovian valence × *OPRM1* polymorphism | 0.426 | 0.808 |
| Instrumental action × *OPRM1* polymorphism | 11.706 | **0.001** |
| Pavlovian valence × relapse | 0.513 | 0.774 |
| Instrumental action × relapse | 12.786 | **<0.0001** |
| *OPRM1* polymorphism × relapse | 0.042 | 0.838 |
| Pavlovian valence × instrumental action × *OPRM1* polymorphism | 16.786 | **0.001** |
| Pavlovian valence × instrumental action × relapse | 13.647 | **0.001** |
| Pavlovian valence × *OPRM1* polymorphism × relapse | 0.571 | 0.752 |
| Instrumental action × *OPRM1* polymorphism × relapse | 1.988 | 0.159 |
| Pavlovian valence × instrumental action × *OPRM1* polymorphism × relapse | 30.347 | **<0.0001** |
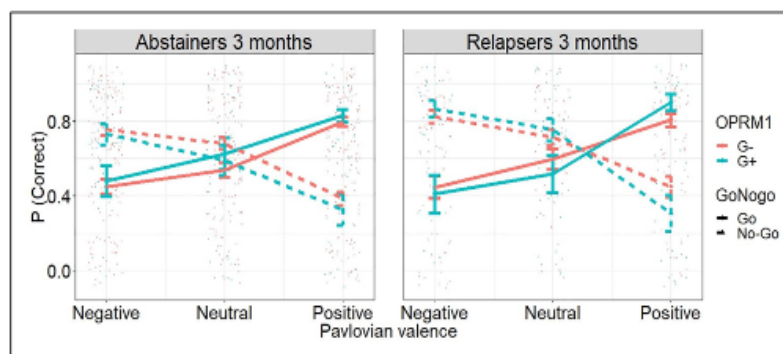


**Figure 3.** Results of the PIT phase as a function of treatment outcome (abstainers vs. relapsers) and *OPRM1* polymorphism (analysis 3). Patients with AD who relapsed showed a stronger interaction between the PIT effect and the *OPRM1* polymorphism compared to patients with AD who remained abstinent. Moreover, G+ carriers showed a strong and significant interaction between the PIT effect and treatment outcome, whereas G− carriers did not.

*Analysis 3: Relapse-related group differences for the association between the PIT effect and the* OPRM1 *polymorphism.* Last, we tested whether the observed interaction between the *OPRM1* polymorphism and the PIT effect was associated with relapse. Again, we found a three-way interaction between the *OPRM1* polymorphism, Pavlovian valence and instrumental action (Table 4). In addition, we observed an interaction between relapse status and instrumental action, and a three-way interaction between Pavlovian valence, instrumental action and relapse. This interaction was further modulated by the *OPRM1* polymorphism, resulting in the expected four-way interaction between Pavlovian valence, instrumental action, *OPRM1* polymorphism and relapse status (Figure 3 and Table 4). Thus, the interaction between the *OPRM1* polymorphism and the PIT effect was statistically different between patients with AD who prospectively relapsed and those who

remained abstinent. Post hoc tests indicated that the interaction between Pavlovian valence, instrumental action and the *OPRM1* polymorphism was only significant for relapsers ($p < 0.0001$) but not for abstainers ($p = 0.328$). Moreover, the interaction between Pavlovian valence, instrumental action and relapse was significant for G+ carriers ($p < 0.0001$) but not for G− carriers ($p = 0.09$).

## Discussion

To explore and further understand the behavioural and genetic underpinnings of 'wanting' as an expression of incentive salience attribution in humans and to bridge the gap to preclinical results, we investigated the association between the *OPRM1* polymorphism, PIT effect and relapse across a large cohort of patients with AD and two independent cohorts of HCs.

We demonstrate that (a) in all three independent cohorts, G+ carriers showed an increased PIT effect; (b) there is no difference between patients with AD and HCs in the interaction between *OPRM1* and PIT; but (c) when merely investigating AD, relapsing patients carrying the G+ allele showed an increased PIT effect as opposed to abstaining patients, who did not show an association between *OPRM1* genotype and PIT. We henceforth discuss these three main results.

## Analysis 1: Association between the PIT effect and the OPRM1 *polymorphism across cohorts*

The first analysis demonstrated a clear association between the *OPRM1* genotype and PIT in three independent human cohorts. Two studies have previously investigated the role of the human opioid system in PIT-like effects in healthy human subjects. By using a pharmacological challenge, Weber et al. (2016) demonstrated that naltrexone reduces PIT effects for primary reinforcers (e.g. food rewards). We here demonstrate that the opioid system is also involved in modulating PIT effects for secondary reinforcers (e.g. monetary rewards). Beyond this, the experimental design from Weber et al. (2016) also differed in several other aspects from our study. Weber et al. (2016) focused on the positive 'limb' of the PIT effect (the extent to which positive stimuli affect responses), whereas our paradigm also enabled us to examine the negative 'limb' of the PIT effect (the extent to which negative stimuli affect responses). Moreover, our instrumental task included both 'Go' and 'No-Go' responses, whereas the instrumental task by Weber et al. (2016) merely included a 'Go' component. Thus, in line with previous investigations (Guitart-Masip et al., 2011, 2014; Swart et al., 2017), our experimental manipulation enabled us to test for more complex valence–action interactions. These previous tasks in line with our results have identified a potentially phylogenetically induced bias for congruent action–valence responses (e.g. better performance when a 'Go' response was acquired to win) compared to incongruent action valence (e.g. when a 'No-Go' response was acquired to win).

A second study published by Wiers et al. (2009) investigated automatic appetitive action tendencies in male heavy-drinking carriers of the *OPRM1* G allele. Heavy-drinking G+ carriers showed increased automatic approach tendencies not only to alcohol-associated stimuli but also to other appetitive stimuli (Wiers et al., 2009). This is in line with our finding of increased behavioural modulation in the presence of appetitive cues in AD G+ carriers. However, Wiers et al. did not include a control group in their study design and only included male sex, which limits generalisability and comparability to our results.

In summary, our data support the notion that the *OPRM1* polymorphism serves as one biological agent associated with human PIT effect in both AD patients and HCs.

## Analysis 2: Alcohol-related group differences for the association between the PIT effect and the OPRM1 *polymorphism*

We did not find a significantly different association between the PIT effect and the *OPRM1* polymorphism between patients with AD and HCs, which partly reflects the ongoing debate and contradictory results published so far on the association between the *OPRM1* genotype and AD (Hendershot et al., 2016; Kong et al., 2017; Ray and Hutchison, 2004; Sloan et al., 2018). Instead, we found that AD and the *OPRM1* polymorphism are independent factors that both increase the PIT effect. Moreover, we found an interaction between instrumental action, *OPRM1* polymorphism and group, indicating that the opioid system differently affects instrumental responses in AD patients and HCs. Exploratory post hoc analyses (Supplementary Information 4) indicated that AD G+ carriers showed increased 'Go' responses compared to 'No-Go' responses, whereas HC G+ carriers showed increased 'No-Go' responses compared to 'Go' responses. Of note, a positive PIT effect is accompanied by an overall increase of 'Go' responses, while a negative PIT effect is accompanied by an overall increase in 'No-Go' responding. Thus, the *OPRM1* polymorphism may influence the positive PIT effect in patients with AD and the negative PIT effect in HC. A core feature of AD is the persistent substance consumption despite the negative consequences of consumption (Stacy and Wiers, 2010). We speculate that this paradox might partly be explained by an increased responsiveness of patients with AD to positively conditioned cues, which is stronger in G+ carriers. On the other hand, an increased responsiveness to negative stimuli might reveal a protective mechanism of healthy G+ carriers (S3 and S4). Clearly, future studies need to validate this speculation.

## Analysis 3: Relapse-related group differences for the association between the PIT effect and the OPRM1 *polymorphism*

Only relapsers but not abstainers showed a significant interaction between the PIT effect and the *OPRM1* polymorphism. Moreover, only relapsing G+ carriers showed an increased PIT effect compared to abstainers, whereas there was no difference between the PIT effect in relapsers and abstainers in G− carriers. One speculative interpretation of these findings is that there may be two pathways to relapse, and that these fundamentally differ with regard to the *OPRM1* polymorphism and the PIT effect. On the one hand, in G+ carriers, the mechanisms driving PIT might also be related to relapse, whereas in G− carriers, these mechanisms could be less related to relapse. Our finding of an increased PIT effect in relapsing AD G+ carriers might also be relevant for precision medicine, particularly in the light of the ongoing discussion of the *OPRM1* polymorphism as a potential biomarker for the effectiveness of naltrexone treatment (Chamorro et al., 2012; Hartwell et al., 2020; Oslin et al., 2003; Setiawan et al., 2012; Ziauddeen et al., 2016). Strikingly, treatment response to naltrexone was also particularly high in patients with AD classified as reward drinkers (Mann et al., 2018; Witkiewitz et al., 2019) and reduced craving, most notably in social drinkers, who had high positive alcohol expectancies (Palfai et al., 1999).

Similar considerations might be relevant to nalmefene, the MOP antagonist and partial κ-agonist, recently approved for the treatment of AD (Gual et al., 2013), with similarly conflicting results. According to a meta-analysis, the drug is able to improve behavioural outcomes in patients with AD (Mann et al., 2016),

while others show that it has a limited efficacy in AD therapy (Palpacuer et al., 2015; Soyka and Muller, 2017). Nalmefene administered in a modified 'Go'/'No-Go' paradigm mildly reduced vigor to alcoholic cues in patients with AD (Gal et al., 2019). However, no major differences were observed between the treatment group and the placebo group with respect to behavioural and neural correlates of approach/avoidance tendencies. Given our data, future studies could investigate whether naltrexone and/or nalmefeme might be particularly effective in alcohol-dependent patients who are G+ carriers and additionally show large PIT effects.

## Outlook: How does OPRM1 *influence neural reward processing?*

The neural correlates of PIT have been associated with relapse in AD within the mesolimbic reward system (Garbusow et al., 2016; Sekutowicz et al., 2019; Sommer et al., 2020) and could predict future drinking behaviour in adolescents (Sekutowicz et al., 2019). Recent studies have suggested a direct link between the *OPRM1* polymorphism and the mesolimbic dopaminergic system. For instance, by using a mouse model of the *OPRM1* A118G SNP, Popova et al. (2019) demonstrated that A- and G-allele carriers show significantly different regulation of mesolimbic dopaminergic firing. One potential underlying mechanism is that MOP receptors (which are affected by the *OPRM1* polymorphism) mediate opioid-induced disinhibition of midbrain dopaminergic neurons (Jalabert et al., 2011; Jhou et al., 2012; Matsui et al., 2014). Recent work in rodents has proven that optogenetic manipulations of those dopaminergic neurons can bidirectionally modulate online action selection (Howard et al., 2017). Thus, we speculate that the *OPRM1* polymorphism is associated with the extent to which Pavlovian stimuli functionally activate the mesolimbic dopaminergic system in AD. This speculation is in line with functional magnetic resonance imaging studies using cue reactivity paradigms in substance-dependent individuals. For instance, some studies suggest that AD G+ carriers display increased neural responses to alcohol-associated stimuli in mesocorticolimbic areas (Bach et al., 2015; Courtney et al., 2015; Filbey et al., 2008; but see Schacht et al., 2013). In line with this, humanised mice carrying the G+ allele of the *OPRM1* polymorphism displayed increased striatal dopamine release in response to an intravenously infused alcohol dose (Ramchandani et al., 2011). Clearly, future studies should further investigate how the *OPRM1* polymorphism affects the underlying neural mechanisms of the PIT effect in humans.

