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Prefrontal-Striatal Functional Pathology is Associated with Alcohol Abstinence Days at Treatment Initiation and Heavy Drinking After Treatment Initiation

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Abstract

Objective: Alcohol use disorders (AUDs) are associated with neuroadaptations in brain stress and reward circuits. It is not known whether such neuroadaptations are affected by alcohol abstinence days and also influence heavy drinking during the early treatment phase. A novel Functional Magnetic Resonance Imaging (fMRI) approach was utilized to assess brain responses during sustained exposure to standardized visual stimuli of stressful, alcohol cue and control, neutral images combined with *prospective* assessment of drinking outcomes during early outpatient treatment.

Methods: In study 1, 44 treatment entering AUD patients and 43 demographically matched healthy controls (HC) participated in the fMRI experiment to identify dysfunctional responses associated with chronic alcohol abuse. In study 2, 69 AUD patients entering treatment assessed for whether fMRI responses at treatment initiation were influenced by alcohol abstinence and were *prospectively* predictive of early heavy drinking outcomes.

Results: AUD patients, relative to HC showed hyperreactivity in the Vento-medial Prefrontal Cortex (VmPFC) to neutral images, but VmPFC and ventral striatum hypoactivation to stress, and to alcohol cues relative to response to neutral images (whole brain, voxel based, $p < .001$, cluster corrected $\alpha = .05$). In study 2, this specific prefrontal-ventral striatal dysfunction was associated with shorter days of alcohol abstinence (corr $p < 0.05$), and also predicted greater number heavy drinking days during subsequent 2 weeks of treatment engagement (corr $p < 0.05$).

Conclusions: Alcohol abstinence days at treatment initiation significantly affects functional disruption of the prefrontal-striatal responses to stress and alcohol cues in AUD patients, and severity of this disruption, in turn, predicts greater heavy drinking during early treatment.

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Treatments that target this functional prefrontal-striatal pathology could improve early treatment outcomes in AUD.

Introduction

Individuals in recovery from Alcohol Use Disorders (AUD) struggle with poor treatment adherence, early dropout, and high relapse risk [1, 2]. Furthermore, AUD patients enter outpatient treatment with varying lengths of abstinence, from less than 24 hours up to a few weeks [3]. Little is known about the neurobiological state during early abstinence, a critical time of outpatient treatment initiation. Current research on alcohol brain recovery with abstinence includes evidence from animal models [4, 5, 6] and data from a select group of AUD patients who can abstain for months or years at a time [7–10]. However, over 35% of AUD patients entering outpatient treatment relapse within 30 days, and over 65% relapse within 90 days [1, 11, 12]. Furthermore, the effects of each day of alcohol abstinence on functional brain recovery and the impact of such recovery on lapses to heavy drinking during early treatment are not known.

Previous research indicates that chronic alcohol intake is associated with neuroadaptations in functional brain responses in stress and reward circuits [for review, see 13, 14]. These neuroadaptations interfere with adaptive emotion processing, stress regulation, and cognition [15] and are associated with high alcohol craving, thereby increasing susceptibility to continued heavy drinking and high relapse risk after treatment [11]. Specifically, we have shown that in 4-week abstinent AUD patients, disrupted activity of the ventromedial prefrontal cortex (VmpFC), encompassing the orbitofrontal cortex (OFC) and the rostral anterior cingulate cortex (rACC), and ventral striatal (VS) responses to stress, alcohol and neutral-relaxed cues was associated with higher stress- and cue-induced craving and increased relapse risk after inpatient treatment [16]. While the VmpFC and VS regions make up the salience network and are identified as a key circuit in reward prediction [17, 18] in addiction models [19], the VmpFC and VS have been shown to be responsive to stress and negative mood cues [20] and predictive of resilient and adaptive coping under stress [21]. Importantly, the effect of days of alcohol abstinence on the integrity of brain functional responses of this key prefrontal-striatal circuit known to predict alcohol relapse has not been systematically investigated thus far.

To address this gap, we conducted two related studies utilizing a unique multi-method functional magnetic resonance imaging (fMRI) experimental approach combined with a *prospective* clinical outcome design. A novel sustained stress and reward provocation task employed a series of standardized and matched visual cues for alcohol, stress, or neutral stimuli over a brief period in a block design [22]. Study 1 assessed the brain functional responses to stress and to alcohol cues relative to an active neutral-control cue block in 44 treatment entering AUD patients and 43 demographically matched controls to specifically examine possible functional disruption of the prefrontal-striatal circuit. Study 2 utilized an expanded group of the treatment entering AUD patients (N=69) to assess whether the fMRI responses to stress and alcohol cues in AUD patients are influenced by abstinence days at treatment entry, and whether such abstinence-related neurobiological functioning is predictive of subsequent heavy drinking during the 2 week period after treatment initiation.

