Received: 28 September 2018 | Revised: 15 December 2018 | Accepted: 8 January 2019

DOI: 10.1111/adb.12717

ORIGINAL ARTICLE

WILEY Addiction Biology SSA

Incubation of neural alcohol cue reactivity after withdrawal and its blockade by naltrexone

Patrick Bach[1,2] | Georg Weil[1] | Enrico Pompili[1] | Sabine Hoffmann[1,2] | Derik Hermann[1,2] | Sabine Vollstädt-Klein[1] | Karl Mann[1] | Ursula Perez-Ramirez[5] | David Moratal[5] | Santiago Canals[6] | Serdar M. Dursun[7] | Andrew J. Greenshaw[7] | Peter Kirsch[3] | Falk Kiefer[1,2] | Wolfgang H. Sommer[1,4]

[1] Department of Addictive Behavior and Addiction Medicine, Central Institute of Mental Health, University of Heidelberg, Medical Faculty Mannheim, Mannheim, Germany

[2] Feuerlein Center on Translational Addiction Medicine (FCTS), University of Heidelberg, Heidelberg, Germany

[3] Department for Clinical Psychology, Central Institute of Mental Health, University of Heidelberg, Medical Faculty Mannheim, Mannheim, Germany

[4] Institute of Psychopharmacology, Central Institute of Mental Health, University of Heidelberg, Medical Faculty Mannheim, Mannheim, Germany

[5] Center for Biomaterials and Tissue Engineering, Universitat Politècnica de València, Valencia, Spain

[6] Instituto de Neurociencias, Consejo Superior de Investigaciones Científicas and Universidad Miguel Hernández, San Juan de Alicante, Spain

[7] Department of Psychiatry, University of Alberta, Edmonton, Canada

Correspondence
Patrick Bach, Department of Addictive Behavior and Addiction Medicine, Central Institute of Mental Health, Heidelberg University, Medical Faculty Mannheim, Square J5, Mannheim D-68159, Germany.
Email: patrick.bach@zi-mannheim.de

Funding information
Deutsche Forschungsgemeinschaft, Grant/Award Number: SFB636; Horizon 2020 Framework Programme, Grant/Award Number: 668863-SyBil-AA; ERA-Net NEURON program, Grant/Award Number: FKZ 01EW1112-TRANSALC; European Union's Horizon 2020 research and innovation program, Grant/Award Number: 668863-SyBil-AA

Abstract

During the first weeks of abstinence, alcohol craving in patients may increase or "incubate." We hypothesize that Naltrexone (NTX) blocks this incubation effect. Here, we compared NTX effects on neural alcohol cue reactivity (CR) over the first weeks of abstinence and on long-term clinical outcomes to standard treatment. Male alcohol-dependent patients (n = 55) and healthy controls (n = 35) were enrolled. Participants underwent baseline psychometric testing and functional magnetic resonance imaging (fMRI) assessment of mesolimbic alcohol CR. Patients participated in a standard treatment program with the option of adjuvant NTX. They received another scan after 2 weeks of treatment. We found higher CR in several brain regions in patients versus healthy controls. CR significantly increased over 2 weeks in the standard treatment group (n = 13) but not in the NTX group (n = 22). NTX significantly attenuated CR in the left putamen and reduced relapse risk to heavy drinking within 3 months of treatment. Additionally, increased CR in the left putamen and its course over time predicted both NTX response and relapse risk. Carrier status for the functional OPRM1 variant rs1799971:A > G was considered but had no effect on NTX efficacy. In conclusion, NTX was most effective in patients with high CR in the left putamen. While the results from our naturalistic study await further confirmation from prospective randomized trials, they support a potential role of neural CR as a biomarker in the development of precision medicine approaches with NTX.

KEYWORDS

alcohol addiction, cue reactivity, fMRI, naltrexone, relapse

1 | INTRODUCTION

The consumption of alcohol is a major risk factor for death, disease, and disability.[1] Reducing alcohol-related harm is therefore a major public health priority. Despite the high prevalence, only few medications are available for treatment, with the opioid antagonist naltrexone (NTX) as a prototypical example, providing proof-of-concept for efficacy of alcohol use disorder (AUD) pharmacotherapy. However, these treatments suffer from modest effect sizes with numbers of patients needed to treat (NNT) ranging above 10 for one successful outcome.[2] Understanding the neural and behavioral mechanisms underlying the highly variable treatment response to antirelapse medications will be a key factor for improving individual treatment success and enhancing impact on clinical practice based on the principles of precision medicine[3,4]

Animal studies suggest that NTX blocks mu-opioid receptors (MORs) within the dopaminergic reward system and thereby produces its antirelapse effects (recently reviewed in the study of Hansson et al.[5] A meta-analysis of human laboratory studies found that NTX relative to placebo reduces the extent of subjective craving as well as the amount of alcohol consumption.[6] However, the factors that might account for the highly heterogeneous clinical outcome of NTX treatment are largely unknown. Functional magnetic resonance imaging (fMRI) has shown that alcohol cues can induce brain activity in striatal regions of both healthy consumers and AUD patients.[7-9] Patients with high neural cue reactivity (CR) were shown to respond favorably to NTX treatment.[10] Furthermore, persistent high CR in striatum and orbitofrontal cortex during the first 2 weeks of treatment was associated with an increased relapse risk.[11] Further, fMRI studies found significant interactions between NTX treatment and reduction of mesolimbic CR in the striatum,[12,13] prefronto-cortical areas, and the inferior frontal gyrus.[14] In addition, a recent study[13] indicated that patients with greater reduction in striatal CR during the first 2 weeks of NTX treatment showed improved clinical outcomes. Thus, neural alcohol CR changes during early abstinence could comprise a potential biomarker of treatment response. However, little is known about the dynamics of neural CR during this period.

Notably, the preclinical literature describes a robust phenomenon of time-dependent increase in drug seeking during the first weeks of withdrawal, termed "incubation of craving."[15,16] Specifically, it refers to the observation that conditioned drug-seeking behavior—a widely used animal model of craving—increases over time compared with the first day of withdrawal following a period of extensive drug taking. The phenomenon was also observed in an animal model of alcoholism.[17] Here, rats showed increased alcohol seeking after 4 weeks of forced abstinence compared with seeking behavior on the first day after a period of alcohol self-administration. Interestingly, a recent study in AUD patients reported an increase in craving over 2 months of treatment that was interpreted as an incubation effect.[18]

A critical mechanism underlying incubation of craving in animals involves enhanced activity of striatal medium spiny neurons in response to glutamatergic input from corticolimbic structures.[19,20] Given that the activity of striatal neurons is also controlled by MORs, which are targeted by NTX, we hypothesized that NTX treatment may also block incubation effects during early abstinence. To test this assumption and to better understand the factors that predict NTX treatment efficacy, we studied male, treatment-seeking, hospitalized alcohol-dependent patients in early abstinence, a period during which many suffer from enhanced alcohol cravings and therefore are at high risk to relapse. We twice measured the neural response to alcohol-related cues—an objective proxy measure of craving—using a well-validated fMRI task.[8] After the first measurment, patients were offered NTX treatment in a naturalistic, longitudinal open-label setting, and neural CR was measured again following 2 weeks of treatment.