## Limitations

The generalisability of our results is limited by the lack of preregistration, additional analyses designed after study protocol and the use of single gene analyses. The correlational nature of the analyses only allows speculation about causal relationships and needs to be further validated in a longitudinal design. Even though candidate genes as opposed to large-scale GWAS studies have come into disrepute, we believe that there is still a high relevance in connecting single genes and their respective pathways to specific neurocognitive processes and thus providing the opportunity for more specific interventions in precision medicine (Deb et al., 2010; Di Martino et al., 2020). Another limitation of our design is that the procedure used here to indicate Pavlovian learning (task phase 4) was not designed to detect between-group effects but instead served to identify subjects who did not learn the Pavlovian contingencies (Supplemental Information 8). Across all cohorts, subjects could almost perfectly identify the best Pavlovian stimuli, and these ceiling effects potentially lowered statistical power to detect differences in Pavlovian learning. Several studies across humans and animals have demonstrated that individuals who attribute incentive salience to reward predicting stimuli through Pavlovian conditioning (so called sign-trackers) will also show an increased PIT effect (Garofalo and di Pellegrino, 2015; Schad et al., 2019b). Future studies should therefore use more sensitive methods to identify sign-tracking humans (such as eye-tracking; Schad et al., 2019b) and test the role of the *OPRM1* polymorphism in this phenomenon. One further limitation is the relatively small sample size of relapsers versus abstainers in analysis 3. Importantly, the group of G+ carriers that relapsed versus abstained was 16 versus 14, respectively. Thus, future stratification studies need to replicate our findings in larger sampling sizes, for example by oversampling G+ carriers in AD.

## Summary

This study presents strong evidence for an association between the *OPRM1* polymorphism and the PIT effect in both patients with AD and HCs. It is the first to show that the *OPRM1* polymorphism modulates the extent to which Pavlovian stimuli exert control over behaviour and suggests a functional difference of this gene–behaviour interaction between relapsers and abstainers.

### Declaration of conflicting interests

### Funding

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## Supplemental material

Supplemental material for this article is available online.

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# Supplementary Information: The association of the OPRM1 A118G polymorphism and Pavlovian-to-instrumental transfer: clinical relevance for alcohol dependence

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## SI 1: Sample selection

Originally, we had behavioral data of 542 subjects (221 alcohol-dependent (AD) patients, 129 middle-aged controls, 192 young controls). However, due to missing data in the genetic information (27 AD patients, 4 middle-aged controls, 19 young controls) or due to insufficient performance in the forced choice task (indicating low Pavlovian learning: 8 AD patients, 17 middle aged controls, 12 young controls), we had to exclude several subjects (Figure S1), resulting in the final data sets reported throughout the manuscript for the respective analsysis.

**Behavioral data**
alcohol-dependent patients n = 221
middle-aged controls n = 129
young controls n = 192

**No genetic information**
alcohol-dependent patients n = 27
middle-aged controls n =7
young controls n = 19

**Behavioral + genetic data**
alcohol-dependent patients n = 194
middle-aged controls n = 122
young controls n =173

**No Pavlovian learning**
alcohol-dependent patients n = 8
middle-aged controls n = 17
young controls n = 12

**Final data set analysis 1 and 2**
alcohol-dependent patients n = 186
middle-aged controls n = 105
young controls n = 161

analysis 1: effects presented in all three cohorts, respectively
analysis 2: comparison between alcohol-dependent patients and middle-aged controls

**No follow up information**
Missing relapse infromation n = 41

**Final data set analysis 3**
relapsers n = 51
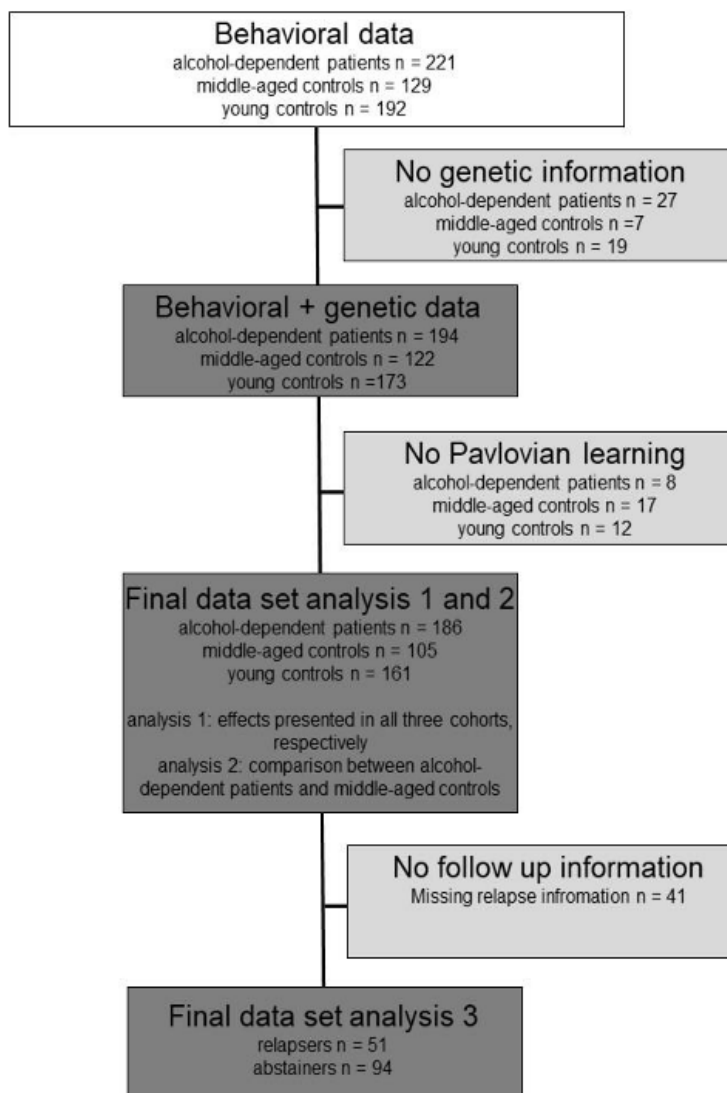abstainers n = 94

*Figure S1: Sample selection procedure.*

## SI 2: Model Comparisons

We performed several generalized linear mixed models (across all subjects) and compared model fits. The first model (henceforth referred to *"1. linear-accuracy"*) included Pavlovian valence as a linear contrast (-2,-1,0,1,2), instrumental action (Go, No-Go, coded as 0.5 and -0.5) and OPRM1 polymorphism (G-, G+, coded as -0.5 and +0.5) on the participant's accuracy (Correct, Incorrect). This model (without the OPRM1 polymorphism) has been used to model performance of the PIT paradigm in a recent sample of the LeAD study (Sommer et al., 2017). The next model (henceforth referred to as *"2. non-linear-accuracy"*) included Pavlovian valence as non-linear contrast (Negative, Neutral, Positive, dummy coded with neutral as reference), instrumental action (Go, No-Go, coded as 0.5 and -0.5) and OPRM1 polymorphism (G-, G+, coded as -0.5 and +0.5) on the participant's accuracy (Correct, Incorrect). We additionally fitted reduced versions of these two models: a linear accuracy model without the OPRM1 polymorphism, henceforth referred as *"1a: linear accuracy no OPRM1"*, a linear accuracy model without the instrumental action: henceforth referred as *"1b: linear accuracy no Instrumental action"* and the same for the non-linear accuracy model: a non-linear accuracy model without the OPRM1 polymorphism, henceforth referred as *"2a: non-linear accuracy no OPRM1"*, a non-linear accuracy model without the instrumental action: henceforth referred as *"2b: non-linear accuracy no Instrumental action"*. Last, we fitted an accuracy model without Pavlovian valence, henceforth referred as *"3: accuracy no Pavlovian valence"*

We used the *anova* function of the *stats* package to perform model comparisons. This analysis indicated that the non-linear accuracy model was the best fitting model (lowest AIC score and highest Log Likelihood, Table S1).

*Table S1: Results of the model comparisons*

| Model | AIC | Log Likelihood |
|---|---|---|
| 1. linear-accuracy | 42164 | -21073 |
| 1a: linear accuracy no OPRM1 | 42182 | -21086 |
| 1b: linear accuracy no Instrumental action | 45479 | -22735 |
| **2. non-linear-accuracy** | **41851** | **-20911** |
| 2a: non-linear accuracy no OPRM1 | 41900 | -20943 |
| 2b: non-linear accuracy no Instrumental action | 45471 | -22728 |
| 3: accuracy no Pavlovian valence | 45470 | -22730 |

## SI 3: Group comparisons for demographic and clinical characteristics
*Table S2: OPRM1 independent group comparisons for alcohol dependent patients, middle aged controls and young healthy controls*

| Cohort | Alcohol-dependent patients | Middle-aged controls | Young controls | Test Statistics | | | |
|---|---|---|---|---|---|---|---|
| | mean (sd) | mean (sd) | mean (sd) | Anova statistics | Alcohol-dependent patients vs. Middle-aged controls | Alcohol-dependent patients vs. Young controls | Middle-aged controls vs. Young controls |
| **Demographic Variables** | | | | | | | |
| Age | 46.33 (10.56) | 44.24 (10.96) | 18.36 (0.2) | F = 521.96, **p <.0001** | t = 1.58, p = 0.11 | t = 36.11, **p <.0001** | t = -24.18, **p <.0001** |
| Sex (% male) | 84% | 83% | 100% | $X^2$ = 29.78, **p < .0001** | $X^2$ = .01, *p* = .93 | $X^2$ = 26.24, **p < .0001** | $X^2$ = 26.95, **p < .0001** |
| Years of education | 14.85 (3.84) | 15.84 (3.24) | 11.65 (0.93) | F = 77.612, **p <.0001** | t = -2.28, **p = .02** | t = 10.75, **p <.001** | t = -12.67, **p < .0001** |
| **Clinical Characteristics** | | | | | | | |
| Anxiety [a] | 4.44 (3.39) | 2.21 (2.08) | 2.46 (2.39) | F = 30.033, **p <.0001** | t = 6.83, **p < .0001** | t = 6.28, **p <.0001** | t = 0.88, p = 0.38 |
| Depression [b] | 3.64 (3.64) | 1.57 (2.15) | 1.7 (1.81) | F = 27.724, **p <.0001** | t = 6, **p < .0001** | t = 6.31, **p < .0001** | t = 0.51, p = 0.61 |
| Craving [c] | 12.72 (8.03) | 2.73 (2.93) | 3.77 (3.16) | F = 129.89, **p < .0001** | t = 14.31, **p < .0001** | t = 13.58, **p < .0001** | t = 2.46, **p = 0.02** |
| Impulsivity [d] | 31.66 (6.48) | 29.2 (5.35) | 30.45 (5.06) | F = 6.12, **p = 0.002** | t = 3.45, **p = 0.001** | t = 1.93, p = 0.05 | t = 1.89, p = 0.06 |
| **Neuropsychological Testing** | | | | | | | |
| Cognitive Speed [e] | 9.31 (2.76) | 10.67 (3.07) | 11.37 (2.31) | F = 25.78, **p <.0001** | t = -3.73, **p = 0.001** | t = -7.4965, **p <.0001** | t = 2.02, **p = 0.04** |
| Working Memory [f] | 6.55 (1.87) | 7.46 (2.07) | 8.04 (2.01) | F = 24.60, **p <.0001** | t = -3.69, **p = 0.001** | t = -7.038, **p < .0001** | t = 2.26, **p = 0.03** |

## SI 4: Post-hoc analyses based on visual inspection of the 3-way interaction: Pavlovian valence x Instrumental action x OPRM1 polymorphism (analysis 1)

Interestingly, our graphical illustrations of the interaction between the PIT effect and the OPRM1 polymorphism (Figure S2) indicated that the direction of this association was group dependent. More precisely, Figure S2 suggests that AD G+ carriers showed increased modulation of Go and No-Go responses in the context of positive stimuli, whereas G+ carriers in both healthy control samples (middle aged controls and young controls) seemed to show increased modulation of Go and No-Go responses in the context of negative stimuli.