Based on previous research [16, 23], we hypothesized in Study 1 that the treatment entering AUD patients would show altered VmPFC and ventral striatal response to alcohol, stress, and neutral cues, relative to demographically matched, socially drinking healthy controls (HC). In Study 2, we hypothesized that the extent of functional brain alterations in VmPFC and ventral striatal regions to stress and to alcohol cues would be significantly associated with alcohol abstinence days, and that abstinence days-related brain changes would *prospectively* predict heavy drinking days during the first 2 weeks of treatment engagement.

Methods

Participants:

Participants were recruited from the greater New Haven, CT area via advertising on social media and with flyers in community locations and treatment facilities. In Study 1, treatment seeking men and women (N=44) ages 21– 60, meeting current criteria for moderate to severe AUD, as determined by the Structured Clinical Interview for DSM-V (SCID)[24] were studied during acute abstinence (1–12 days), along with 43 demographically matched healthy, non-binging, social drinking men and women (HC). The Study 1 AUD and HC participants were matched for age, sex, race, IQ, and education, but not for drinking measures and nicotine use (Table 1). In Study 2, 42 of the Study 1 AUD sample were included and an additional 27 AUD patients were enrolled to comprise 69 treatment seeking men and women with alcohol abstinence days varying between 24 hours and 35 days pre-scan. All AUD patients were scanned prior to outpatient treatment initiation during their intake phase in both Study 1 and 2 and all subjects were only scanned once for this research (Figure 1a). Across both studies, participants were required to have a negative breathalyzer on scan day. Exclusion criteria included history of psychotic disorders, pregnancy, any non-removable metal in their bodies, loss of consciousness for longer than 30 minutes, and individuals with current other medical, psychiatric, or substance use disorders (excluding nicotine) and taking medications for such illnesses. We screened 398 individuals which included 176 patients with AUD. Of the 176 AUD patients, 10.79% (19/176) were on psychiatric medications and an additional 4% (7/176) were on medications for medical conditions and were excluded. Individuals requiring medical detoxification from alcohol were also excluded.

Weekly Behavioral Counseling in Study 2:

After the fMRI scan at treatment entry, all AUD patients started weekly behavioral counseling with a training Master's level alcohol and drug abuse counselor who utilized the standardized, 12-step facilitation from NIAAA [25] as part of their standard of care, which was not compared to any other behavioral treatment.

The study was approved by the Human Investigation Committee of the Yale University School of Medicine.

Functional magnetic resonance imaging (fMRI) provocation procedure:

The novel fMRI paradigm involved brief successive exposure to three conditions: (1) a block of highly appetitive images of alcoholic beverages and people drinking alcoholic beverages

in social or alone contexts, (2) a block of highly threatening, aversive and stressful images and (3) a no-cue, control block involving neutral images (Figure 1b[22]). Pictures included images from the International Affective Picture System [26] and those developed at the Yale Stress Center; select alcohol cue images from the web were downloaded and rated using the same rating method as the IAPS pictures. The alcohol cues included images of wine, beer, cocktail, champagne, and hard liquor. The stress condition involved highly aversive images of terror, violence, mutilation, fear, disgust, and desperation. The neutral condition consisted of neutral images of mountains, grass, trees, streams, stones, and trails (Figure 1b).

Each image within a block was presented for 5 seconds (s), with a 1-s inter-stimulus interval (ISI), over six successive runs of 66 s each (11 images per run; 396 images per 6-minute block). Each alcohol cue, stress, and neutral condition was counter-balanced across participants and presented in randomized order to prevent order effects in the responses. Baseline brain responses were collected within each Condition block where provocation stimuli runs were preceded by three 66-s runs of gray fixation baseline blocks for comparison with the respective alcohol cue, stress, or neutral provocation runs (Figure 1b). There was a 5-minute post-provocation recovery period after each condition during which participants were provided progressive relaxation instructions via audiotape. Visual analog scales (rating 1–9) were used for assessing alcohol craving and subjective stress after each 6-6-s run within each block.

fMRI Methods and Procedures:

Subjects arrived at the Yale Magnetic Resonance Research Center (MRRC) at 7:00 AM under fasting conditions. Transportation was provided if it was unavailable. Breathalyzer and urine toxicology screenings were used to confirm drug and alcohol abstinence for each intake session and the day of the MRI scan. All metal was removed and subjects were settled in a hospital bed in a preparation/recovery room where an intravenous (IV) line was inserted in the non-dominant forearm of subjects by the nurse for repeated blood drawing for cortisol data collection during the scan. Subjects then completed a practice task consisting of 10 trials using stimuli that were not used for the in-scan fMRI task. After a 45-minute adaptation period, the subject entered the MRI to complete the scan, and a pulse oximeter was placed on their non-dominant forefinger to obtain heart rate and blood samples during the scan.