2 | METHODS AND MATERIALS

2.1 | Study design

The study constructed as a naturalistic, longitudinal open-label trial and conducted at the Central Institute for Mental Health (CIMH) in Mannheim, Germany. The local ethics committee approved all study procedures, and participants provided informed written consent. Patients and controls underwent a baseline fMRI alcohol CR task.[8] For patients, the baseline scans were scheduled after about 2 to 4 weeks of controlled abstinence (M = 23.2 days, SD = 15.2). This time frame was chosen to ensure that acute symptoms of physical and psychic withdrawal had subsided. All participants completed a series of psychometric assessments before the fMRI session, including the Beck Depression Inventory (BDI, Beck et al[21]), the Fagerstrom Test for Nicotine Dependence (FTND[22]), the Alcohol Dependence Scale (ADS[23]), the Alcohol Use Disorders Identification Test (AUDIT[24]), the Obsessive Compulsive Drinking Scale (OCDS[25]), and the Clinical Institute Withdrawal Assessment scale (CIWA-Ar[26]). Baseline drinking data were collected using the Form 90 semistructured interview,[27] covering the 90-day time frame before admission to the clinic. After the baseline MRI scan, ie, after about 3 weeks of controlled abstinence, patients either participated in a treatment program that runs about 21 days consisting of a daily multiprofessional medically supervised therapy schedule—here termed Intensive Withdrawal Treatment (IWT)—including occupational therapy, physical activation, psychoeducation, psychological group therapy, and psychological one-on-one sessions, as well as multiple medical rounds,[28] or IWT plus adjuvant oral NTX (IWT + NTX) in a naturalistic open-label free-choice design. The adherence to medication during inpatient treatment was ensured by daily supervised intake of medication. After discharge, adherence was monitored during the follow-up interviews by patient report and monitoring of prescription frequency. A follow-up fMRI scan was scheduled for all patients 2 weeks into treatment with NTX (M = 15.5 days, SD = 3.5). Drinking and relapse data were collected for 3 months following the experiment using the Form 90.[27] Relapse was defined as return to heavy drinking if patients' alcohol consumption exceeded 60 g per day for men. In accordance with our earlier studies, we used time to relapse to heavy drinking as outcome variable in our survival analyses.[29-31] As previous studies and meta-analysis indicated that the genetic variant rs1799971:A > G at the

mu-opioid receptor gene locus may affect the efficacy of NTX treatment, this genotype was determined for all participants.[32-35]

2.2 | Participants

Alcoholdependent patients (n = 55) were recruited from an inpatient setting at the CIMH in Mannheim, Germany. Healthy controls (n = 37) were recruited by newspaper advertisement. Only male participants were included in the study, in order to reduce heterogeneity of the sample and because these comprise the vast majority of alcohol-dependent patients admitted to our inpatient care unit. Patients had to meet the following inclusion criteria: (1) diagnosis of alcohol dependence according to the Diagnostic Statistical Manual of Mental Disorders (DSM-IV), (2) age between 18 and 65 years, (3) abstinence from any substance for two to 5 weeks, except tobacco and caffeine (controlled by negative urine drug screening), and (4) average minimum consumption of at least six drinks per day (84-g alcohol, one standard drink = 14 g) in the last 90 days before admission to the clinic and initiation of controlled abstinence. Exclusion criteria were (1) comorbid axis-I disorders (other than nicotine dependence) in the last year, (2) treatment with psychotropic or anticonvulsive medications in the last 3 months, (3) severe neurological or medical condition (ie, liver cirrhosis), (4) positive drug screening, (5) ineligibility for MRI scanning (eg, metal implants), (5) history of severe head trauma, or (6) changes in vasoactive or antihypertensive medication during the last 7 days. Healthy control participants were only included if they (1) were aged between 18 and 65 years, (2) did not meet diagnosis of alcohol dependence or any other axis-I disorder, (3) had an average alcohol consumption below one drink per day (14 g), and (4) did not meet any of the exclusion criteria (see above). See Figure 1 for a depiction of the study flow and data availability.

2.3 | fMRI alcohol cue-reactivity task

During the fMRI session, participants underwent a previously validated alcohol cue-reactivity task.[8] The task consisted of 12 blocks featuring a series of five alcohol pictures each and 12 blocks featuring

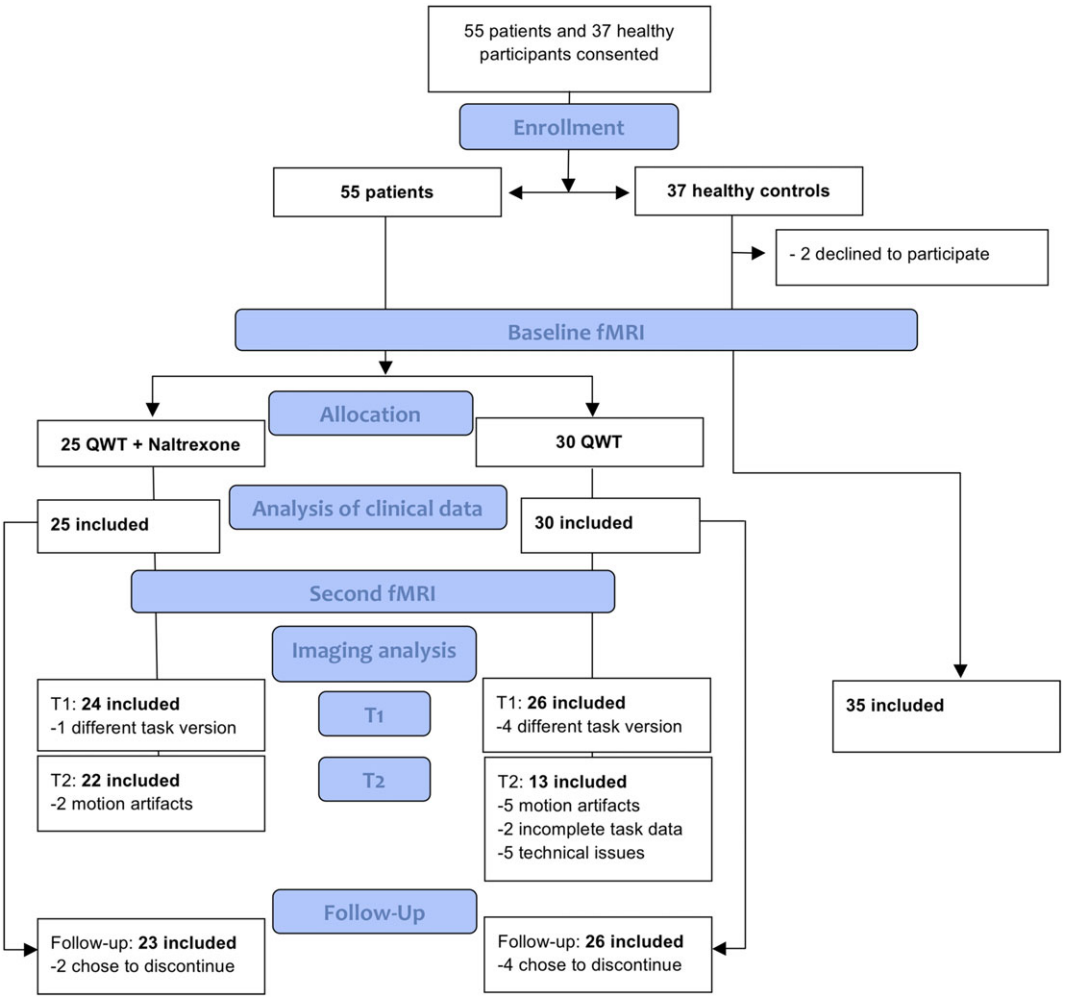

FIGURE 1 CONSORT diagram of subject flow through the study. Data sets of 50 patients (24 of whom later received naltrexone [NTX]), and 35 controls were available for baseline imaging analyses. For the imaging analyses comparing baseline and follow-up scan, data from 22 NTX patients and 13 patients with standard treatment could be included. Follow-up relapse data were available for 23 NTX patients and 26 patients receiving standard treatment

series of five neutral pictures. All pictures were presented for 4 seconds, and individual blocks were separated by 10-second intervals.