We thus performed exploratory post hoc analyses for all three cohorts, respectively, where we tested instrumental action*OPRM1 interactions separately for the negative (Negative vs. neutral) and positive (positive vs. neutral) limb of the PIT effect. In AD patients, we found a significant interaction between instrumental action and OPRM1 for positive values (p < .0001), but not for negative values (p = .29). In young and middle aged healthy controls, we found the reverse patterns, namely an instrumental action*OPRM1 interaction for negative values (young controls, p < .0001, middle aged controls, p < .0001) but no significant interaction between instrumental action and OPRM1 for positive values (young controls, p = .59, middle aged controls, p = .08).

## SI 5: Post-hoc analyses for the 3-way interaction: Instrumental action x group x OPRM1 polymorphism (analysis 2)

We performed additional post-hoc analyses for AD patients and healthy controls separately, where we analyzed how the OPRM1 polymorphism would differentially affect Go and No-Go responses. This analysis revealed that AD G+ carriers showed increased Go responses compared to No-Go responses compared to G- carriers p = .01) whereas healthy controls showed increased No-Go responses compared to Go responses compared to G- carriers (p < .0001).

## SI 6: Performance of the instrumental learning phase

448 out of the initial 452 subjects had complete data of the instrumental learning phase (phase 1 of the paradigm).

On average subjects performed 87.2 trials (sd = 25.7) until they reached the criterion in the instrumental learning phase. OPRM1 polymorphism did not covary with the number of trials that G- and G+ carriers performed (young controls: W = 2542.5, p = .74, middle-aged controls: W = 884.5, p = 0.48, AD patients: W = 2403.5, p = 0.91). Thus, overall performance in the instrumental learning phase was not dependent on OPRM1 polymorphism. Adapting our analysis approach from the PIT phase we also performed a binomial mixed effect regression, where we regressed instrumental action (Go, No-Go) and the OPRM1 polymorphism on accuracy (correct, incorrect) in the instrumental learning phase.

Overall, we found a main effect of instrumental action that was positive across all cohorts, indicating that subjects were better in Go learning compared to No-Go learning. Beyond this, the OPRM1 polymorphism did not interact with instrumental action in young healthy controls ($p = .26$), AD patients ($p = .17$), but was statistically significant in middle-aged healthy controls ($p = <.0001$). Post-hoc analyses indicated that in this cohort (middle-aged controls), G- carriers showed significantly better performance in Go compared to No-Go trials ($p < .0001$), whereas this difference was not significantly different in G+ carriers ($p = .06$), see Table S3, Figure S2.

Table S3: Results from the generalized linear mixed effects model of the instrumental training data

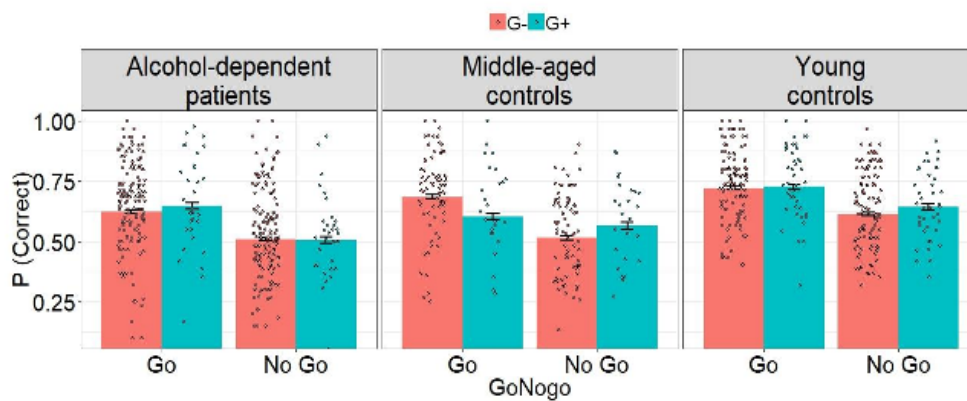| | Alcohol-dependent patients (n = 184) | | Middle-aged controls (n = 103) | | Young controls (n =161) | |
|---|---|---|---|---|---|---|
| | Estimate | p-value | Estimate | p-value | Estimate | p-value |
| Intercept | 0.59 | **<.0001** | 0.88 | **<.0001** | 1.11 | **<.0001** |
| Instrumental action | 0.48 | **<.001** | 0.74 | **<.0001** | 0.51 | **<.0001** |
| OPRM1 polymorphism | 0.08 | .39 | -0.36 | **.002** | -0.01 | 0.94 |
| Instrumental action * OPRM1 polymorphism | -0.11 | 0.17 | 0.57 | **<.0001** | 0.10 | 0.26 |



Figure S2: Correct responses in the instrumental learning phase as a function of action (Go/No-go), group (alcohol dependent, middle age controls, young controls) and OPRM1 polymorphism (G-/G+). For display purposes only responses larger than 10% correct are displayed.

## SI 7: Stay/switch behavior of the instrumental learning phase

Some previous studies have suggested a profound effect of the opioid system on reinforcement learning (Efremidze et al., 2017; Lee et al., 2011). Moreover, our analyses of the overall correct responses from the instrumental learning phase revealed that at least in one cohort (middle aged controls) Go and No-Go learning was different across G+ and G- carriers (see SI 5). Thus, we additionally tested, whether OPRM1 polymorphism additionally affected choice behavior depending on outcome. More precisely we asked, whether G+-carriers would show increased learning from reward or punishment. To this end, we carried out an analysis previously introduced by Huys et al. (2011), where we analyzed the immediate consequences of a trial's outcome on subsequent behavior. We performed a binomial mixed effect regression, where we regressed outcome of the previous trial (reward$_{(t)}$, punishment$_{(t)}$) and the OPRM1 polymorphism on repetition behavior of a specific stimulus (stay = Go$_{(t)}$ & Go$_{(t+1)}$ | No-Go$_{(t)}$ & No-Go$_{(t+1)}$, switch = Go$_{(t)}$ & No-Go$_{(t+1)}$ | No-Go$_{(t)}$ & Go$_{(t+1)}$). This analysis revealed increased stay behavior after reward compared to switch behavior after punishment (see Figure S3, Table S4) across all cohorts. This is in line with Huys et al. (2011) and has previously been interpreted as increased reward sensitivity opposed to punishment sensitivity. OPRM1 polymorphism did not influence the impact that outcome of the previous trial (reward/punishment) had on stay/ switch behavior. However, in middle aged controls, the main effect of OPRM1 polymorphism was on the border of significance

(*p* = .07), indicating that in this cohort, G+ carriers tended to show deficits in adapting their behavior according to the outcomes of their action, which is in line with the finding that in this cohort G+ carriers showed overall less correct responses in the instrumental learning phase (see Figure S3, middle panel).

*Table S4: Results from the generalized linear mixed effects model of the instrumental training data regarding stay/switch behavior as a function of previous outcome and OPRM1 polymorphism*

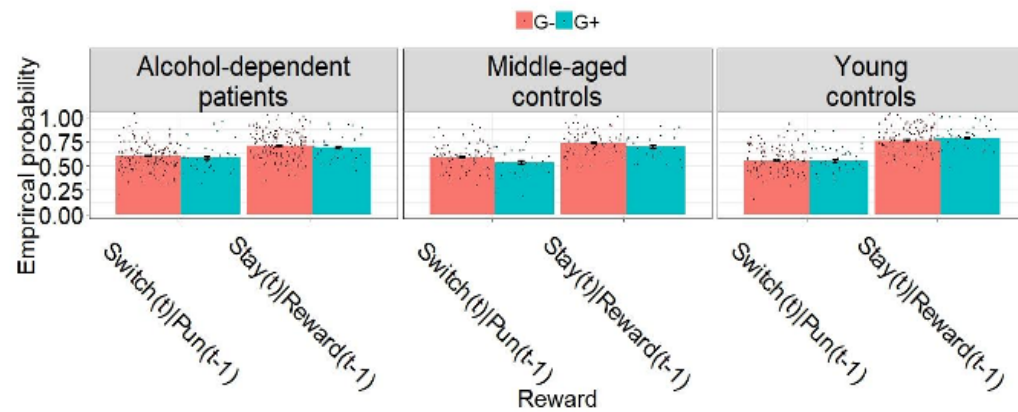|  | Alcohol-dependent patients (n = 184) | | Middle-aged controls (n = 103) | | Young controls (n =161) | |
|---|---|---|---|---|---|---|
|  | Estimate | p-value | Estimate | p-value | Estimate | p-value |
| Intercept | 0.46 | **<.0001** | 0.42 | **<.0001** | 0.30 | **<.0001** |
| Reward/Punishment of the previous trial | 0.47 | **<.001** | 0.65 | **<.0001** | 0.91 | **<.0001** |
| OPRM1 polymorphism | 0.01 | .97 | -0.24 | 0.07 | -0.01 | 0.90 |
| Reward/Punishment * OPRM1 polymorphism | -0.03 | 0.69 | 0.02 | 0.82 | 0.15 | 0.12 |



*Figure S3: Mean overall probability of repeating an action in the instrumental part given that it was last rewarded in the presence of the current stimulus, or the probability of switching given a previous punishment as a function of Group (Alcohol dependent, middle age controls, young controls) and OPRM1 polymorphism (G+/G- carriers).*

## SI 8: Performance of the forced choice phase

448 out of the initial 452 subjects had complete data of the forced choice phase (phase 4 of the paradigm). Based on prior work (Garbusow et al., 2016; Garbusow et al., 2014), we only included subjects in all analyses, who responded better than chance in the query trials (see SI 1). We did this to ensure that subjects had sufficiently acquired the Pavlovian associations, which is a prerequisite of the Pavlovian-to-instrumental transfer effect.

To mirror our behavioral analyses of the PIT phase and the instrumental learning phase, we analyzed performance from the query trials in the same way. Thus we regressed correct responses on Pavlovian valence by using a linear mixed model. Note that in each trial two Pavlovian values are displayed and subjects have to indicate the better one. Thus, if subjects give a correct response, this trial is coded as correctly for both Pavlovian values, whereas if the subject

gives a wrong response, this trial is coded as false for both Pavlovian values. This results in 60 correct/false responses for each subject from 30 trials.