Physiological measurements:

Heart rate was obtained using a pulse oximeter during the baseline and provocation runs in each condition. Pulse was estimated and averaged for baseline and each provocation run and change from baseline within each condition was computed for each condition. Blood sampling was performed for each Condition block at baseline, after the 6 provocation runs, and after a 5-minute recovery period for each condition for the assessment of cortisol levels. The nurse obtained blood samples from an indwelling catheter on the subjects' forearm which was accessible without moving the subject before and after each condition[22]. Blood was collected in Ethylenediaminetetra- acetic acid (EDTA) coated tubes (BD Biosciences, NJ, USA). Blood samples were immediately stored on ice, plasma was separated by centrifugation at 4°C for 10 minutes at 1000X g. Aliquots of plasma was stored in

polypropylene tubes at -80°C until assayed. Cortisol were assessed using core laboratory radioimmunoassay (RIA) procedure at the Yale Center for Clinical Investigation. Within-person change in cortisol responses were computed by subtracting Recovery-Provocation for each Condition, and then further assessing change in stress and in alcohol cue responses relative to neutral condition, as well as an assessment of group differences in Study 1 for the Neutral Provocation – Recovery (see Supplemental methods).

fMRI Acquisition:

The MRI scan was conducted on a 3-T multiband Siemens Trio or Prisma MRI system equipped with a standard quadrature head coil, using T1 MPRAGE sequence for structural scanning and T2*-sensitive gradient-recalled single shot echo planar pulse sequence for functional scans. Functional, blood oxygen level dependent (BOLD) signals were acquired with a 64-channel head coil with a multi-band accelerated, echo planar imaging sequence. Seventy-five axial slices parallel to the AC-PC line covering the whole brain were acquired with TR = 1000 msec, TE = 30 msec, bandwidth = 1894 Hz/pixel, flip angle = 55 degrees, field of view = 220×220 mm, slice thickness = 2 mm and no gap.

fMRI Data Preprocessing:

Data was converted from Digital Imaging and Communication in Medicine format to Analyze format using XMedCon. General Linear Models (GLM) were used for individual-level analysis on each voxel in the entire brain volume with a regressor (time during picture viewing) for each run per condition using BioImageSuite. Trials with linear motion in excess of 1.5 mm or rotation greater than 2 degrees were discarded. The regressor was each visual image run relative to the baseline gray fixation period, resulting in a relative stress images - stress baseline, alcohol cue images-alcohol cue baseline, and neutral images - neutral baseline contrast for each run. Temporal filtering was carried out by including drift correction in the GLM. Each trial was spatially smoothed using a 6 mm Gaussian kernel and individually normalized to generate beta-maps ($3.44\text{mm} \times 3.44 \text{ mm} \times 4\text{mm}$). To account for individual anatomical differences, three sequential registrations were performed using BioImageSuite: linear registration of raw data into 2D anatomical image, the 2D to 3D ($1 \times 1 \times 1 \text{ mm}$) linear registration, and a non-linear registration to a reference 3D image, the Colin27 Brain. The overall preprocessing approach did not utilize any pre-whitening correction to address potential serial autocorrelations of time series data, as additional preprocessing with pre-whitening correction using the autoregressive moving average (ARMA) model from AFNI yielded remarkably similar second level results (see Supplemental Figure 2).

fMRI Statistical Parameters:

In both Study 1 and Study 2, to correct for multiple comparisons, we used family-wise errors (FWE) correction determined by Monte Carlo simulation using the AFNI 3dClustSim version (16.3.05, October 2016) program. A value of $p = 0.001$ was considered statistically significant for whole brain FWE correction and a cluster correction of $p = 0.05$ was used. The cluster forming threshold was 320 mm^3 .