2.4 | fMRI acquisition and preprocessing

Functional neuroimaging was conducted using a 3 T whole-body tomograph (MAGNETOM Trio, Siemens, Germany). For each subject, we acquired a total of 303 T2*-weighted echo-planar images (EPI) in transversal orientation of 30° clockwise to the AC-PC line covering the entire brain (TR = 2.41 s, TE = 25 ms, flip angle = 80°, 42 slices, slice thickness = 2 mm, 1-mm gap, voxel dimensions 3 × 3 × 3 mm^3, FOV = 192 × 192 mm^2, 64 × 64 in-plane resolution). Visual stimuli were presented using Presentation software (Version 16.0, Neurobehavioral Systems Inc., Albany, California) and MRI-compatible goggles (MRI Audio/Video Systems, Resonance Technology Inc., Los Angeles, California).

All imaging data were processed and analyzed using SPM8 (preprocessing and individual statistics) and SPM12 (second-level group analyses; Wellcome Centre for Human Neuroimaging, Institute of Neurology, University College London, United Kingdom). In order to avoid artifacts because of magnetic saturation, the first five scans were excluded from the analyses. The remaining 298 scans were corrected for residual geometric distortion on the basis of the acquired magnetic field map, spatially realigned, normalized to a standardized EPI template from MNI (Montreal Neurological Institute, Quebec, Canada), and smoothed using an isotropic Gaussian kernel for group analyses (full width at half maximum: 8 mm). Prior to normalization, distortion correction was applied by affine transformation followed by a nonlinear registration of imaging data to the EPI template. Rigid quality checks were implemented for every participant. Data were excluded if there was excessive motion (>3 degrees of rotation or >3-mm movement in any axis) or if visual inspection indicated poor fitting to the standard EPI template. Additionally, SPM-based analyses of VBM data, controlling for age and total intracranial volume, indicated no significant family-wise error-corrected differences in whole-brain gray matter volumes as well as in striatal regions between patients and controls or between treatment groups (IWT vs IWT + NTX), indicating that there were no gross morphological gray matter differences between groups (see Supporting Information), supporting the use of the standard EPI template. First-level contrast images were computed for all participants by modeling the different task conditions (alcohol and neutral) as explanatory variables within a general linear model and including motion variables as covariates of no interest.

2.5 | DNA preparation and genotyping

Genomic DNA was isolated from blood using the QIAamp DNA micro kit (Qiagen, Maryland) according to the manufacturer's protocol. The *OPRM1* A118G SNP (rs1799971) was detected by a TaqMan SNP Genotyping Assay (C_8950074_1; Applied Biosystems, Carlsbad, California) on an ABI 7900 HT RT-PCR system with SDS 2.2.2 software (10 µl reaction volume containing 10 ng genomic DNA, 40 cycles of 95°C for 15 s, and 60°C for 1 min).

2.6 | Statistical analyses

Group characteristics and clinical data were analyzed using *t* tests, analysis of variance test, and Fisher exact tests where appropriate, using the Statistical Package for the Social Sciences (SPSS, IBM Corp., Somers, New York) version 24.0. Imaging data were analyzed using one-sample *t* tests to investigate alcohol cue-induced activation compared with neutral stimuli (contrast: "alcohol - neutral stimuli") across groups. Baseline differences between patient and control groups were investigated by applying a two-sample *t* test for independent groups. As both groups differed with regards to age, this variable was included as a covariate. In addition, medication effects and interactions between medication and time were investigated by implementing the first-level contrast images (contrast: "alcohol - neutral stimuli") in a 2 × 2 full factorial model with the factors (1) treatment (NTX vs standard treatment) and (2) time (baseline vs 2 weeks). In order to satisfy a family-wise error rate correction of *p*FWE < 0.05, we determined a combined voxel-wise- ($p < 0.001$) and cluster-extent-threshold ($k \geq 33$ voxels) by running 10 000 permutations by Monte Carlo simulations (estimated smoothness was *x*/*y*/*z* = 10.06/9.97/10.40 mm, 66 × 52 × 56 volume with 66 703 voxels of 3-mm resolution, restricted to a modified standardized whole brain EPI template, limiting the search space to relevant mesocorticolimbic areas) using the Neuroelf analysis package (www.neuroelf.net).[36] For detailed information, see Supporting Information. In order to further investigate associations between alcohol CR and external variables (eg, relapse risk), beta values of neural activation in the left putamen were extracted using a functional region of interest (ROI) extracted with the MarsBar software package (http://marsbar.sourceforge.net/) and imported into SPSS for further analyses. The left putamen was chosen as ROI over the other brain areas that showed a significant whole-brain interaction effect because previous studies demonstrated significant associations between left putamen CR and relapse risk,[29] and a meta-analysis (Noori et al,[37] Supporting Information) demonstrated that alcohol cues compared with other cues (eg, neutral and food) generated distinct activation patterns in the left putamen (peak activation voxel *x*/*y*/*z* = −18/4/−8). Furthermore, alcohol-dependent patients showed increased brain activation specifically in the left posterior putamen during habitual responding.[38] Thus, ROI analyses were restricted to the left putamen. The functional ROI mask for the left putamen was derived by computing the intersection between the anatomical mask of the left putamen from the Automated Anatomical Labeling atlas (AAL) and areas of the interaction contrast (medication × time) activation map that showed significant whole group effects. The resulting mask is displayed in Figure S1. Cox regression models were implemented to test the main effect and interaction of NTX and CR in the left putamen ROI on time to first severe relapse. Previous studies reported better treatment efficacy of NTX in patients with a positive cue reactivity (contrast: "alcohol – neutral"), compared with those with negative cue reactivity[10] and better treatment response in patients that showed a decrease in alcohol cue reactivity between baseline and after 2 weeks of treatment.[13] Hence, effects of dichotomized (positive vs negative) CR in the left putamen on relapse risk as well as effects of an increase in CR in the left putamen from baseline to second scan (increase vs decrease) were investigated using

Cox regression models in addition to testing cue reactivity as continuous variable.

3 | RESULTS

3.1 | Group characteristics

Demographic and clinical data of healthy participants and of patients at baseline before choosing IWT + NTX or IWT only are displayed in Table 1. There was a marginally significant difference in age between patient groups. Hence, this variable was included as a covariate in all subsequent models comparing the patient groups. *OPRM1* genotype data were available for 92% of patients and 100% of the control sample. Analyses indicated no significant difference between the two treatment groups (see Tables 1 and 2). The number of G-allele carriers in the IWT-only group was very low; thus, meaningful comparison between patient groups was not feasible. Exploratory factorial analyses in the IWT + NTX group showed no significant main effects on neural CR (see Supporting Information). Hence, the genotype was not considered in further analyses.

3.2 | Imaging outcomes

As expected (ie, in the study of Yalachkov et al[9]), data demonstrated a significant main effect of stimulus category (alcohol vs neutral pictures) in a network of frontal, temporal, occipital, and mesolimbic brain areas, including the superior, middle, and inferior frontal gyri, parts of the temporal and parietal gyri, as well the cerebellum, caudate, putamen, thalamus, and hippocampus (see Table S1). Patients relative to healthy controls had increased alcohol CR in right and left superior and middle temporal gyri (see Table 3), while healthy controls did not show higher-cue reactivity than patients in any brain area.

Factorial whole-brain analyses among patients revealed a significant interaction between treatment (IWT vs IWT + NTX) and time (baseline vs week 2) in the putamen, pallidum, bilateral thalami, and hippocampus (see Table 4a). Subsequent post-hoc tests indicated that the interaction effect was driven by an increase in CR in the left putamen, bilateral pallidum, and right thalamus among patients receiving IWT only (see Figure 2A and Table 4b). No main effect of time or medication was found. Further analyses of brain activation supported this interaction. Following previous results on CR and relapse risk association[29] and the distinct activation pattern to alcohol cues in a meta-analysis,[37] we selected the left putamen as ROI. We found that an increase in CR in the putamen was only present in patients receiving standard treatment ($F_{(1,33)} = 6.823$, $p = 0.013$; see Figure 2B). In addition, the main effect of time was highly significant in the standard treatment group ($F_{(1,12)} = 23.526$, $p = 0.001$), ie, there was an increase in CR from baseline to week 2 in this group, whereas there was no increase in CR in the NTX + IWT group. In addition, at week 2, there was a main effect of group, specifically, the IWT group had higher CR in the putamen compared to patients also receiving NTX ($F_{(1,33)} = 4.601$, $p = 0.039$; see Figure 2B).