Results from this analysis indicated that Pavlovian valence influenced correct choices in AD patients ($p < .0001$) and in middle-aged controls ($p < .0001$) but not in young controls ($p = .88$, probably due to ceiling effects in this group). This effect was not modulated by the OPRM1 polymorphism in AD patients ($p = 0.07$), nor middle-aged controls ($p = 0.16$) or young controls ($p = 0.8$, Table S5, Figure S4)

*Table S5: Results from the linear mixed effects model of the forced choice data*

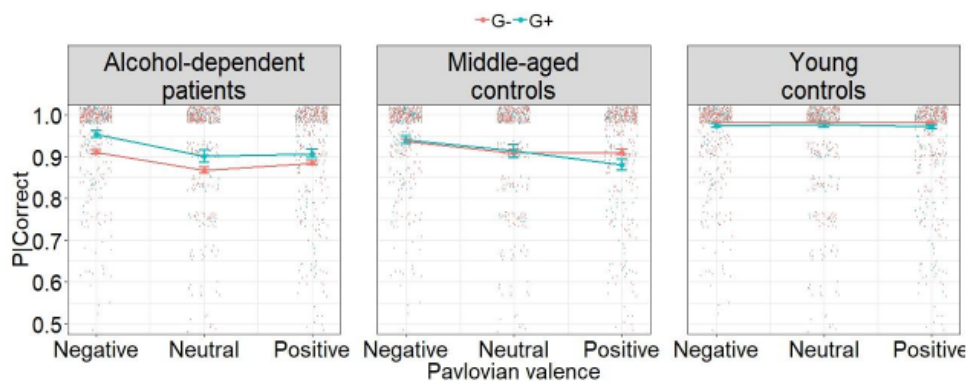| | Alcohol-dependent patients (n = 184) | | Middle-aged controls (n = 103) | | Young controls (n =161) | |
|---|---|---|---|---|---|---|
| | Chisq | p-value | Chisq | p-value | Chisq | p-value |
| Pavlovian valence | 46.24 | **<.0001** | 24.95 | **<.0001** | 0.26 | 0.88 |
| OPRM1 polymorphism | 2.56 | .11 | 0.92 | 0.34 | 0.38 | 0.54 |
| Pavlovian valence* OPRM1 polymorphism | 5.20 | 0.07 | 3.66 | 0.16 | 0.45 | 0.8 |



*Figure S4: Correct responses for the forced choice task as a function of Pavlovian valence (Negative, Neutral, Positive) OPRM1 polymorphism (G-,G+ carriers) and group (alcohol-dependent, middle-age controls, young controls). For display purposes individual correct responses are clustered, in the way that subjects with the same correct responses for a Pavlovian valence are displayed as scatter.*

## SI 9: Reaction times of the PIT phase

In the behavioral analyses, we did not see a OPRM1 polymorphism*Pavlovian valence interaction across all subjects, However, we found evidence that Pavlovian valence interacted more strongly with action across G+ carriers. We performed additional analyses to see how Pavlovian valence influenced reaction times.

Thus we regressed reaction times of first button presses on Pavlovian valence and OPRM1 polymorphism on reaction times of first button presses. First button responses were cleaned for reaction times faster than 50 milliseconds (0.006 % of all trials) or slower than 2950 ms (0.001% of all events). Note that even if a subject performs a response in a No-Go trial, this can be a correct response, because the coding of correct/ incorrect depends of the number of button presses. Thus we collapsed all trials across Go and No-Go responses.

Mirroring our behavioral analyses, we performed all analyses separately for the three groups. Our analyses indicated that across all groups, Pavlovian valence influenced RTs (Table S6, Figure S5). Moreover, in AD patients, G+ carriers showed a stronger modulation of Pavlovian valence than G- allele carriers. This result was mirrored by young controls, but it failed to reach statistically significance in middle aged healthy controls.

*Table S6: Results from the linear mixed effects model of the Reaction time data of the first button press during the PIT phase*

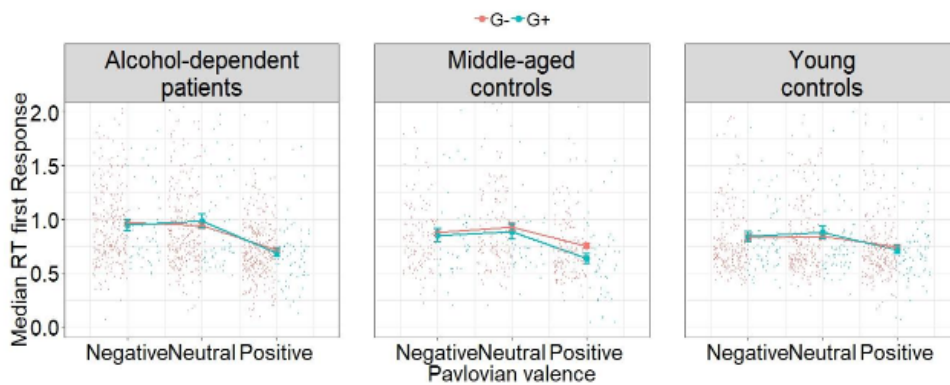| | Alcohol-dependent patients (n = 186) | | Middle-aged controls (n = 105) | | Young controls (n =161) | |
|---|---|---|---|---|---|---|
| | Chisq | p-value | Chisq | p-value | Estimate | p-value |
| Pavlovian valence | 342.19 | **<.0001** | 84.71 | **<.0001** | 45.22 | <.0001 |
| OPRM1 polymorphism | 0.01 | .93 | 3.36 | .07 | 0.18 | 0.67 |
| Pavlovian valence* OPRM1 polymorphism | 7.11 | **0.03** | 4.06 | 0.11 | 2.93 | **0.05** |



*Figure S5: Reaction times of the first Response in the PIT phase as a function of Pavlovian valence (Negative, Neutral, Positive), OPRM1 polymorphism and group. For display purposes only individual responses < 2 seconds are displayed.*

## SI 10: Subjective Pleasantness of the rating data

446 out of the initial 452 subjects had complete data of the rating part (phase 5 of the paradigm). We here additionally tested the hypothesis, that pleasantness ratings of instrumental and Pavlovian stimuli were affected by OPRM1 polymorphism across groups. With regard to the instrumental stimuli, we thus performed a linear mixed model, where we regressed OPRM1 polymorphism and instrumental action on pleasantness ratings. With regard to the Pavlovian stimuli, we performed a linear mixed model, where we regressed OPRM1 polymorphism and Pavlovian valence on pleasantness ratings.

Table S7: Results from the linear mixed effects model of the pleasantness ratings of the instrumental stimuli (above) and the Pavlovian stimuli (below).

| | Alcohol-dependent patients (n = 182) | | Middle-aged controls (n = 104) | | Young controls (n =160) | |
|---|---|---|---|---|---|---|
| *Instrumental stimuli* | Estimate | p-value | Estimate | p-value | Estimate | p-value |
| Intercept | 4.04 | **<.0001** | 4.08 | **<.0001** | 4.01 | **<.0001** |
| Instrumental action | 1.29 | **<.0001** | 2.13 | **<.0001** | 2.43 | **<.0001** |
| OPRM1 polymorphism | -0.10 | 0.53 | -0.14 | 0.48 | -0.01 | 0.94 |
| Instrumental action * OPRM1 polymorphism | -0.12 | 0.71 | -0.27 | 0.48 | -0.05 | 0.87 |
| *Pavlovian stimuli* | Chisq | p-value | Chisq | p-value | Chisq | p-value |
| Pavlovian valence | 127.95 | **<.0001** | 138.94 | **<.0001** | 311.84 | **<.0001** |
| OPRM1 polymorphism | 1.87 | 0.17 | 3.90 | 0.05 | 0.17 | 0.68 |
| Pavlovian valence * OPRM1 polymorphism | 3.06 | 0.22 | -0.13 | 0.24 | 2.52 | 0.28 |

With regard to the ratings of the instrumental stimuli, we found a main effect of instrumental action across groups (AD patients ($p < .0001$), middle aged controls ($p < .0001$) in young controls ($p < .0001$), demonstrating that subjects rated Go-trials as more pleasant compared to No-Go trials. Likewise, with regard to the ratings of the Pavlovian stimuli, we found a main effect of Pavlovian valence across groups (AD ($p < .0001$), middle aged controls ($p < .0001$) and in young controls ($p < .0001$), demonstrating that subjects rated the stimuli in accordance with their Pavlovian valence. However, neither ratings of instrumental nor Pavlovian stimuli were additionally modulated by OPRM1 polymorphism (Table S7 & Figure S6).
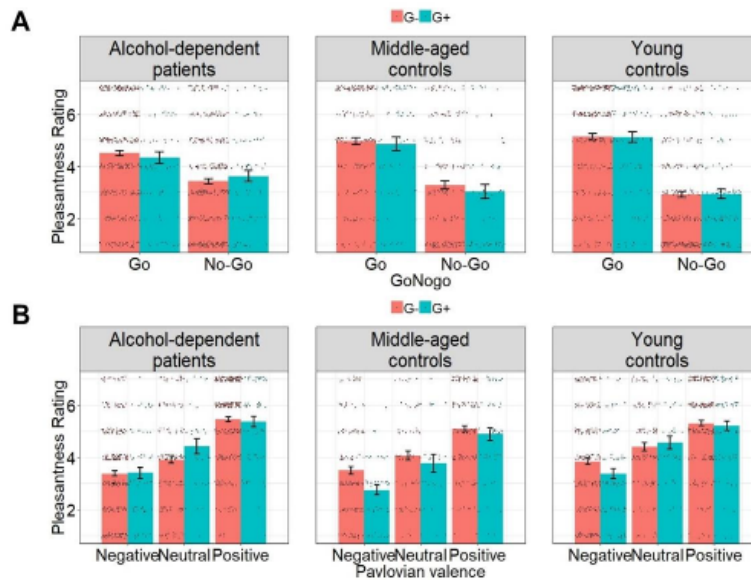
*Figure S6: Pleasant ratings of **A.) Instrumental stimuli** and **B.) Pavlovian valence** as a function of OPRM1 polymorphism (G+,G- carriers) and group (Alcohol dependent, middle age controls, young controls)*

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**Alcohol approach bias is associated with both behavioral and neural Pavlovian-to-instrumental transfer effects in alcohol-dependent patients.**

**Chen K**, Garbusow M, Sebold M, Kuitunen-Paul S, Smolka MN, Huys QJ, Zimmermann US, Schlagenhauf F, Heinz A. Alcohol approach bias is associated with both behavioral and neural Pavlovian-to-instrumental transfer effects in alcohol-dependent patients. Biological Psychiatry Global Open Science. 2022 Apr 14. https://www.bpsgos.org/article/S2667-1743(22)00053-2/fulltext

Biological Psychiatry: Global Open Science has not been included in Journal Citation Reports (JCR) yet (new open access journal launched in 2020).

# Archival Report

Biological Psychiatry: GOS

## Alcohol Approach Bias Is Associated With Both Behavioral and Neural Pavlovian-to-Instrumental Transfer Effects in Alcohol-Dependent Patients

Ke Chen, Maria Garbusow, Miriam Sebold, Sören Kuitunen-Paul, Michael N. Smolka, Quentin J.M. Huys, Ulrich S. Zimmermann, Florian Schlagenhauf, and Andreas Heinz

### ABSTRACT

**BACKGROUND:** Even after qualified detoxification, alcohol-dependent (AD) patients may relapse to drinking alcohol despite their decision to abstain. Two mechanisms may play important roles. First, the impact of environmental cues on instrumental behavior (i.e., Pavlovian-to-instrumental transfer [PIT] effect), which was found to be stronger in prospectively relapsing AD patients than in abstaining patients. Second, an automatic approach bias toward alcohol stimuli was observed in AD patients, and interventions targeting this bias reduced the relapse risk in some studies. Previous findings suggest a potential behavioral and neurobiological overlap between these two mechanisms.

**METHODS:** In this study, we examined the association between alcohol approach bias and both behavioral and neural non–drug-related PIT effects in AD patients after detoxification. A total of 100 AD patients (17 females) performed a PIT task and an alcohol approach/avoidance task. Patients were followed for 6 months.