Study 1 Group Analyses:

Second-level group differences in terms of age, gender, IQ, and race were examined using chi-square tests and independent samples t-tests for continuous measures. Linear mixed effect (LME) models were utilized to assess subjective stress, alcohol craving, heart rate, cortisol responses during each condition to ensure validity of the stress and emotion provocation manipulation. For brain responses, voxel-wise whole brain data were analyzed using LME models with Subjects as a Random effect. Condition was treated as a Within subjects fixed-effect factor and, group as the between-subjects factor. Covariates included gender, age and Days Abstinent from alcohol. Condition effects represent activation to the active cue-baseline. The neutral condition served as an active control comparison condition for the stress and alcohol cue challenge conditions, because it does not increase alcohol craving, and it controls for the nonspecific effects of the experimental manipulation. Thus, group differences are presented in the form of alcohol cue-neutral and stress-neutral contrasts and also responses in the neutral control condition alone.

Study 2 Abstinence and fMRI:

Second level voxel-wise whole brain LME analyses was used with Abstinence Days as the predictor variable of functional brain responses to cues. Condition was treated as a within subjects fixed-effect factor and Subject as a random factor. Covariates included gender and age. Any significant Abstinence Days interaction with Condition (stress, alcohol cues and neutral) was further assessed using alcohol cue and stress relative to neutral contrasts. Beta values were extracted from the significant regions of interest (ROIs) for illustration purposes only.

Study 2: Prospective Daily Assessment of Early Treatment Outcome:

At the first intake, AUD patients completed an orientation to the Daily Diary assessments via a smartphone application (MetricWire, Inc). The daily surveys assessed how many glasses of beer, wine, and mixed drinks were consumed per day during the first two weeks of treatment engagement (14 days). The quantity of each type of drink was summed for each day to create an index of total drinks consumed per day.

Measurements were collected during an evening survey that was available from 8:00 pm - 2:00 am. Participants also completed a morning survey that was available 8:00 am-2:00 pm, which was used to estimate drinking if the participants missed the previous day's evening survey.

Compliance with Daily Diaries:

Participants completed a total of 1026 logs, of which 37.5% were drinking days. Participants drank, on average, 5.20 ± 4.36 days (range 0–14) during the two-week period. The average number of drinks consumed on drinking days were 4.58 ± 4.52 . Consistent with previous patient treatment-based daily diary research[27], adherence to daily reporting was 71.2%. Information about daily alcohol consumption was supplemented using a weekly Substance Use Calendar, based on the Timeline Follow-back assessment method [28], for any patient that missed daily drinking diary entries on a particular day.

Study 2 Craving, Cortisol and fMRI Prospective Early Treatment Outcome Analyses:

First, the effects of Abstinence Days at treatment entry were prospectively evaluated for impact on HDD during early treatment using Cox Proportional Hazard regression. The Wald test was performed to determine if the number of days of abstinence at treatment initiation predicted a swifter relapse, or heavy drinking day (HDD), during treatment. Second, we assessed whether Abstinence Days predicted subjective alcohol craving, stress, and Cortisol responses using multiple regression models. Finally, LME models covarying for gender, age, and Abstinence Days assessed the effects of alcohol craving, stress and cortisol on early treatment response and on the interaction of functional brain response (voxel-wise, whole brain) with Condition, and Drinking Outcome during early treatment. A-priori hypothesized ROIs that significantly predicted HDD during treatment were extracted to illustrate scatterplots of abstinence-related responses and HDD during early treatment.

Results

Demographic Characteristics for Study Participants (Table 1):

In Study 1, the AUD and HC groups are matched for age, sex, race, IQ, and years of education, and years of regular alcohol consumption, but as expected, differed vastly in frequency of drinking alcohol ($t(85)=57.5$, $p<0.0001$), and usual amount of alcohol consumed ($t(85)=46.4$, $p<0.0001$). The AUD samples in Study 1 and Study 2 contained significantly more nicotine smokers than the healthy controls, ($\chi^2(2, N=153)=22$, $p<0.0001$), but did not differ significantly from each other ($\chi^2(1, N=113)=0.074$, $p=0.79$.) The AUD sample in Study 1 also had significantly more lifetime diagnoses of mood disorders ($\chi^2(1, N=85)=5.78$, $p<0.016$), lifetime anxiety disorders ($\chi^2(1, N=85)=36.79$, $p<0.0001$), and other lifetime substance use disorders (SUD; $\chi^2(1, N=85)=6.28$, $p<0.01$), than the Study 1 healthy controls. Healthy controls did not differ in lifetime diagnoses of Post-Traumatic Stress Disorder (PTSD) from the AUD samples.

The AUD participants in Study 2 did not differ from the AUD participants in Study 1 in age, years of education, IQ, race, alcohol use variables, number of smokers, or lifetime diagnoses of mood disorders, PTSD, or other SUDs. The AUD sample in Study 1 had significantly higher rates of lifetime anxiety disorder diagnoses than the sample in Study 2 ($\chi^2(1, N=113)=7.99$, $p<0.005$). Among both HC and AUD groups for Study 1 and Study 2, those with and without a lifetime history of co-morbid diagnoses were not different in abstinence days, craving, stress, or cortisol responses.