Analyses of craving scores obtained before the first and second fMRI scans indicated a significant interaction between the course of putamen CR (ie, increase vs decrease) and the course of the scores obtained by the OCDS ($F_{(1,19)} = 4.892$, $p = 0.039$), such that patients with a decrease in putamen CR showed a significant reduction in OCDS scores from baseline to week 2 ($OCDS_{baseline} = 14.8$ [SD = 6.7], $OCDS_{week2} = 8.9$ [SD = 6.6], $t = 4.170$, $p = 0.002$).

We also investigated whether the choice of NTX treatment was associated with the extent of CR in the left putamen. There was no significant difference between groups with negative CR (50% chose NTX) and those with positive CR (50% chose NTX) with regard to clinical scales and substance use patterns ($p > 0.05$).

TABLE 1 Demographic and clinical data for healthy controls and patients

	Control ($n = 35$)	Patients ($n = 50$)	Statistics	Significance
Demographic variables				
Age (years)	42.0 (9.8)	45.6 (8.9)	$t_{(83)} = 1.723$	$p = 0.089$
Education (no post secondary educ./apprenticeship only/attended college or higher)	0/5/30	2/14/32	$Chi^2_{(2)} = 15.047$	**$p = 0.002$***
Genotype data				
OPRM1 (AA vs. any G)	27:8	39:7	$Chi^2_{(1)} = 0.769$	$p = 0.381$
Substance use patterns				
Ethanol (g/day; mean of last 90 days)	6.4 (5.9)	202.7 (134.6)	$t_{(48)} = 8.479$	**$p < 0.001$***
Smoker (yes/no)	7:28	40:10	$Chi^2_{(1)} = 29.983$	**$p < 0.001$***
Clinical scales				
OCDS (sumscore)	1.5 (1.4)	16.6 (6.8)	$t_{(53)} = 14.856$	**$p < 0.001$***
STAI (trait sumscore)	30.3 (6.9)	40.5 (10.3)	$t_{(79)} = 5.329$	**$p < 0.001$***
FTND (sumscore)	4.8 (3.9)	6.0 (2.6)	$t_{(40)} = 0.882$	$p = 0.383$
ADS (sumscore)	2.1 (2.4)	14.2 (6.6)	$t_{(62)} = 11.648$	**$p < 0.001$***
BDI (sumscore)	2.0 (2.5)	10.9 (8.3)	$t_{(59)} = 7.047$	**$p < 0.001$***

Abbreviations: ADS, Alcohol Dependence Scale; BDI, Beck Depression Inventory; FTND, Fagerstroem Test for Nicotine Dependence; OCDS, Obsessive-Compulsive Drinking Scale; SD, standard deviation; STAI, State-Trait-Anxiety Inventory; * = significant differences between $p < 0.05$.

TABLE 2 Demographic data, alcohol use, and severity measures for patient groups with available imaging data for both time points (baseline and week 2 scan)

	IWT (n = 13)	IWT + naltrexone (n = 22)	Statistics	Significance
Demographic variables				
Age (years)	41.2 (8.7)	48.6 (8.6)	$t_{(33)} = -2.472$	**$p = 0.019$***
Education (no post secondary educ./ apprenticeship only/attended college/higher education)	0/5/4/4	1/5/10/6	$Chi^2_{(3)} = 1.447$	$p = 0.695$
Genotype data				
OPRM1 (AA vs. any G)	12:1	16:6	$Chi^2_{(1)} = 1.958$	$p = 0.162$
Substance use patterns				
Duration of alcohol dependence (years)	11.0 (10.0)	14.4 (10.1)	$t_{(32)} = 0.865$	$p = 0.394$
Ethanol (g/day; mean of last 90 days)	218.2 (185.5)	179.62 (132.7)	$t_{(29)} = 1.348$	$p = 0.184$
Drinks per day (mean of last 90 days)	21.3 (15.5)	15.0 (11.1)	$t_{(32)} = 1.375$	$p = 0.179$
Abstinent days (% in last 90 days)	18.5 (30.1)	15.9 (21.3)	$t_{(32)} = 0.290$	$p = 0.774$
Heavy-drinking days (% in last 90 days)	79.9 (29.9)	76.4 (27.0)	$t_{(32)} = 0.353$	$p = 0.726$
Smoker (yes/no)	10:3	15:7	$Chi^2_{(1)} = 0.306$	$p = 0.580$
Clinical scales				
OCDS (sumscore)	17.6 (8.2)	14.2 (5.8)	$t_{(32)} = 1.409$	0.168
FTND (sumscore)	6.9 (1.8)	5.1 (2.9)	$t_{(22)} = 1.690$	0.105
ADS (sumscore)	15.0 (5.7)	12.6 (5.9)	$t_{(32)} = 1.140$	0.263
STAI (trait sumscore)	34.6 (10.8)	37.6 (8.4)	$t_{(30)} = 0.851$	0.401
BDI (sumscore)	7.4 (7.9)	10.1 (7.7)	$t_{(32)} = 0.957$	0.346
Follow-up				One-tailed
Drinks per day (mean of last 90 days)	0.9 (2.1)	0.1 (0.1)	$t_{(32)} = 1.667$	$p = 0.067$
Drinks per drinking day (mean of last 90 days)	3.4 (2.1)	1.2 (3.3)	$t_{(32)} = 1.411$	$p = 0.075$
Abstinent days (% during follow-up)	88.3 (21.7)	98.2 (5.0)	$t_{(32)} = 1.834$	**$p = 0.042$***
Heavy-drinking days (% during follow-up)	8.1 (20.2)	0.6 (2.1)	$t_{(32)} = 1.570$	$p = 0.063$

Abbreviations: ADS, Alcohol Dependence Scale; BDI, Beck Depression Inventory; FTND, Fagerstroem Test for Nicotine Dependence; IWT, Intensive Withdrawal Treatment; OCDS, Obsessive-Compulsive Drinking Scale; SD, standard deviation; STAI, State-Trait-Anxiety Inventory; * = significant differences between $p < 0.05$.

TABLE 3 Depiction of areas that show greater alcohol cue-induced (contrast: alcohol-neutral) activation in patients compared with control participants at baseline of neural activation (combined voxel-wise- [$p < 0.001$] and cluster-extent-threshold [k > 33 voxel], corresponding to pFWE < 0.05)

Two sample t test at baseline (n = 80)							
Side	**Lobe**	**Brain Areas**	**Cluster size**	**MNI Coordinates (X, Y, Z)**			**Statistic**
Patients > healthy controls							**T_{max}**
R	Temporal lobe	Middle temporal gyrus, superior temporal gyrus	75	64	−40	10	3.68
L	Temporal lobe	Middle temporal gyrus	28	−68	−38	4	3.67
R	Occipital/temporal lobe	Middle occipital gyrus, middle temporal gyrus	32	42	−66	8	3.66
L	Temporal lobe	Middle temporal gyrus	27	−38	−66	12	3.51
R	Temporal lobe	Middle temporal gyrus, superior temporal gyrus	21	50	−48	12	3.48

L or R, Left or right hemisphere, respectively.

3.3 | Relapse to heavy drinking

Within 3 months after the baseline scan, 25 out of 29 (86%) patients receiving standard IWT versus 13 out of 23 (57%) NTX-treated patients relapsed to heavy drinking. Cox regression analyses indicated that NTX treatment was associated with lower risk to relapse to heavy drinking (hazard $ratio_{(NTX)}$ [HR] = 0.407, 95%CI , 0.195-0.849, $p = 0.017$; see Figure 3A). Based on the procedure suggested by Altman and Andersen,[39] a number needed to treat (NNT) of 3.2 [95%CI, 1.8–20.7] can be computed from the HR data for the fixed time point of 90 days after the experiment. Furthermore, NTX-treated patients had a significantly higher proportion of abstinent days during follow-up ($t = 1.834$, $p = 0.042$) and a trend towards a lower percentage of heavy drinking days (%HDD) compared with IWT-only patients ($t = 1.570$, $p = 0.067$, see Table 2).