**RESULTS:** A stronger alcohol approach bias was associated with both a more pronounced behavioral PIT effect and stronger PIT-related neural activity in the right nucleus accumbens. Moreover, the association between alcohol approach bias and behavioral PIT increased with the severity of alcohol dependence and trait impulsivity and was stronger in patients who relapsed during follow-up in the exploratory analysis.

**CONCLUSIONS:** These findings indicate partial behavioral and neurobiological overlap between alcohol approach bias and the PIT effect assessed with our tasks. The association was stronger in patients with more severe alcohol dependence.

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Alcohol-dependent (AD) patients frequently relapse after detoxification despite their intention to remain abstinent (1). Pavlovian conditioning has been hypothesized to contribute to relapse, because environmental cues associated with alcohol intake can become conditioned stimuli (CS) that elicit drug craving and may bias instrumental behavior toward drug seeking [e.g., (2–4)]. This phenomenon—the impact of environmental cues on instrumental behavior (i.e., Pavlovian-to-instrumental transfer [PIT] effect)—has been investigated in animal and human studies. Ethanol-associated cues can promote seeking behavior for not only ethanol but also non–ethanol-related reward in ethanol-treated rats (5). Repeated drug intake can further enhance non–drug-related reward-seeking elicited by nondrug cues in rats (6–10). Comparable alterations in motivational processes have also been observed in humans: enhanced non–drug-related PIT effects have been observed in AD patients compared with healthy control subjects and in prospectively relapsing patients compared with abstaining patients (11–13). Functional magnetic resonance imaging (fMRI) studies revealed that PIT effects can induce activation in the nucleus accumbens (NAcc) (14–17). This brain area has long been associated with reinforcement learning (18,19), processing of alcohol cues, and craving (20).

It has been suggested that relapse can be triggered by an automatic approach tendency to alcohol stimuli, which was observed to be stronger in AD patients and heavy drinkers in some studies [e.g., (21–23)]. An alcohol approach bias in a laboratory can be operationalized as a shorter response latency to approach alcohol cues than to avoid them, even though the content of alcohol cues is task irrelevant. Applying an alcohol approach/avoidance task (aAAT), there is evidence that alcohol approach bias was positively associated with past hazardous drinking and future drinking (24,25). Inconsistently, there are studies using a stimulus-response compatibility task that found no approach bias or even an avoidance bias toward alcohol in AD patients (26,27) and a predictive role of the avoidance bias in future drinking or relapse (27,28). The discrepant findings could partly be explained by differences in the tasks (29). Cognitive bias modification (CBM) intervention adapted from the aAAT to retrain the approach bias has shown promising effects on decreasing relapse risk in AD patients [e.g., (30–33)].

The previous findings indicate that both alcohol approach bias and PIT effect are closely associated with alcohol dependence. Although the association between alcohol approach bias and PIT effect in AD patients has so far not been directly investigated, previous theories and findings indicated a potential overlap between these two phenomena. Theoretically, in view of a dual-process model, an automatic approach bias was suggested to occur when the appetitive stimulus activates an impulsive or automatic system, which cannot be overridden by cognitive control processes because this reflective system is weakened (34). Automatic or impulsive approach biases may then drive addictive behavior (35), promoting drug seeking despite long-term harm (36). The strength of a non–drug-related PIT effect in AD patients has also been associated with impulsivity as assessed by a delay-discounting task (12). Moreover, impairments in inhibiting automatic approach biases to appetitive Pavlovian stimuli in a non–drug-related PIT task predicted relapse risk in AD patients (13). Based on those findings, we hypothesize that the impulsivity process could contribute to linking alcohol approach bias with PIT effects. On the neural level, neuroimaging studies also suggest that the underlying neurobiological mechanisms of the two effects overlap at least partly, because functional NAcc activation relates to both alcohol approach bias (37) and PIT effects (14–17).

This study tested the hypothesis that alcohol approach bias is associated with behavioral and neural correlates of the non–drug-related PIT effect in recently detoxified AD patients. We hypothesized that patients with a stronger alcohol approach bias would show a more pronounced PIT effect behaviorally and in the NAcc. Furthermore, we hypothesized that the association between alcohol approach bias and PIT effect increases with the severity of alcohol dependence and trait impulsivity. In addition, we explored if the association between PIT and alcohol approach bias differed between prospective relapsers and abstainers with a 6-month follow-up.

## METHODS AND MATERIALS

### Participants

AD patients were assessed in a bicentric research project conducted in Berlin and Dresden, Germany (ClinicalTrials.gov identifier: NCT02615977). The study was approved by local ethics committees of Charité Universitätsmedizin Berlin (EA1/268/14) and Technische Universität Dresden (EK 300082014). All participants gave written informed consent before participation.

Patients fulfilled the criteria of alcohol dependence according to the DSM-IV-TR, assessed by the Munich Composite International Diagnostic Instrument (38,39). After data cleaning (Supplement), 100 AD patients (age [mean ± SD] = 46.86 ± 10.30 years; 17 females; abstinence before study participation [mean ± SD]: 22.44 ± 12.77 days) were included for behavioral analyses and a subcohort of 72 patients (age [mean ± SD] = 44.97 ± 9.64 years; 10 females; abstinence before study participation [mean ± SD]: 23.33 ± 12.54 days) for imaging analyses.

The severity of alcohol dependence was measured by the Alcohol Dependence Scale (ADS) (40). Trait impulsivity was assessed by the Barratt Impulsiveness Scale-15 (BIS-15) (41).

This study was conducted within a research consortium (DFG FOR 1617 and CRC-TRR 265), which also applied other tasks not reported in this paper. In addition, a subset of the participants underwent CBM training after the task assessments (nonsignificant training effects will be reported elsewhere). Patients had follow-ups 6 months (at weeks 6, 10, 14, 18, 22, and 26) after study participation and retrospectively reported their alcohol consumption since the last follow-up interview using the Timeline Follow-Back (42). We applied an intention-to-treat analysis (43) and classified all patients who relapsed to heavy drinking (i.e., ≥5 standard drinks [e.g., one standard drink = 0.33 L beer] for males and ≥4 standard drinks for females consumed on one drinking occasion), did not respond, or had incomplete follow-up information as belonging to the relapser group [as in (30,31,44)], while the rest of the patients were categorized as abstainers. We additionally conducted explorative analyses that included only patients with clear relapse status and reported results in the Supplement.

### The aAAT

In this task, images of drink (alcohol drink or soft drink) were randomly presented inclined to the left or the right, and participants pulled or pushed a joystick (approach or avoid) according to the inclination of the image (see detailed description in the Supplement).

### PIT Task

Participants performed an instrumental task (pressing the button to collect shells) while monetary CS learned from Pavlovian training were presented in the background [see the Supplement and (11–13) for a detailed description].

### MRI Acquisition

Functional imaging was performed on Siemens Trio 3T MRI scanners at both study centers using echo-planar imaging sequences (repetition time: 2410 ms; echo time: 25 ms; flip angle: 80°; field of view: 192 × 192 mm²; voxel size: 3 × 3 × 2 mm³) comprising 42 slices approximately −25° to the bicommissural plane. We acquired a three-dimensional magnetization-prepared rapid gradient echo image (repetition time: 1900 ms; echo time: 5.25 ms; flip angle: 9°; field of view: 256 × 256 mm²; 192 sagittal slices; voxel size: 1 × 1 × 1 mm³) for coregistration and normalization during fMRI data preprocessing. A field map was collected before functional scanning to account for individual homogeneity differences of the magnetic field.

### Data Analysis

Data were analyzed using MATLAB R2020b (MATLAB version 9.9, 2020; The MathWorks, Inc.) and the R System for Statistical Computing version 4.0.3 (R Development Core Team, 2020). SPM12 software package (http://www.fil.ion.ucl.ac.uk/spm/; Wellcome Centre for Human Neuroimaging) was used for fMRI data analyses.

### Behavioral Analyses

For the aAAT, 6 patients were excluded because of excessive errors (>35%) (30,31). To exclude extreme outlier response times, the 1% fastest and 1% slowest responses were excluded

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in the overall response time distribution, consistent with the method used in previous studies (33,45–47). Trials with incorrect responses on the first try were also discarded. In line with Wiers et al. (31), D scores were calculated to reflect the approach bias to each stimulus category (see below). We further calculated a D-diff score to reflect an approach bias to alcohol relative to soft drink:

$$D\ score_{alcohol} = (Push\ median\ RT_{alcohol}$$
$$-Pull\ median\ RT_{alcohol})/Personal\ SD$$

$$D\ score_{soft\ drink} = (Push\ median\ RT_{soft\ drink}$$
$$-Pull\ median\ RT_{soft\ drink})/Personal\ SD$$

$$D-diff\ score = D\ score_{alcohol}-D\ score_{soft\ drink}$$

where RT is the response time and personal SD is the standard deviation of all response times including alcohol trials and soft drink trials, per participant.

For the PIT task, 10 patients who did not successfully learn the correlation between Pavlovian CS and unconditioned stimuli (i.e., performance in the forced choice task was not above chance) were excluded from analyses. A generalized linear mixed-effects model (GLMM) [R package lme4 (48)] with Poisson distribution was used to predict the number of button presses in each trial in the transfer part. The number of button presses depending on Pavlovian CS value was used to assess the behavioral PIT effect to be consistent with the imaging analyses (see Imaging Analyses). Parameters of Pavlovian CS value (i.e., the monetary value of Pavlovian CS in the background: +2, +1, 0, −1, −2), trial type of the instrumental condition (go and no-go; coded as +0.5 vs. −0.5), the individual alcohol approach bias (i.e., D-diff score in the aAAT), the interaction of Pavlovian CS value and D-diff score, and the interaction of instrumental condition and D-diff score were included as fixed effects in the GLMM. Subject IDs, instrumental stimuli (shells), and Pavlovian CS (fractal combined with pure tone) were treated as random effects to be controlled.

In addition, we further established GLMMs with additional parameters of ADS score and BIS-15 score separately and their interaction with other predictors (i.e., Pavlovian CS value and D-diff score) to examine if the association between alcohol approach bias and PIT effect interacts with those factors. We applied another GLMM to explore if this association differed between patients who abstained from alcohol and those who relapsed in follow-up.

### Imaging Analyses

Nipype (49) was used for preprocessing the PIT fMRI data. First, correction for differences in slice time acquisition was performed to the middle slice as reference. Based on acquired field maps, voxel displacement maps were estimated. Images were realigned to correct for head motion, distortion, and their interaction. Coregistration of the individual structural T1 image to the individual mean echo-planar imaging was conducted. Then, 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 echo-planar imaging images. Finally, images were partially smoothed with a Gaussian kernel of 8-mm full width at half maximum. Before statistical analysis, data were high-pass filtered with a cutoff of 128 seconds to remove low-frequency fluctuation in the blood oxygen level–dependent signal.

After preprocessing, individual general linear models were established in SPM12. Non–drug-related PIT trials were modeled as one condition with three parametric modulators: the Pavlovian CS value, the transformed number of button presses [calculated as ln(the original number of button presses + e)], and the PIT parameter, which is the product of the Pavlovian CS value and the transformed number of button presses. We added e (Euler's number) to the log transformation function for the number of button presses so that 0 button presses would be transformed to 1, resulting in different numerical values in the PIT parametric modulator after weighing by different Pavlovian CS values. In the end, a higher number of button presses to a higher Pavlovian CS value leads to a higher numerical value in the PIT parametric modulator. To account for variance caused by motor responses associated with button pressing, button presses of all trials were modeled in an additional regressor as stick functions. Drug-related PIT trials with similar parametric modulators as a separate condition and the realignment parameters with derivatives were included as regressors of no interest. The individual neural PIT effect was measured with a contrast in which the non–drug-related PIT parametric modulator was weighted with 1 and other regressors weighted with 0.