Study 1

Manipulation Check for Stress and Alcohol Cue Provocation

Alcohol Craving: AUD show greater baseline alcohol craving than HC group ($F(1,255)=56.54$, $p<0.0001$) across conditions. After correction for baseline levels of craving in each group, there was a group x condition interaction ($F(6,595)=3.36$, $p=0.003$), such that AUD also showed significantly higher craving responses to alcohol cues ($F(1,595)=286.2$, $p<0.0001$) and stress cues ($F(1,595)=209.3$, $p<0.0001$) than HC (Figure 1c). There was no significant group difference in craving increases elicited by the neutral stimuli.

Subjective Stress: AUD show greater baseline subjective stress relative to HC group, ($F(1,255)=41.92$, $p<0.0001$). After correcting for baseline differences, there was a Condition main effect such that both AUD and HC show significantly greater subjective response to stress cues ($F(2,903)=160.1$, $p<0.0001$) and to alcohol cues ($F(2,882)=206.8$, $p<0.0001$) relative to the neutral cues (Figure 1d). Within the AUD group, there was an interaction between condition and timepoint, such that relative to neutral, subjective stress was greater at the beginning and end of the alcohol cue block ($p's<0.05$) (see Figure 1d).

Cortisol: With-in person Cortisol responses (provocation-recovery) to conditions were significantly different in the AUD versus HC groups. In the Neutral Control condition, cortisol was higher in the AUD versus HC group ($t=2.29$, $p<.03$). Assessing stress and alcohol cue responses relative to neutral response, we found significant Group ($F[1,58]=6.40$, $p<.01$) and Condition ($F[1,58]=4.13$, $p<.05$) main effects but no interactions. The group effect results from HC group showing increased cortisol response to stress and cue relative to neutral response, but not in the AUD group ($t(85)=2.1$, $p=0.04$, Supplemental Figure 1a). The condition effect was due to greater overall increases with Alcohol Cue than with Stress relative to Neutral change across groups ($t(85)=2.03$, $p<.05$). These data suggested significantly altered Cortisol responses in the AUD relative to the HC group.

Heart Rate: AUD participants show elevated basal heart rate relative to HC group across conditions ($t(85)=6.68$, $p<0.0001$). The AUD group also showed blunted heart rate response to alcohol cues relative to neutral, compared to the HC group ($F(1,170)=5.013$, $p=0.03$), but similar increases in heart rate responses to stress relative to neutral cues across both groups ($F(1,170)=3.329$, $p=0.07$). (Supplemental Figure 1b).

Whole Brain Voxel-based Analyses: Highly significant differences between AUD and HC were found in cortico-striatal-limbic regions in response to stress (S), alcohol cues (AC) and neutral (N) in whole brain analyses ($p<.001$, alphasim correction at $p<.05$) (Supplementary Table 1.1). As hypothesized and shown in Figure 2, AUD participants show hyperactivation of the VmPFC, including the rostral Anterior Cingulate Cortex (rACC) in the neutral condition, but hypoactivation in VmPFC/rACC in the stress-neutral contrast and the alcohol cue - neutral contrast relative to HC group. Additionally, HC show increased VS activation in response to alcohol cues, which was absent in the AUD group (alcohol-neutral contrast). Finally, AUD show VmPFC hypoactivation and both VS and dorsal striatal (DS) hyperactivation in the stress-alcohol cue contrast, while HC do not.

Study 2

Alcohol Abstinence Days and Craving, Stress and Cortisol Responses: Lower Abstinence Days was significantly associated with higher mean alcohol craving during stress provocation ($r=-.28$, $p<.02$) and higher maximum alcohol craving during neutral condition ($r=-.22$, $p<.05$), but not with alcohol cue-provoked craving. Abstinence Days was not significantly associated with subjective stress ratings. Cortisol responses to stress and alcohol cue relative to neutral and in the neutral condition were also not significantly associated with alcohol Abstinence Days.

Alcohol Abstinence Days and Brain Functional Responses to Cues: Abstinence Days were significantly associated with brain responses in several corticostriatal-limbic regions during stress and alcohol cue relative to neutral cues (see Supplementary Table 1.2). More importantly, as hypothesized, Abstinence Days significantly influenced extent of disrupted functional responses in the VmPFC/rACC and VS across stress, alcohol and neutral cues. Specifically, lower abstinence days were associated with greater VmPFC/rACC hypoactivation in response to stress and greater VS hypoactivation in response to alcohol cues, but greater VmPFC/rACC and VS hyperactivation in the neutral condition (Figure 3).