Cox regression analyses found that higher CR in the left putamen at baseline was associated with a shorter time to heavy relapse (HR = 2.641, 95%CI = 1.069-6.524, $p = 0.035$). In addition, increasing

TABLE 4 Imaging results for the longitudinal comparison (baseline vs week 2) of cue-induced activation in patients with and without naltrexone, showing (a) an interaction effects of time and medication on alcohol cue-induced brain activation that is driven by (b) a significant increase in cue reactivity in the putamen, pallidum, and thalamus in the patient group without medication (n = 13) (contrast "alcohol – neutral," combined voxel-wise-[p < 0.001] and cluster-extent-threshold [k > 33 voxel], corresponding to pFWE < 0.05)

Full factorial model time x treatment (n = 35)							
Side	**Lobe**	**Brain Areas**	**Cluster Size**	**MNI Coordinates (X, Y, Z)**			**Statistic**
a) Interaction treatment x time (n = 35)							**F_{max}**
L		Thalamus	57	−2	−16	−6	16.92
L	Limbic lobe	Parahippocampal gyrus Hippocampus	34	−20	−4	−32	16.12
L		Putamen, pallidum	33	−26	−10	2	16.11
L & R		Thalamus	44	8	−10	12	14.55
b) Baseline < week 2: IWT group (n = 13)							**T_{max}**
R		Pallidum, thalamus	43	14	−4	−2	4.51
L		Putamen, pallidum	36	−26	−10	0	3.79

L or R, Left or right hemisphere, respectively. IWT = standard psychosocial "qualified withdrawal treatment" program

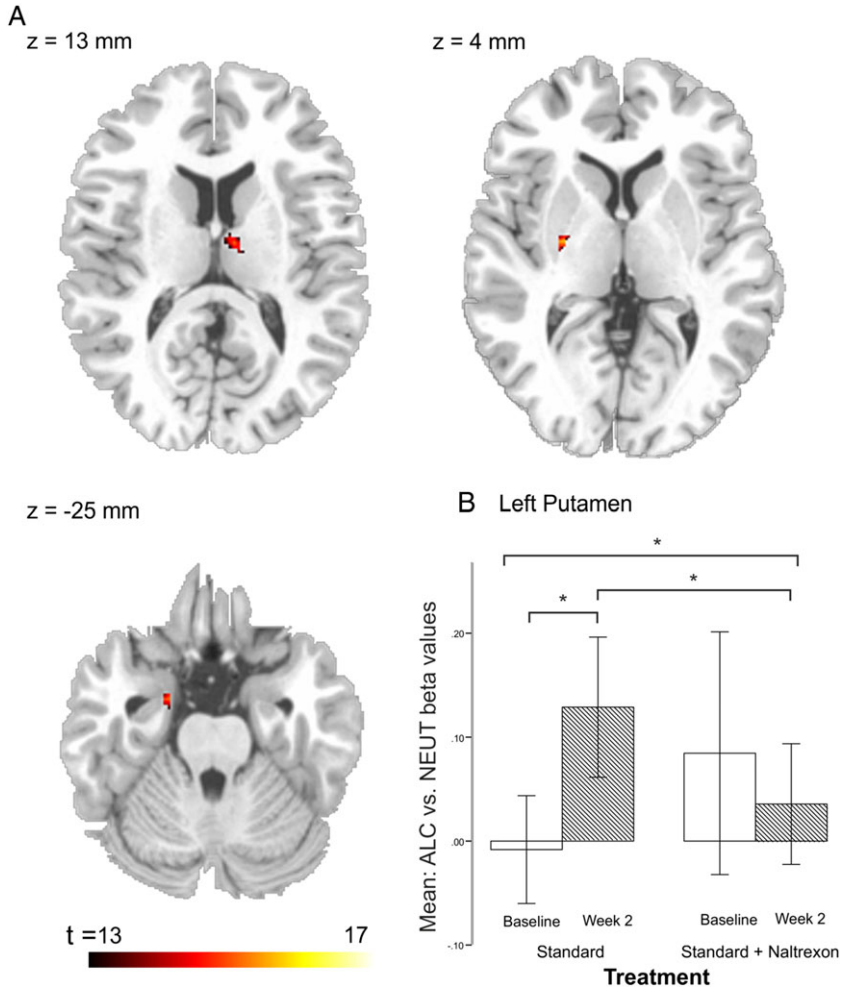

FIGURE 2 Significant interaction between time (baseline, week 2) and treatment (standard vs standard + naltrexone) on (A) alcohol cue-induced brain response (alcohol vs neutral) in the left putamen, hippocampus, and left and right thalamus. (B) Comparison of alcohol cue-elicited activation in the left putamen region of interest (ROI) at baseline and week 2 for each treatment group. While there was no significant difference in baseline CR, the standard treatment group significantly increased left putamen activation between baseline and week 2 and had a significantly greater putamen activation at week 2 compared with the naltrexone-treated patients. Figures show estimated means +2x standard error of the mean (SE). * = significant (p < 0.05) interaction between medication and time and post hoc: effect of group at week 2, and effect of time in the standard treatment group

CR from baseline to week 2 predicted faster relapse, while a decrease was associated with longer time to relapse into heavy drinking ($HR_{Decrease}$ = 0.253, 95%CI , 0.084-0.760, p = 0.014; see Figure 3B). Furthermore, we found a significant interaction between putamen CR at baseline and medication on time to relapse to heavy drinking (HR = 0.255, 95%CI , 0.084-0.775, p = 0.016), such that NTX-treated patients with high CR at baseline, comparedwith patients with low CR, had a longer time to heavy relapse. Separate survival analyses showed that in the group of patients with a positive putamen CR (alcohol > neutral) (n = 21), there was a highly significant NTX effect (HR = 0.174, 95%CI, 0.055-0.556, p = 0.003; see Figure 3C) that corresponds to a NNT of 1.8 (95%CI, 1.3-6.2), whereas no NTX effect was found in the patients with negative CR (n = 24, HR = 1.083, 95%CI, 0.402-2.921, p > 0.05; see Figure 3D).