At the second-level analysis, a one-sample t test was established with individual contrast images. Individual alcohol approach bias (i.e., D-diff score) was treated as a covariate of interest in the model. In addition, participants' age, sex, and study center were taken as additional covariates to control their potential impact on the results. Consistent with Garbusow et al. (11), a region of interest analysis was conducted with an a priori–defined compound region of interest in the left and right NAcc (NAcc_L, NAcc_R) (derived from the Wake Forest University PickAtlas software; http://www.fmri.wfubmc.edu/download.htm). Moreover, we performed an explorative whole-brain analysis for the main PIT effect on a significance level of uncorrected $p < .001$ and with $k \geq 20$ activated voxels per cluster (Supplement). In addition, similar to the behavioral analysis, we also explored if retrospective relapsers and abstainers differ in the association of alcohol approach bias and neural PIT effect.

### RESULTS

#### Behavioral Results

Patients showed a significant behavioral PIT effect—more button presses in the presence of higher monetary value–associated Pavlovian CS (main effect of Pavlovian CS value: estimate = 0.28, $z = 77.81$, $p < .001$) (Table 1). Moreover, there was a significant association of PIT effect elicited by the Pavlovian CS value with aAAT D-diff score (Pavlovian CS value × D-diff score: estimate = 0.14, $z = 11.34$, $p < .001$). Patients with a stronger alcohol approach bias in the aAAT task showed a more pronounced PIT effect (Figure 1). For a visual inspection of the raw D-diff scores and individual PIT slopes, see the Supplement.

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**Table 1. Results of the Generalized Linear Mixed-Effects Model Regarding Effects of the Different Variables (Pavlovian CS Value and Instrumental Condition) and Association of Alcohol Approach Bias With Number of Button Presses in the PIT Task**

| Parameter | Estimate | SE | z | p |
|---|---|---|---|---|
| Intercept | 1.44 | 0.05 | 28.80 | <.001 |
| D-Diff Score | 0.01 | 0.13 | 0.09 | .93 |
| Pavlovian CS Value | 0.28 | 0.004 | 77.81 | <.001 |
| Instrumental Condition (Go vs. No-Go) | 0.56 | 0.04 | 15.93 | <.001 |
| D-Diff Score × Pavlovian CS Value | 0.14 | 0.01 | 11.34 | <.001 |
| D-Diff Score × Instrumental Condition | −0.06 | 0.04 | −1.74 | .083 |

CS, conditioned stimulus; PIT, Pavlovian-to-instrumental transfer.

When including alcohol dependence severity (i.e., ADS score) in the GLMM, the results showed a significant interaction effect (Pavlovian CS value × D-diff score × ADS score: estimate = 0.02, $z$ = 12.51, $p$ < .001). Specifically, the more severe the alcohol dependence of the patient, the stronger the association between aAAT score and PIT effect (Figure 2A). Similar results also showed in the model with trait impulsivity (i.e., BIS-15 score) (Pavlovian CS value × D-diff score × BIS-15 score: estimate = 0.04, $z$ = 14.58, $p$ < .001) (Figure 2B). The association between alcohol approach bias and PIT effect increased with trait impulsivity. It should be noted that ADS score was positively correlated with BIS-15 score (rho = 0.24, $p$ = .026, Spearman rank correlation) (see the Supplement for a visual inspection of the raw data).

For the exploratory analysis regarding aAAT D-diff score and PIT association between abstainers ($n$ = 21) and relapsers ($n$ = 79) using the intention-to-treat analysis approach, results yielded a significant interaction of Pavlovian CS value, aAAT D-diff score, and relapse group (estimate = 0.08, $z$ = 2.34, $p$ = .020). Follow-up analyses examined the Pavlovian CS value × D-diff score interaction in abstainers and relapsers separately and showed a higher parameter estimate of Pavlovian CS value × D-diff score interaction in relapsers (estimate = 0.15, $z$ = 10.57, $p$ < .001) compared with abstainers (estimate = 0.07, $z$ = 2.43, $p$ = .015).

### Functional MRI Results

We observed a significant activation elicited by PIT in $NAcc_L$ (x = −10, y = 8, z = −10; $t_{67}$ = 2.92, small volume–corrected [SVC] and familywise error–corrected [FWE] $p_{SVC-FWE}$ = .035; voxel-based analysis) and $NAcc_R$ (x = 8, y = 8, z = −12, $t_{67}$ = 3.29, $p_{SVC-FWE}$ = .014).

More importantly, we observed a significant effect of aAAT D-diff score on PIT-related blood oxygen level–dependent signals in $NAcc_R$ (x = 16, y = 14, z = −12; $t_{67}$ = 3.40, $p_{SVC-FWE}$ = .010) (Figure 3) and a trendwise effect in $NAcc_L$ (x = −14, y = 12, z = −12; $t_{67}$ = 2.74, $p_{SVC-FWE}$ = .053).

Exploratory analyses with 17 abstainers and 55 relapsers did not find a significant difference between the two subgroups in the association between aAAT D-diff score and neural PIT effect in either $NAcc_R$ (relapsers > abstainers: x = 14, y = 4, z = −14, $t_{65}$ = 0.98, $p_{SVC-FWE}$ = .667; abstainers > relapsers: x = 16, y = 14, z = −12, $t_{65}$ = 0.42, $p_{SVC-FWE}$ = .815) or $NAcc_L$ (relapsers > abstainers: x = −14, y = 8, z = −8, $t_{65}$ = −0.04, $p_{SVC-FWE}$ = .870; abstainers > relapsers: x = −12, y = 8, z = −12, $t_{65}$ = 1.33, $p_{SVC-FWE}$ = .523).

### DISCUSSION

This study examined the association between a non–drug-related PIT effect and automatic alcohol approach bias in AD patients. These two paradigms were chosen because both may reflect an impulsive approach bias (12,34), one alcohol cue–related and one reflecting an effect of nondrug Pavlovian cues, and because approach effects assessed in both paradigms have been associated with poor treatment outcomes or greater future drinking (11,13,25). Our key finding is that detoxified AD patients who had a stronger alcohol approach bias (relative to soft drinks) displayed a higher behavioral PIT effect (i.e., a stronger effect of Pavlovian CS in the background on unrelated instrumental behavior, indicated by more button presses) and a stronger PIT-related functional activation of $NAcc_R$. Furthermore, as expected, the association between alcohol approach bias and behavioral PIT effect increased with the severity of alcohol dependence and trait impulsivity. These findings link two well-established paradigms in alcohol research and indicate at least partially shared underlying mechanisms between alcohol approach bias and behavioral PIT effect.
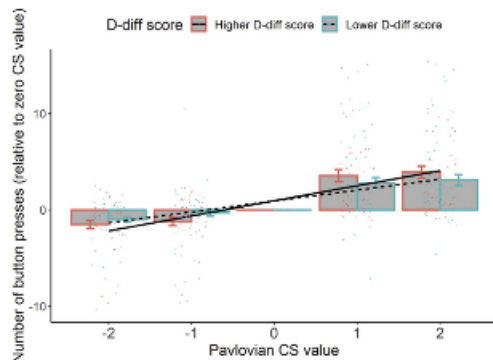


**Figure 1.** Patients who displayed a stronger alcohol approach bias (a higher D-diff score in the alcohol approach/avoidance task) showed a higher Pavlovian-to-instrumental transfer effect (a steeper slope) than patients who had a lower alcohol approach bias (a lower D-diff score). The continuous D-diff score was transferred to a factor with two levels with a median split in this figure for illustration. Group means and SEMs are shown with bars and error bars. Individual values (mean number of button presses) are represented by colored dots. CS, conditioned stimulus.

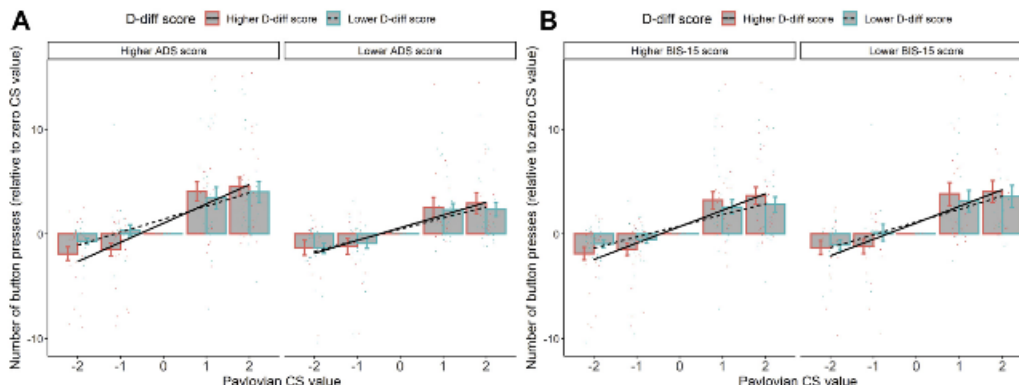The Association of Alcohol Approach Bias With PIT

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**Figure 2.** **(A)** Patients with higher Alcohol Dependence Scale (ADS) scores had a stronger association between alcohol approach bias (i.e., D-diff score) and the Pavlovian-to-instrumental transfer effect. **(B)** Patients with higher Barratt Impulsiveness Scale-15 (BIS-15) scores showed a stronger association between alcohol approach bias and the Pavlovian-to-instrumental transfer effect. Alcohol approach bias, ADS score, and BIS-15 score in this figure were all transferred to factors with two levels with a median split for illustration. Group means and SEMs are shown with bars and error bars. Individual values (mean number of button presses) are represented by colored dots.

From the perspective of dual-process accounts, alcohol approach bias is mainly driven by a system associated with impulsive and automatic decision making (34), and PIT effect has also been associated with choice impulsivity in AD patients



**Figure 3.** Strength of the alcohol approach bias was associated with Pavlovian-to-instrumental transfer–related neural activation in the nucleus accumbens. The bilateral nucleus accumbens region of interest is marked in blue, and functional Pavlovian-to-instrumental transfer activation associated with alcohol approach bias is marked in yellow (uncorrected $p < .005$ for illustration).

(12). In this study, the association between the two effects was indeed larger in patients who reported higher trait impulsivity. Conditioned cues in the PIT paradigm were associated with monetary reward, while conditioned cues in the aAAT task reflect drug versus nondrug cues. These findings suggest that impulsive decision making can be triggered by the impact of drug-related and drug-unrelated cues on approach behavior in AD patients.