Relationship between Abstinence Days and Early Treatment Outcome: The Cox proportional hazards regression analysis indicated that Abstinence days significantly predicted HDD outcome (Wald test=6.1, $p<.01$), with less Abstinence Days, associated with greater likelihood of subsequent heavy drinking during early treatment. For each additional day of abstinence at treatment initiation, the risk of relapse to heavy drinking in the first two weeks decreased by 14% at a hazards ratio of 0.873 (Figure 4a).

Relationship between Craving, Stress and Cortisol Response and HDD During Early Treatment: While high alcohol craving in response to alcohol cues at trend level ($p<.10$), high stress during the neutral condition ($p<.03$), and high cortisol response during neutral condition ($p<.05$) were each associated with HDD outcome independently, these responses were not significantly predictive of HDD outcome when Abstinence Days was added to a multivariate regression model (see Supplemental Table 2). Therefore, only abstinence days was used as a predictor variable in the following fMRI and outcome analyses.

Disrupted Brain Responses to Stress and Cues and Heavy Drinking Outcome: Abstinence Days-related disrupted functional responses in the hypothesized VmPFC/rACC and ventral striatal regions, as well as several corticostriatal-limbic regions of the brain, during stress, alcohol cue relative to neutral and neutral cues alone were significantly predictive of HDD during early treatment (Supplementary Table 1.3). ROIs for the hypothesized regions of the VmPFC/rACC and VS regions were extracted from the significant voxel-based interaction effects of Condition x Outcome (HDD) analyses to illustrate the prediction of HDD during early treatment. Findings indicate that both lower the VmPFC/rACC hypoactivation to stress cues (stress-neutral contrast) and greater VmPFC/rACC prefrontal hyperactivity in the neutral state (neutral-baseline contrast) each predicted increased future HDD during early treatment. Furthermore, lower ventral striatal hypoactivation in response to alcohol cues (alcohol cues-neutral contrast) was predictive of higher HDD during early treatment (see Figure 4b–c).

Discussion

In two related studies, using a novel, brief sustained stress, alcohol cue and neutral control visual stimuli paradigm, we provide previously unreported evidence of, (1) disrupted prefrontal-striatal functional responses to stress and alcohol cue provocation and to neutral control condition in AUD participants entering treatment relative to healthy social drinkers, and (2) a significant influence of days of alcohol abstinence on extent of disrupted

prefrontal-striatal function in response to stress, alcohol cue and neutral cue provocation, that in turn, had a significant *prospective* effect on early treatment drinking outcomes. In Study 1, AUD patients, upon treatment entry, showed highly significant dysfunctional blunted VmPFC and VS responses to stress and to alcohol cues relative to neutral cue as compared to demographically matched controls, but VmPFC/rACC and VS hyperactivation to neutral cues. These results supported our hypothesis of significant prefrontal-striatal functional pathology during early abstinence in treatment entering AUD individuals. In Study 2, we found alcohol abstinence days at treatment initiation significantly affected the altered VmPFC/rACC and VS functional response, such that less days of abstinence prior to the scan was associated with greater disruption of the VmPFC/rACC and VS activity in response to stress, alcohol and neutral cues. Additionally, after controlling for the effects of abstinence days on brain responses to cues, we found that greater dysfunctional prefrontal and striatal responses to stress and to alcohol cues prospectively predicted greater HDD in the first two weeks of outpatient treatment for AUD.

It is notable that dysfunctional prefrontal-ventral striatal functional responses was found in AUD relative to control group in Study 1, and that such dysfunction was also related to important clinical indicators such as abstinence days at treatment entry and drinking outcome during early treatment engagement. We used a whole brain analytic approach to specifically assess the hypothesized prefrontal-ventral striatal circuit across the two studies. The similarity of brain findings across the two related studies speaks to the strength and robustness of the results. Furthermore, while subjective craving, subjective stress and cortisol were each correlated to HDD outcome, only Abstinence Days and abstinence-related dysfunctional brain responses predicted future drinking outcome in early treatment. These findings suggest that extent of brain functional pathology along with days of abstinence may be an important marker of early treatment outcome and recovery.