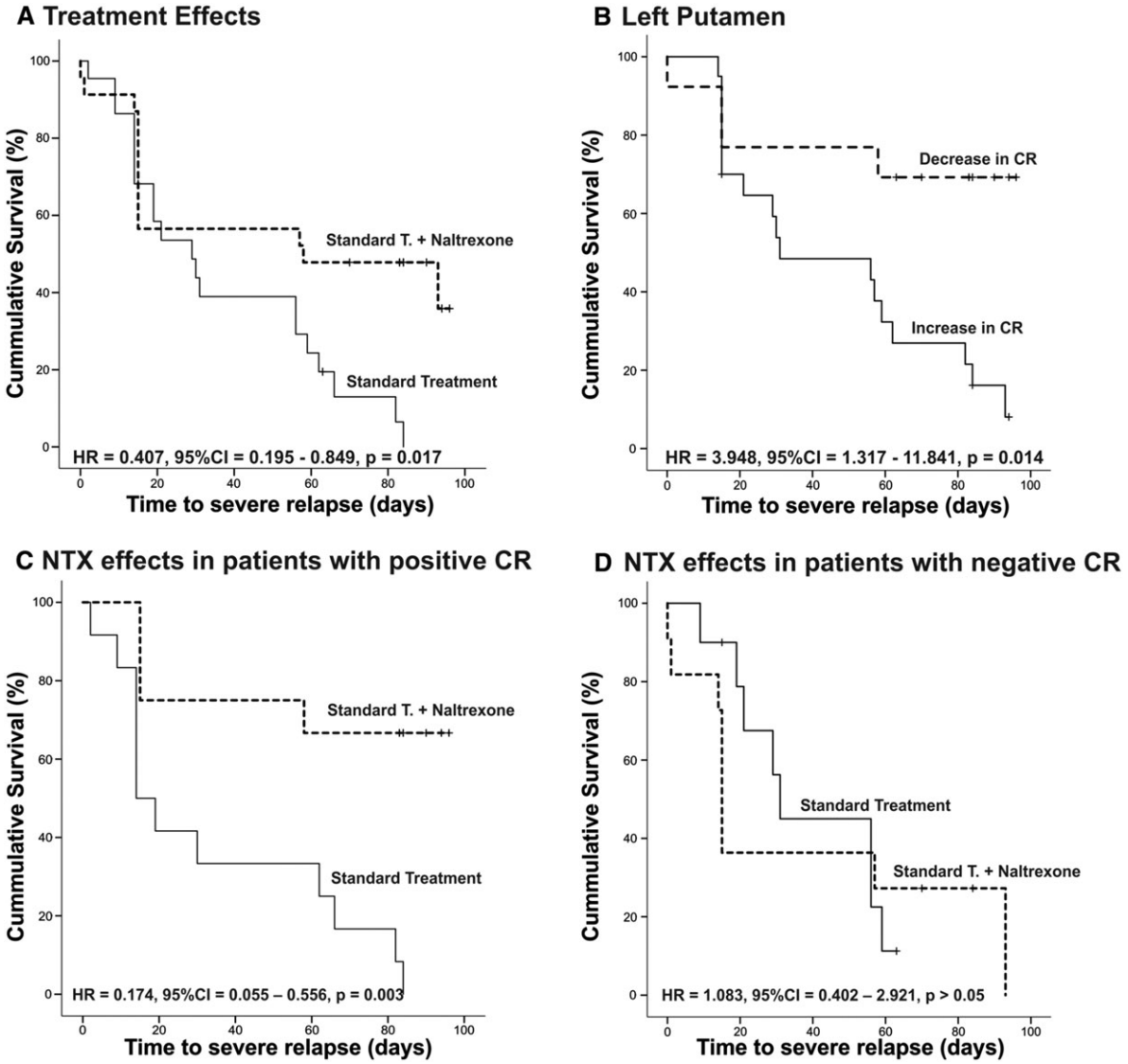

FIGURE 3 Kaplan Meier curves illustrating (A) days to heavy relapse in patients receiving naltrexone treatment (n = 45), (B) the significantly lower-relapse risk in patients with a decrease in putamen CR from baseline to week 2 (dichotomized: nominal increase vs decrease, n = 35), (C) the significant naltrexone effects in patients with positive putamen CR (n = 21), and (D) the absence of a naltrexone effect in patients with negative putamen CR (n = 24) at baseline scan. HR = hazard ratio, CI = confidence interval, CR = cue reactivity

On the basis of previous studies suggesting that smoking and abstinence goals might influence treatment effects, we investigated the potential effects of both in the present study. Results showed no significant main effect or interaction of either on time to heavy relapse (p > 0.05).

4 | DISCUSSION

The present study shows that neural CR to alcohol-associated stimuli increases, or "incubates," over the first weeks of abstinence in alcohol-dependent patients and that suppression of this incubation effect by NTX predicts treatment outcome. These results are in line with previous work of our group, in which we have demonstrated significant associations between CR in the left putamen and time to relapse to heavy drinking.[29] Important to note here is that individual CR at the time of choosing the respective treatment option was not associated with differences in subjective craving, clinical scales, or substance use patterns. Our findings underline the importance of neuroimaging markers for elucidating mechanisms of relapse vulnerability and further development of personalized therapies,[3,4] particularly in the case of NTX treatment, which still suffers from highly heterogeneous outcomes.[2]

Incubation of craving is a well-established phenomenon in preclinical addiction research.[15,16] Specifically, it refers to the observation that conditioned drug-seeking behavior—a widely used animal model of craving—increases over time compared with the first day of withdrawal after a period of extensive drug taking. The neural circuits underlying the incubation effect have so far mostly been studied in models of stimulant addiction and strongly implicate neuroplasticity in dorsal striatal regions.[19] Interestingly, a recent study found increasing alcohol craving over a period of 2 months of treatment indicating an incubation effect in male AUD patients.[18] Our findings of increased mesolimbic CR during early abstinence and the observation that patients with a decrease

in neural activation in the left putamen from baseline to week 2 show a reduction in subjective alcohol craving concur with the incubation construct. In an earlier study, we found increased striatal CR to alcohol cues over time during early abstinence, specifically from 1 week after withdrawal to 4 weeks, which mirrors current results.[40] The increase in alcohol CR in these studies was detected during a relatively brief, but for patients, highly vulnerable period. In both studies, IWT was used as the control condition. During this standardized treatment, patients learn to recognize craving and to deal with it (eg, skill training). Whether this cognitive therapy interferes with the incubation effect remains elusive and further research is necessary to clarify time course, extent and clinical relevance of this phenomenon.

Converging lines of evidence from prospective studies suggest that NTX effects in parts of the putamen, caudate, and ventral striatum seem to be strongly related to treatment outcome.[10,13] Previous studies demonstrated associations between CR in ventral striatum and NTX treatment response using a priori selected ROI. While this ROI-based approach might be suitable for studies with strong assumptions about the location of neural CR, the current study opted for a whole-brain analytic strategy acknowledging the fact that brain areas outside the ventral striatum are relevant to alcohol addiction and might gain increasing importance during the course of the disorder according to a model of allostatic dysregulation of the reward system.[41] This model is supported by previous findings where exposure to alcohol-related stimuli was found to be correlated with reward craving or neural CR in reward-related brain areas such as the ventral striatum in nondependent alcohol consumers but not alcohol-dependent patients.[8,42,43] Nevertheless, results also indicated modulation of CR by NTX in other brain areas, such as the bilateral thalamus and ventral pallidum. Recent reviews suggest that hyperactivation of the thalamus in addiction reflects increased drug and stress reactivity that contributes to increased relapse risk.[44] However, the exact contribution of the thalamus to addictive behavior has yet to be determined. The pallidum, and especially parts of the ventral pallidum, has been identified as reward structures with major outputs to limbic regions whose function is necessary for reward-driven behavior.[45] Activation patterns in the pallidum encode reward to hedonic stimuli and stimulation of the structure results in enhancement of the rewarding value of a stimulus. Attenuation of CR in the thalamus and the pallidum might therefore reflect NTX-related attenuation of drug reward and drug reactivity.

The current study measured the effects of NTX on CR in the putamen. The choice of this region was guided by meta-analysis[37] and previous findings on association of alcohol CR in AUD patients with relapse risk[29,46] and habitual responding.[38] The latter findings support the idea that dorsal striatal regions including the putamen are strongly implicated in loss of control over drug taking, reflected in a shift away from goal-directed behavior to outcome-insensitive habitual responding, which has been conceptualized as an important neurobehavioral mechanism in the development of addiction.[47] The fact that alcohol CR was not correlated with subjective measures of craving or other clinical variables in our study further supports the notion of the habit system as a key player.