The severity of alcohol dependence might modulate the association of these two effects, because stronger associations between PIT and aAAT effects were found among patients with more severe alcohol dependence. Correlations are not causations, and potential explanations for these observations indicate two directions of further research. First, a stronger effect of nondrug Pavlovian background cues on approach behavior was already observed in young adults with higher versus lower levels of alcohol intake (50) and may reflect a risk factor for excessive consumption. Second, higher levels of alcohol intake can impact monoaminergic neurotransmission and promote associative learning of drug-related and contextual cues (51,52), thus potentially modifying cue-induced approach biases. With more severe alcohol dependence and higher levels of drug intake, the impact of conditioned cues on fast and impulsive decision making can increase, which may then lead to the observed, stronger association between cue effects assessed with both aAAT and the PIT paradigm. In this study, we observed a positive correlation between the severity of alcohol dependence (i.e., ADS score) and trait impulsivity (i.e., BIS-15 score), which emphasizes the role of impulsive decision making in more severe forms of alcohol dependence. Again, impulsive decision making can be both a cause and a consequence of excessive alcohol intake, because alcohol is known to impact not only monoaminergic systems but also prefrontal cortical brain areas associated with impulse control (53). Future studies in nonclinical high risky drinkers are needed to longitudinally assess the development of associations between impulsive
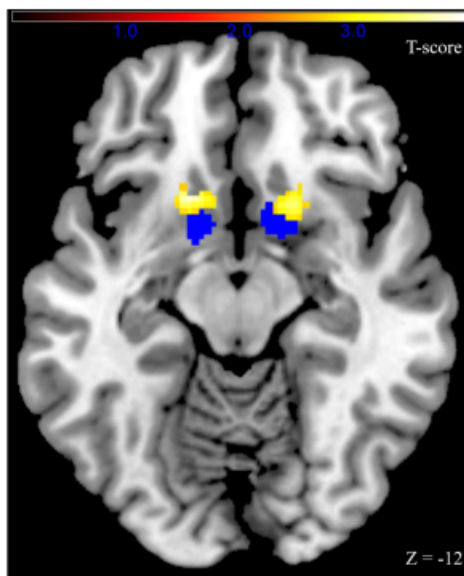
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decision making, conditioned cues responses, and alcohol intake.

On the neural level, functional NAcc activation has been associated with both PIT effect (14–17) and alcohol approach bias (54). In this study, the strength of the behavioral alcohol approach bias was associated with the PIT-related functional activation of NAcc$_R$. Previous literature suggested a lateralized dopamine function in the NAcc, with dopamine release in NAcc$_R$ reflecting the impact of drink-related CS (i.e., beer flavor) (55). In this study, NAcc$_R$ was related to the association between alcohol approach bias and neural PIT effect, which underlies the role of this brain area in mediating the effects of Pavlovian conditioned cues as assessed in both paradigms. In the aAAT, Pavlovian conditioning to alcohol stimuli has been established during prolonged alcohol consumption, while in the PIT task, the Pavlovian conditioning was drug-unrelated and had been established in a laboratory setting. The correlation of the two effects is likely to reflect a more general alteration in the Pavlovian learning processes in alcohol dependence. Future longitudinal research can help assess changes in Pavlovian conditioning across the addiction cycle.

Some research has shown the predictive role of alcohol approach bias in drinking behavior (24,25). The CBM intervention targeted on retraining alcohol approach bias showed evidence of reducing the relapse risk in AD patients [e.g., (30,31)]. In contrast, the instrumental go/no-go responses in PIT can also be understood as an approach/no-approach behavior. The stronger impact of environmental cues on approach/no-approach behavior in the PIT task was particularly pronounced in prospective relapsers compared with abstainers (13). Our exploratory analysis compared subsequent relapsers with abstainers and observed a stronger association between alcohol approach bias and PIT effect in relapsers, indicating a potential role of the association of two approach behaviors in predicting treatment outcome.

Several limitations should be addressed. First, we lost track of a substantial number of patients during follow-up, which limits the interpretation of our exploratory analysis regarding the treatment outcome. Our study categorized patients with missing follow-up information as relapsers, in accordance with the method used in previous studies under the assumption that missing data is indicative of relapse [e.g., (30,31,44)]. When excluding patients who had unclear relapse status ($n$ = 49) from the analysis, there was no more significant group difference between relapsers and abstainers in the association between alcohol approach bias and behavioral PIT (Supplement). We suspect that this null effect could be due to the insufficient statistical power of the small sample size. Future studies are warranted to elucidate the predictive role of the association between alcohol approach bias and PIT regarding relapse. Second, abstaining in our study was defined as not relapsing to heavy drinking. Other studies with different abstaining definitions (e.g., no alcohol consumption at all) might have different results. Third, most of the participants in this study underwent a CBM training procedure after conducting the aAAT and PIT, which we expected to reduce the relapse risk in AD patients. However, the relapse ratio did not differ between the training and placebo groups (results will be reported elsewhere). Considering that the null effect of training on relapse status could be due to insufficient statistical power

(56), we included the training condition as a covariate in additional analyses. By doing that, we still observed a statistically significant interaction of treatment outcome (relapsers versus abstainers, categorizing patients lost to follow-up as relapsers) with the association between D-diff score and behavioral PIT and no difference between relapsers and abstainers in the association between D-diff score and neural PIT in either NAcc$_R$ or NAcc$_L$. There is no indication that the training impacted findings regarding relapse in this study.

In conclusion, our study observed a significant association between alcohol approach bias and behavioral and neurobiological non–drug-related PIT effect in AD patients, and the behavioral association was correlated with the severity of alcohol dependence and trait impulsivity. These findings indicate at least a partial overlap of the underlying mechanisms of learning and decision making assessed in both paradigms and emphasize their relevance for severe alcohol use disorders.

### ARTICLE INFORMATION

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# Alcohol Approach Bias Is Associated With Both Behavioral and Neural Pavlovian-to-Instrumental Transfer Effects in Alcohol-Dependent Patients

## *Supplement*

### Participant recruitment and exclusion criteria

Alcohol-dependent (AD) patients were recruited during detoxification treatment in addiction-specific, psychiatric wards of university hospitals. All participants were aged between 18 to 65, and were fluent in German. Exclusion criteria were: other substance dependence (except nicotine dependence); current substance use (assessed by drug urine test); alcohol intoxication (assessed by alcohol breath test); major psychiatric disorders assessed by M-CIDI; neurological disorders; medications that are known to interact with the central nervous system (less than four half-lives post last intake). Patients had no or low alcohol withdrawal symptoms for 3 days before fMRI as assessed by Clinical Institute Withdrawal Assessment for Alcohol revised version (CIWA-Ar score < 4; 1). The sample sizes for different analyses are shown in Figure S1.
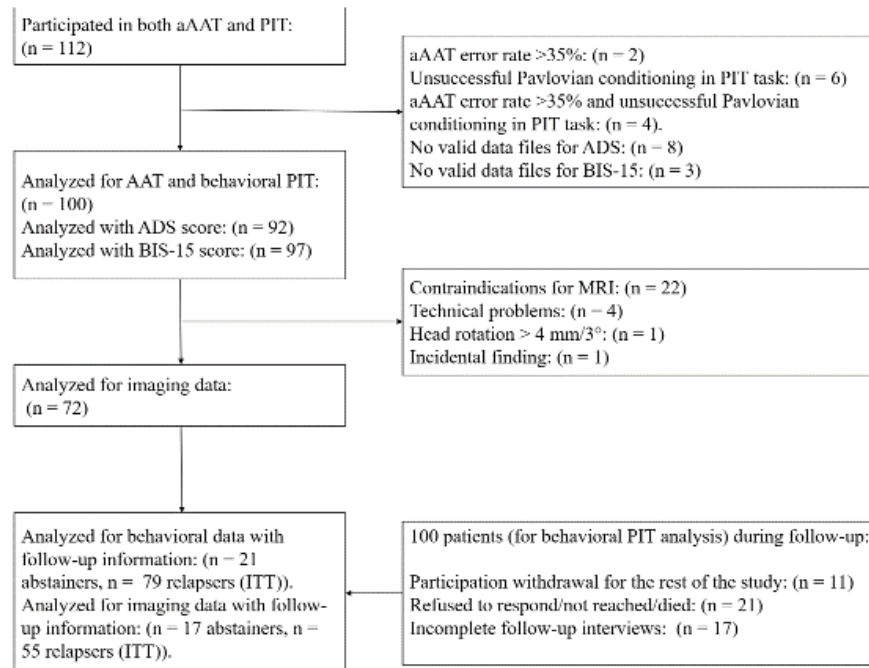
**Figure S1:** the flow chart of sample sizes for behavioral and imaging analyses. aAAT: alcohol approach/avoidance task; ADS: Alcohol Dependence Scale (2); BIS-15: Short German version of the Barrat Impulsiveness Scale-15 (3); ITT: intention-to-treat; PIT: Pavlovian-to-instrumental transfer task. Details regarding data exclusion based on aAAT and PIT performances are illustrated in the data analysis part in the main text.

**Alcohol approach/avoidance task**

Twenty-one alcohol drink images and 21 soft drink images were used in this task. In each trial, one of those images randomly presented inclined to the left or to the right on the screen. Pictures of each stimulus category were presented equally often as inclined to either side, and participants responded with a joystick movement according to the inclination of the image. For example, they had to pull the joystick towards themselves (approach) if the image inclined to the left and to push the

joystick away (avoidance) if it inclined to the right (see Figure S2). The

correspondence between left/right inclination and push/pull responses was

counterbalanced across participants. There was no response time limitation, and

participants had to correct their response in case of a wrong action. Only when the

trial was accurately responded or corrected, a new trial started. Pulling the joystick

enlarged the image while pushing the joystick minimized the image with a zooming

motion. Participants conducted 26 practice trials with drink-unrelated neutral images

and then 168 experimental trials. The aAAT was conducted outside the fMRI scanner.



**Figure S2**: A push trial in alcohol approach/avoidance task (aAAT). An alcohol drink
image tilted to the right and thus needed to be pushed away. By pushing the joystick,
the picture was minimized.

**Pavlovian-to-instrumental transfer task**

There were four experimental phases in the PIT task.

   *(1) Instrumental training*. Participants underwent a probabilistic instrumental

training and learned to emit a go or a no-go response for each of six instrumental shell

stimuli. For a "good" shell, collecting it (i.e., a "go" response) by repeatedly pressing

the button for five or more times would lead to a monetary reward of 20 cents in 80%

of the trials and a loss of 20 cents in 20% of the trials, while not collecting it (i.e., a

"no-go" response) by pressing the button less than five times or no button pressing

would lead to the monetary reward with a probablilty of 20% and to the monetary loss

with a probability of 80% (Figure S3 (a)). For a "bad" shell, the probability of

monetary reward/loss corresponding to a go/no-go action was reversed. Participants

should complete a minumum of 60 trials and have 80% correct responses in 16

consecutive trials, or a maximum of 120 trials.

(2) *Pavlovian training.* In each trial, a compound stimulus (conditioned stimulus,

CS) consisting of a fractal picture and a pure tone was presented simulatenously with

an unconditioned stimulus (US: monetary gain or loss) after a delay of 500 ms.

Participants were instructed to passively watch and memorize the pairings. There

were 80 trials in the Pavlovian training phase.

(3) *Pavlovian-to-instrumental transfer (PIT).* In this part, participants performed

the same instrumental task as in the first phase. A CS learned from the Pavlovian

training or a beverage image (i.e., alcohol drink or water) that was not introduced in

the previous phase tiled the background of the instrumental shell in each trial. Ninety

trials with Pavlovian CS background and 72 trials with beverage image background

were implemented. Trials with beverage image background were out of the scope of

the current paper. The instrumental task was independent of the value of the CS. No

feedback was given at the end of each trial in this phase to avoid further instrumental

learning. However, participants were instructed that their actions were counted to the final monetary outcome.

(4) *Forced choice task*. The forced choice task was used to exmaine the efficacy of the Pavlovian training. In each trial, participants chose one CS over another between two CSs that presented sequentially. All possible CS pairings were presented three times in randomized order. Each choice trial was pesented for 2 sec.