Because relapse to drinking and treatment drop out during early outpatient treatment is common, identifying predictors of heavy drinking and relapse during early recovery is critical. Stress and alcohol cues in the environment are two main reasons cited as causing relapse by patients [29, 30]. These results are consistent with current understanding of the underlying neurobiology of AUD, that chronic alcohol-related neuroadaptations in prefrontal-ventral striatal circuits may hamper a person's ability to recruit prefrontal executive control functions in response to stress cues, while also showing hypoactivation in the ventral striatal, appetitive brain regions to respond appropriately to alcohol rewarding stimuli, and this combined dysfunction may increase risk of HDD and relapse [31]. This is also consistent with growing evidence that AUD patients are unable to appropriately regulate responses to stress and alcohol cues in the environment during recovery initiation [29]. Additionally, a number of previous studies have reported downregulation of dopamine and blunted ventral striatal responses to alcohol and to alcohol cues in AUD and binge heavy drinkers [32–34]. Furthermore, we have previously shown VS hyperactivation to neutral cues in AUD patients [16], suggesting a heightened tonic salience of alcohol, and probability of approach behavior in the presence of alcohol cues [35, 36], consistent with previous work in those with AUDs in fMRI and PET studies [13, 37]. Dysfunctional ventral striatal responses predicting future alcohol use outcomes are also consistent with previous data on prediction of drinking outcomes in the laboratory [38], and in real world outcomes [39].

Findings from Study 2 further corroborate those of Study 1 in that disruption of prefrontal-striatal functioning in AUD patients was significantly associated with less days of abstinence, i.e., greater prefrontal hypoactivation during stress, but greater hyperactivity during neutral states, and greater ventral striatal hypoactivity to alcohol cues were associated with fewer days of abstinence prior to treatment entry. These findings support animal studies that suggest the brain structural and functional state is highly dynamic during early recovery [5, 40]. Indeed, it appears that with each day of abstinence, there is improvement in functioning of prefrontal-striatal circuits known to be critical for executive control, resilient coping, and regulation of craving and reward responses [22, 41–43]. Although these data and others [44] suggest longer abstinence periods may be required to reverse disrupted VmPFC and VS functioning, a critical clinical issue is that *such chronic alcohol-related disruptions significantly impact heavy drinking lapses, and thus, stand to jeopardize initiating early recovery by increasing risk of relapse.*

Current findings suggest the need for development of targeted therapeutics to reverse such prefrontal-striatal dysfunction early in treatment, i.e., treatments that target reversing VmPFC/rACC and ventral striatal functional disruptions in order to support successful initiation of recovery. Noradrenergic compounds, e.g., prazosin and guanfacine, which are known to rescue prefrontal stress pathways [45, 46], have the potential to restore VmPFC/rACC functioning and executive control as well as reduce stress and drug craving in patients with substance use disorders [47, 48]. Additionally, intensive outpatient or partial hospitalization programs that provide higher level of support and counseling to maintain alcohol abstinence may also further improve VmPFC functioning to counter high risk stress situations and alcohol-related contexts during early recovery.

This study has several strengths, including careful assessment of the individual variation in length of abstinence and daily mobile health assessment of drinking behavior during initial treatment engagement to obtain daily data on drinking outcome without introducing bias from poor recall [49]. Another important strength was the validation of a novel standardized visual stress and cue provocation paradigm in a patient sample, which can be easily implemented and replicated by other laboratories and in different patient samples.

These novel findings must be considered in light of the study limitations. First, the current sample size limited the ability to systematically assess sex differences in the sample. Additionally, it is important to replicate this research in non-smoking patients with AUDs, as different patterns of co-occurring drug use are common among AUDs and may limit generalizability of current findings. Also, because AUDs are associated with higher levels of chronic stress and traumatic experiences, future samples with varying levels of trauma and stress exposure may elucidate differential functional dysfunction in AUD patients with and without trauma. Finally, given the challenges of achieving and maintaining alcohol abstinence, future studies also need to consider the effects of significant reductions in alcohol use versus complete abstinence on early brain recovery. Despite these limitations, current findings are the first to show a direct relationship between fewer alcohol abstinence days and greater disruption in prefrontal-striatal function in response to stress, alcohol and neutral cues, that in turn, are predictive of subsequent heavy drinking during early treatment. These findings support the critical need for developing targeted medications and