In summary, our study extends current literature by demonstrating that NTX has beneficial effects as an add-on option that can be freely chosen by patients within the setting of an established and independently validated multiprofessional treatment program.[28] Neural response to alcohol cues during early abstinence is increased in a number of patients. They seem to benefit strongly from NTX treatment, which is reflected by a NNT of 1.8 for oral NTX to prevent return to heavy drinking at 90 days in the group with positive CR in the putamen, compared with an overall NNT of 12 for NTX to prevent return to heavy drinking as previously reported by systematic meta-analyses.[2] Thus, high-alcohol CR in striatal areas and the ability of NTX to reduce this neural response appear to be characteristics of a subgroup of patients with a favorable response to NTX treatment. Of note, this observation independently replicates results from a previous prospective randomized trial from our lab.[10]

4.1 | Limitations

The naturalistic open-label design limits the inference that can be made from this study. We opted for an open-label design over the gold-standard randomized control trial (RCT) based on several factors beyond economic constraints. First of all, a naturalistic design may closer reflect clinical reality, although it must be noted that our present population is highly selected in terms of meeting basic research inclusion criteria (absence of psychiatric comorbidities, coabuse of illicit drugs, etc). Secondly, NTX is an approved treatment option, and the offering of this adjuvant option was already implemented in the IWT routines. Thirdly, the naturalistic approach takes into consideration that the effectiveness of a medical treatment ultimately depends on patients' compliance. Indeed, a considerable proportion of NTX-treated patients discontinues the medication within a few weeks,[48,49] which strongly emphasizes the importance of a priori motivation for pharmacotherapy as a crucial factor in achieving the treatment goals. In addition, the uncertainty about the medication effect in RCTs may be counterproductive to cognitive-behavioral interventions, which are part of the QWT program. Thus, while the allocation of patients to NTX treatment based on informed choice within the framework of an open-label study carries the risk of selection bias, it also encourages compliance and represents a more accurate picture of clinical practice. Given that patients who received pharmacotherapy did not differ in a number of clinical baseline characteristics compared with those without adjuvant NTX, we believe that the reported effects are attributable to NTX. This interpretation is further supported by the implementation of a clinical sample that took no other psychotropic medication. While the strict exclusion criteria enhanced internal validity, it prevented the acquisition of a larger sample because of monetary and time constraints. In any case, further studies including more RCTs need to confirm our findings.

Another factor that may limit external validity of our results is the inclusion of only male participants. The prevalence of alcohol dependence in women, although lower than in males, is nevertheless significant. However, the vast majority of AUD patients admitted to inpatient care are males. Furthermore, previous work has indicated effects of gender on CBT treatment efficacy[50] and that genetic effects at the OPRM1 locus, at least with regard to smoking, are sex-specific.[51] Thus, the inclusion of only males may have enhanced

internal validity and prevented further segregation of the sample. Nonetheless, gender effects on the functioning of the opioid system need to be investigated more closely in the future.[5]

5 | CONCLUSION

The current study interlinks three findings: (1) NTX significantly attenuates the mesolimbic response to drug-associated cues during early abstinence, (2) relapse risk is significantly associated with this neural response both at baseline and over 2 weeks of treatment, and (3) the modulation of the brain response by NTX reduces relapse risk in patients with high CR. Together, our findings support potential use of the alcohol CR as predictor of treatment response to NTX and the course of CR over the initial treatment period as measure for NTX efficacy. Interestingly, despite the predictive power of their neural responses, patients' subsequent choice of treatment was not associated with craving, clinical scales, or substance use patterns. Therefore, readily available clinical parameters such as scales that differentiate between positive or negative reinforcement by alcohol (Mann et al 2017) may help to guide decisions for clinicians and patients when to choose NTX, ie, to develop individual biomarkers of NTX treatment response. Further research on the topic of precision medicine seem promising.

ACKNOWLEDGMENTS

We thank Michael Rieß for support during data collection.

AUTHORS CONTRIBUTION

WHS, DH, KM, AJG, SMD, DM and SC were responsible for the study concept and design. PB, GW, SH and SVK contributed to the acquisition of the data. PB, SVK, EP and UPR conducted the data analysis and interpretation of findings. PB, EP and WHS drafted the manuscript. WHS, SVK, FK, PK, DH and KM provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved final version for publication.

FUNDING AND DISCLOSURE

This work was supported by the European Union's Horizon 2020 research and innovation program (668863-SyBil-AA) and the ERA-Net NEURON program (FKZ 01EW1112-TRANSALC) as well as the Deutsche Forschungsgemeinschaft (Center Grant SFB636). None of the authors report any competing interests.

ORCID

Patrick Bach https://orcid.org/0000-0001-5962-019X
Enrico Pompili https://orcid.org/0000-0003-2467-7027
David Moratal https://orcid.org/0000-0002-2825-3646
Peter Kirsch https://orcid.org/0000-0002-0817-1248
Falk Kiefer https://orcid.org/0000-0001-7213-0398
Wolfgang H. Sommer https://orcid.org/0000-0002-5903-6521

REFERENCES

1. World Health Organization (2018) Global Status Report on Alcohol and Health.
2. Jonas DE, Amick HR, Feltner C, Bobashev G, Thomas K, Wines R, Kim MM, Shanahan E, Gass CE, Rowe CJ, Garbutt JC (2014) In: Pharmacotherapy for Adults With Alcohol-Use Disorders in Outpatient Settings: Rockville (MD).
3. Heilig M, Sommer WH, Spanagel R. The need for treatment responsive translational biomarkers in alcoholism research. *Curr Top Behav Neurosci*. 2016;28:151-171.
4. Mann K, Roos CR, Hoffmann S, Nakovics H, Leménager T, Heinz A. Witkiewitz K (2018) precision medicine in alcohol dependence: a controlled trial testing pharmacotherapy response among reward and relief drinking phenotypes. *Neuropsychopharmacology*. 2018;43(4):891-899.
5. Hansson AC, Gründer G, Hirth N, Noori HR, Spanagel R, Sommer WH. *Dopamine and Opioid Systems Adaptation in Alcoholism Revisited: Convergent Evidence from Positron Emission Tomography and Postmortem Studies*. Neuroscience and Biobehavioral Reviews InPress; 2018.
6. Hendershot CS, Wardell JD, Samokhvalov AV, Rehm J. Effects of naltrexone on alcohol self-administration and craving: meta-analysis of human laboratory studies. *Addict Biol*. 2017;22(6):1515-1527.
7. Schacht JP, Anton RF, Voronin KE, et al. Interacting effects of naltrexone and OPRM1 and DAT1 variation on the neural response to alcohol cues. *Neuropsychopharmacology: official publication of the American college of Neuropsychopharmacology*. 2013;38(3):414-422.
8. Vollstadt-Klein S, Loeber S, Richter A, et al. Validating incentive salience with functional magnetic resonance imaging: association between mesolimbic cue reactivity and attentional bias in alcohol-dependent patients. *Addict Biol*. 2012;17(4):807-816.
9. Yalachkov Y, Kaiser J, Naumer MJ. Functional neuroimaging studies in addiction: multisensory drug stimuli and neural cue reactivity. *Neurosci Biobehav Rev*. 2012;36(2):825-835.
10. Mann K, Vollstadt-Klein S, Reinhard I, et al. Predicting naltrexone response in alcohol-dependent patients: the contribution of functional magnetic resonance imaging. *Alcohol Clin Exp Res*. 2014;38(11):2754-2762.
11. Reinhard I, Lemenager T, Fauth-Buhler M, et al. A comparison of region-of-interest measures for extracting whole brain data using survival analysis in alcoholism as an example. *J Neurosci Methods*. 2015;242:58-64.
12. Schacht JP, Anton RF, Myrick H. Functional neuroimaging studies of alcohol cue reactivity: a quantitative meta-analysis and systematic review. *Addict Biol*. 2013b;18(1):121-133.
13. Schacht JP, Randall PK, Latham PK, et al. Predictors of naltrexone response in a randomized trial: reward-related brain activation, OPRM1 genotype, and smoking status. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*. 2017;42(13):2640-2653.
14. Lukas SE, Lowen SB, Lindsey KP, et al. Extended-release naltrexone (XR-NTX) attenuates brain responses to alcohol cues in alcohol-dependent volunteers: a bold FMRI study. *Neuroimage*. 2013;78:176-185.
15. Grimm JW, Hope BT, Wise RA, Shaham Y. Neuroadaptation. Incubation of cocaine craving after withdrawal. *Nature*. 2001;412(6843):141-142.
16. Pickens CL, Airavaara M, Theberge F, Fanous S, Hope BT, Shaham Y. Neurobiology of the incubation of drug craving. *Trends Neurosci*. 2011;34(8):411-420.
17. Bienkowski P, Rogowski A, Korkosz A, et al. Time-dependent changes in alcohol-seeking behaviour during abstinence. *Eur Neuropsychopharmacol*. 2004;14(5):355-360.
18. Li P, Wu P, Xin X, et al. Incubation of alcohol craving during abstinence in patients with alcohol dependence. *Addict Biol*. 2015;20(3):513-522.
19. Dong Y, Taylor JR, Wolf ME, Shaham Y. Circuit and synaptic plasticity mechanisms of drug relapse. *J Neurosci Off J Soc Neurosci*. 2017;37(45):10867-10876.