Three phases (i.e., instrumental training, pavlovian training and forced choice task) were conducted outside the fMRI scanner, while the transfer part was conducted inside the scanner.
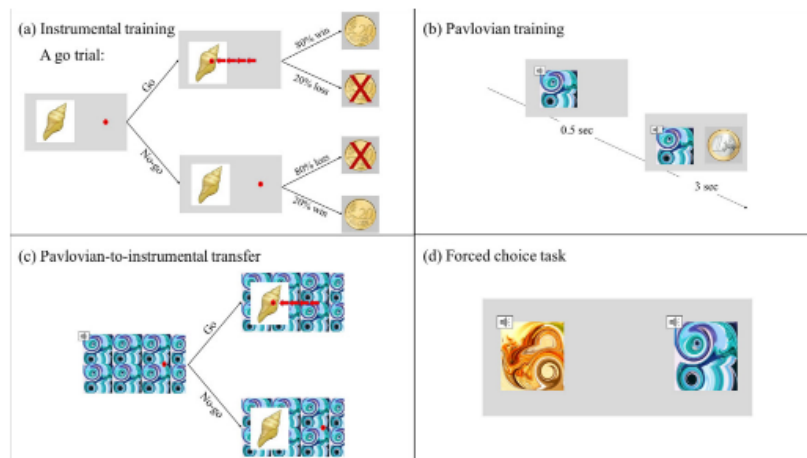


**Figure S3.** Pavlovian-to-instrumental transfer (PIT) task. (a): Instrumental training: participants learn to collect "good" shells (go trials) and leave "bad" shells (no-go trials) with probabilistic outcomes. A go trial was depicted in the figure. Collecting the shell would lead to a reward of 20 cents with 80% probability and to a loss of 20 cents with 20% probability, while vice versa for not collecting it. The probability of reward/loss after an action of go/no-go was reversed for a "bad" shell (not depicted here). (b): Pavlovian training: a Pavlovian conditioned stimulus (CS) consisting of a fractal and a pure tone was paired with an unconditioned cue (US), i.e., a picture of

coin (-2€, -1€, 0€, +1€, +2€). Negative USs were presented as coins with a superimposed red cross. Participants passively viewed the trials and remembered the pairings. (c): Pavlovian-to-instrumental transfer: participants were instructed to perform the instumental actions as learned from instrumental training with a Pavlovian CS tiling the backgroud. (d): Forced choice task: two Palovian CSs simulatenously presented on the screen and participants were instructed to choose the most appealing one.

## Neural PIT effect – whole brain analyses

**Table S1.** Explorative whole-brain analyses: activations for the PIT effect at $p_{unc}$ < .001 with cluster extend k > 20.

| | | Cluster | | | Peak | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $k$ | $p$ (FWE corrected) | $p$ (unc) | $t$ | MNI-Coordinates | | | $p$ (FWE corrected) | $p$(unc) |
| | | | | | | x | y | z | | |
| BA11- Anterior cingulate and paracingulate gyri | L | 441 | .02 | .004 | 4.19 | -6 | 40 | -6 | .33 | < .001 |
| | | | | | 4.05 | 8 | 48 | -4 | .44 | < .001 |
| | | | | | 3.69 | -6 | 46 | 8 | .77 | < .001 |
| BA21- Superior temporal gyrus | R | 105 | .42 | .12 | 4.07 | 66 | -28 | 4 | .43 | < .001 |
| | | | | | 3.79 | 60 | -20 | 4 | .68 | < .001 |
| BA22- Superior temporal gyrus | L | 31 | .83 | .39 | 3.81 | -64 | -32 | 12 | .67 | < .001 |
| BA54- ParaHippocampal gyrus | L | 54 | .69 | .26 | 3.63 | -32 | -36 | -8 | .83 | < .001 |
| | | | | | 3.49 | -40 | -26 | 2 | .91 | < .001 |
| | | | | | 3.47 | -40 | -34 | -4 | .92 | < .001 |
| BA24- Anterior cingulate and paracingulate gyri | L | 21 | .89 | .49 | 3.52 | -2 | 32 | 14 | .90 | < .001 |

*Note.* BA, Brodmann area; FWE, family-wise error; L, left hemisphere; MNI, Montreal Neurological Institute; R, right hemisphere.

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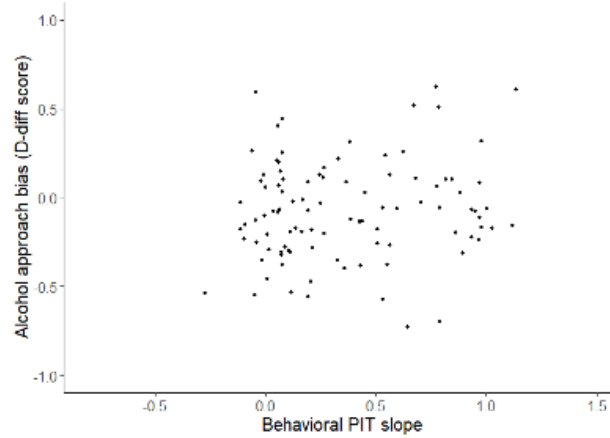## Visual inspection of behavioral PIT slopes and D-diff scores



**Figure S4.** Scatter plot for behavioral PIT slopes and D-diff scores

*Note.* The behavioral PIT slopes were the extracted slopes from a generalized linear mixed-effects model used for the behavioral PIT analysis to reflect the strength of the individual PIT effect.

## The correlation between alcohol dependence severity and trait impulsivity
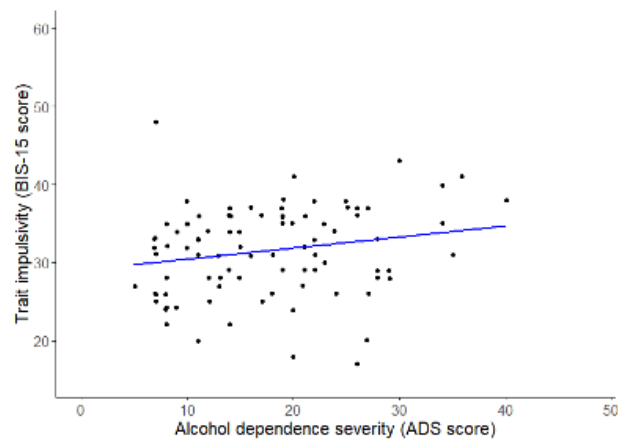


**Figure S5.** The correlation between the ADS score and the BIS-15 score

*Note.* ADS: Alcohol Dependence Scale (2), possible score range is 0 to 48; BIS-15: Barrat Impulsiveness Scale-15 (3), possible score range is 15 to 60. The blue line shows the linear correlation between the ADS score and the BIS-15 score (rho = .24, p = .026, Spearman rank correlation).

**Exploratory analyses including only patients with clear relapse status**

*Behavioral result*

When including only patients with clear relapse status (n = 21 abstainers and 30 relapsers; n = 49 with unclear relapse status were removed here) into analysis, there was no significant interaction of Pavlovian CS value, aAAT D-diff score, and relapse group (estimate = -0.03, z = -0.81, p = .418), which did not support a difference between abstainers and relapsers in the association between the alcohol approach bias and the behavioral PIT effect.

*fMRI results*

When only patients with clear relapse status were included into analysis, as found before, there was no significant group differences in the association between the alcohol approach bias and the neural PIT effect in either the right NAcc or the left NAcc (right NAcc (relapsers > abstainers: x = 6, y = 8, z = -8, t (32) = 0.95, $p_{svc-FWE}$ = .694; abstainers > relapsers: x= 16, y = 14, z = -12, t (32) = 1.13, $p_{svc-FWE}$ = .630; left NAcc (relapsers > abstainers: x = -14, y = 2, z = -12, t (32) = -0.25, $p_{svc-FWE}$ = .892; abstainers > relapsers: x= -10, y = 10, z = -12, t (32) = 2.34, $p_{svc-FWE}$ = .156).

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**Automatic Approach Behaviors in Alcohol Dependence: Does a Cognitive Bias Modification Training Affect Pavlovian-to-Instrumental-Transfer Effects?**

Journal Data Filtered By: **Selected JCR Year: 2021** Selected Editions: SCIE,SSCI
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| 2 | NATURE NEUROSCIENCE | 82,161 | 28.771 | 0.11604 |
| 3 | TRENDS IN COGNITIVE SCIENCES | 36,688 | 24.482 | 0.03441 |
| 4 | Nature Human Behaviour | 11,204 | 24.252 | 0.04187 |
| 5 | BEHAVIORAL AND BRAIN SCIENCES | 12,872 | 21.357 | 0.00816 |
| 6 | BRAIN BEHAVIOR AND IMMUNITY | 31,770 | 19.227 | 0.03902 |
| 7 | Molecular Neurodegeneration | 8,377 | 18.879 | 0.01206 |
| 8 | NEURON | 121,714 | 18.688 | 0.15024 |
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| 10 | ACTA NEUROPATHOLOGICA | 30,046 | 15.887 | 0.03384 |
| 11 | Annual Review of Neuroscience | 15,839 | 15.553 | 0.00985 |
| 12 | BRAIN | 69,241 | 15.255 | 0.05988 |
| 13 | MOLECULAR PSYCHIATRY | 33,324 | 13.437 | 0.04914 |
| 14 | BIOLOGICAL PSYCHIATRY | 51,087 | 12.810 | 0.03831 |
| 15 | NEUROPSYCHOBIOLOGY | 3,757 | 12.329 | 0.00343 |
| 16 | PSYCHIATRY AND CLINICAL NEUROSCIENCES | 6,445 | 12.145 | 0.00577 |
| 17 | JOURNAL OF PINEAL RESEARCH | 13,422 | 12.081 | 0.00692 |
| 18 | SLEEP MEDICINE REVIEWS | 12,620 | 11.401 | 0.01356 |
| 19 | Neurology-Neuroimmunology & Neuroinflammation | 5,161 | 11.360 | 0.01049 |
| 20 | ANNALS OF NEUROLOGY | 45,647 | 11.274 | 0.03862 |
| 21 | PROGRESS IN NEUROBIOLOGY | 15,980 | 10.885 | 0.00886 |

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**Chen K**, Garbusow M, Sebold M, Zech HG, Zimmermann U, Heinz A. Automatic Approach Behaviors in Alcohol Dependence: Does a Cognitive Bias Modification Training Affect Pavlovian-to-Instrumental-Transfer Effects? Neuropsychobiology. 2022;81(5):387-402. DOI: 10.1159/000526805

## Curriculum Vitae

My curriculum vitae does not appear in the electronic version on my paper for reasons of data protection.

## Publication List

1. **Chen K**, Schlagenhauf F, Sebold M, Kuitunen-Paul S, Chen H, Huys QJ, Heinz A, Smolka MN, Zimmermann US, Garbusow M. The association of non-drug-related Pavlovian-to-instrumental transfer effect in nucleus accumbens with relapse in alcohol dependence: a replication. Biological Psychiatry. 2023 Mar 15;93(6):558-65.
   Impact Factor (2021): 12.810

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   Impact Factor (2021): 4.093

3. **Chen K\***, Garbusow M\*, Sebold M, Kuitunen-Paul S, Smolka MN, Huys QJ, Zimmermann US, Schlagenhauf F, Heinz A. Alcohol approach bias is associated with both behavioral and neural Pavlovian-to-instrumental transfer effects in alcohol-dependent patients. Biological Psychiatry Global Open Science. 2022 Apr 14.
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