20. Luis C, Cannella N, Spanagel R, Kohr G. Persistent strengthening of the prefrontal cortex - nucleus accumbens pathway during incubation of cocaine-seeking behavior. *Neurobiol Learn Mem*. 2017;138:281-290.
21. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. *Arch Gen Psychiatry*. 1961;4(6):561-571.
22. Fagerstrom KO, Schneider NG. Measuring nicotine dependence: a review of the Fagerstrom tolerance questionnaire. *J Behav Med*. 1989;12(2):159-182.
23. Kivlahan DR, Sher KJ, Donovan DM. The alcohol dependence scale: a validation study among inpatient alcoholics. *J Stud Alcohol*. 1989;50(2):170-175.
24. Saunders JB, Aasland OG, Babor TF, De la Fuente JR, Grant M. Development of the alcohol use disorders identification test (AUDIT): WHO collaborative project on early detection of persons with harmful alcohol consumption-II. *Addiction*. 1993;88(6):791-804.
25. Mann K. Die OCDS-G: Psychometrische Kennwerte der deutschen Version der Obsessive Compulsive Drinking Scale. *Sucht*. 2000;46:10.
26. Sullivan JT, Sykora K, Schneiderman J, Naranjo CA, Sellers EM. Assessment of alcohol withdrawal: the revised clinical institute withdrawal assessment for alcohol scale (CIWA-Ar). *Br J Addict*. 1989;84(11):1353-1357.
27. Tonigan JS, Miller WR, Brown JM. The reliability of form 90: an instrument for assessing alcohol treatment outcome. *J Stud Alcohol*. 1997;58(4):358-364.
28. Loeber S, Kiefer F, Wagner F, Mann K, Croissant B. Treatment outcome after inpatient alcohol withdrawal: impact of motivational interventions: a comparative study. *Nervenarzt*. 2009;80(9):1085-1092.
29. Bach P, Vollstädt-Klein S, Kirsch M, et al. Increased mesolimbic cue-reactivity in carriers of the mu-opioid-receptor gene OPRM1 A118G polymorphism predicts drinking outcome: a functional imaging study in alcohol dependent subjects. *Eur Neuropsychopharmacol*. 2015;25(8):1128-1135.
30. Jorde A, Bach P, Witt SH, et al. Genetic variation in the atrial natriuretic peptide transcription factor GATA4 modulates amygdala responsiveness in alcohol dependence. *Biol Psychiatry*. 2014;75(10):790-797.
31. Kiefer F, Witt SH, Frank J, et al. Involvement of the atrial natriuretic peptide transcription factor GATA4 in alcohol dependence, relapse risk and treatment response to acamprosate. *Pharmacogenomics J*. 2011;11(5):368-374.
32. Anton RF, Oroszi G, O'Malley S, et al. An evaluation of mu-opioid receptor (OPRM1) as a predictor of naltrexone response in the treatment of alcohol dependence: results from the combined pharmacotherapies and behavioral interventions for alcohol dependence (COMBINE) study. *Arch Gen Psychiatry*. 2008;65(2):135-144.
33. Chamorro AJ, Marcos M, Miron-Canelo JA, Pastor I, Gonzalez-Sarmiento R, Laso FJ. Association of micro-opioid receptor (OPRM1) gene polymorphism with response to naltrexone in alcohol dependence: a systematic review and meta-analysis. *Addict Biol*. 2012;17(3):505-512.
34. Oslin DW, Berrettini W, Kranzler HR, et al. A functional polymorphism of the mu-opioid receptor gene is associated with naltrexone response in alcohol-dependent patients. *Neuropsychopharmacology*. 2003;28(8):1546-1552.
35. Ramchandani VA, Umhau J, Pavon FJ, et al. A genetic determinant of the striatal dopamine response to alcohol in men. *Mol Psychiatry*. 2011;16(8):809-817.
36. Bennett CM, Wolford GL, Miller MB. The principled control of false positives in neuroimaging. *Soc Cogn Affect Neurosci*. 2009;4(4):417-422.
37. Noori HR, Cosa Linan A, Spanagel R. Largely overlapping neuronal substrates of reactivity to drug, gambling, food and sexual cues: a comprehensive meta-analysis. *Eur Neuropsychopharmacol*. 2016;26(9):1419-1430.
38. Sjoerds Z, de Wit S, van den Brink W, et al. Behavioral and neuroimaging evidence for overreliance on habit learning in alcohol-dependent patients. *Transl Psychiatry*. 2013;3(12):e337.
39. Altman DG, Andersen PK. Calculating the number needed to treat for trials where the outcome is time to an event. *BMJ (Clinical Research Ed)*. 1999;319(7223):1492-1495.
40. Vollstadt-Klein S, Loeber S, Kirsch M, et al. Effects of cue-exposure treatment on neural cue reactivity in alcohol dependence: a randomized trial. *Biol Psychiatry*. 2011;69(11):1060-1066.
41. Koob GF, Le Moal M. Drug abuse: hedonic homeostatic dysregulation. *Science*. 1997;278(5335):52-58.
42. Bujarski S, Ray LA. Subjective response to alcohol and associated craving in heavy drinkers vs. alcohol dependents: an examination of Koob's allostatic model in humans. *Drug Alcohol Depend*. 2014;140:161-167.
43. Bujarski S, Hutchison KE, Prause N, Ray LA. Functional significance of subjective response to alcohol across levels of alcohol exposure. *Addict Biol*. 2017;22(1):235-245.
44. Sinha R. How does stress lead to risk of alcohol relapse? *Alcohol Res*. 2012;34(4):432-440.
45. Smith KS, Tindell AJ, Aldridge JW, Berridge KC. Ventral pallidum roles in reward and motivation. *Behav Brain Res*. 2009;196(2):155-167.
46. Grusser SM, Wrase J, Klein S, et al. Cue-induced activation of the striatum and medial prefrontal cortex is associated with subsequent relapse in abstinent alcoholics. *Psychopharmacology (Berl)*. 2004;175(3):296-302.
47. Everitt BJ, Robbins TW. Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nat Neurosci*. 2005;8(11):1481-1489.
48. Kranzler HR, Stephenson JJ, Montejano L, Wang S, Gastfriend DR. Persistence with oral naltrexone for alcohol treatment: implications for health-care utilization. *Addiction*. 2008;103(11):1801-1808.
49. Swift R, Oslin DW, Alexander M, Forman R. Adherence monitoring in naltrexone pharmacotherapy trials: a systematic review. *J Stud Alcohol Drugs*. 2011;72(6):1012-1018.
50. Magill M, Ray LA. Cognitive-behavioral treatment with adult alcohol and illicit drug users: a meta-analysis of randomized controlled trials. *J Stud Alcohol Drugs*. 2009;70(4):516-527.
51. Bernardi RE, Zohsel K, Hirth N, et al. A gene-by-sex interaction for nicotine reward: evidence from humanized mice and epidemiology. *Transl Psychiatry*. 2016;6(7):e861.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Bach P, Weil G, Pompili E, et al. Incubation of neural alcohol cue reactivity after withdrawal and its blockade by naltrexone. *Addiction Biology*. 2019;1–11. https://doi.org/10.1111/adb.12717