interventions that will facilitate rescue of disrupted prefrontal-striatal functioning to initiate early brain recovery from AUDs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Hunt WA, Barnett LW, and Branch LG, Relapse rates in addiction programs. *Journal of clinical psychology*, 1971 27(4): p. 455–6. [PubMed: 5115648]
2. Shim RS, et al., Predictors of mental health treatment seeking and engagement in a community mental health center. *Community mental health journal*, 2017 53(5): p. 510–514. [PubMed: 28150080]
3. Rosengren DB, Downey L, and Donovan DM, “I already stopped”: abstinence prior to treatment. *Addiction*, 2000 95(1): p. 65–76. [PubMed: 10723831]
4. Crews FT, Alcohol-related neurodegeneration and recovery: mechanisms from animal models. *Alcohol Research & Health*, 2008 31(4): p. 377. [PubMed: 23584011]
5. Fritz M, Klawonn AM, and Zahr NM, Neuroimaging in alcohol use disorder: From mouse to man. *Journal of neuroscience research*, 2019.
6. Zahr NM and Pfefferbaum A, Alcohol’s effects on the brain: neuroimaging results in humans and animal models. *Alcohol research: current reviews*, 2017 38(2): p. 183. [PubMed: 28988573]
7. Pfefferbaum A, et al., Longitudinal changes in magnetic resonance imaging brain volumes in abstinent and relapsed alcoholics. *Alcoholism: Clinical and Experimental Research*, 1995 19(5): p. 1177–1191.
8. Muuronen A, et al., Influence of improved drinking habits on brain atrophy and cognitive performance in alcoholic patients: A 5-year follow-up study. *Alcoholism: Clinical and Experimental Research*, 1989 13(1): p. 137–141.
9. Beck A, et al., Effect of brain structure, brain function, and brain connectivity on relapse in alcohol-dependent patients. *Archives of general psychiatry*, 2012 69(8): p. 842–852. [PubMed: 22868938]
10. Fein G and Cardenas VA, Neuroplasticity in human alcoholism: studies of extended abstinence with potential treatment implications. *Alcohol research: current reviews*, 2015 37(1): p. 125. [PubMed: 26259093]
11. Sinha R, New findings on biological factors predicting addiction relapse vulnerability. *Curr Psychiatry Rep*, 2011 13(5): p. 398–405. [PubMed: 21792580]
12. Dong Y, et al., Circuit and synaptic plasticity mechanisms of drug relapse. *Journal of Neuroscience*, 2017 37(45): p. 10867–10876. [PubMed: 29118216]
13. Schacht JP, Anton RF, and Myrick H, Functional neuroimaging studies of alcohol cue reactivity: a quantitative meta-analysis and systematic review. *Addiction biology*, 2013 18(1): p. 121–33. [PubMed: 22574861]
14. O’Daly OG, et al., Withdrawal-associated increases and decreases in functional neural connectivity associated with altered emotional regulation in alcoholism. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 2012 37(10): p. 2267–76. [PubMed: 22617355]
15. Le Berre A-P, et al., Readiness to change and brain damage in patients with chronic alcoholism. *Psychiatry Research: Neuroimaging*, 2013 213(3): p. 202–209. [PubMed: 23838052]

16. Seo D, et al., Disrupted ventromedial prefrontal function, alcohol craving, and subsequent relapse risk. *JAMA Psychiatry*, 2013 70(7): p. 727–39. [PubMed: 23636842]
17. Everitt BJ and Robbins TW, Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nature neuroscience*, 2005 8(11): p. 1481–1489. [PubMed: 16251991]
18. Seeley WW, et al., Dissociable intrinsic connectivity networks for salience processing and executive control. *Journal of Neuroscience*, 2007 27(9): p. 2349–2356. [PubMed: 17329432]
19. Koob GF and Volkow ND, Neurocircuitry of addiction. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 2010 35(1): p. 217–38. [PubMed: 19710631]
20. Cooney NL, et al., Alcohol cue reactivity, negative-mood reactivity, and relapse in treated alcoholic men. *J Abnorm Psychol*, 1997 106(2): p. 243–50. [PubMed: 9131844]
21. Charlet K, et al., Imaging resilience and recovery in alcohol dependence. *Addiction*, 2018 113(10): p. 1933–1950. [PubMed: 29744956]
22. Sinha R, et al., Dynamic neural activity during stress signals resilient coping. *Proceedings of the National Academy of Sciences*, 2016 113(31): p. 8837–8842.
23. Milivojevic V and Sinha R, Central and peripheral biomarkers of stress response for addiction risk and relapse vulnerability. *Trends in molecular medicine*, 2018 24(2): p. 173–186. [PubMed: 29396148]
24. First MB, et al., Structured Clinical Interview for DSM-V- Research Version (SCID 5 for DSM-5, Research Version; SCID-5-RV. Version 1.0.0 ed. 2015, Arlington, VA: American Psychiatric Association.
25. Nowinski J, Baker S, and Carroll KM, Twelve step facilitation therapy manual: A clinical research guide for therapists treating individuals with alcohol abuse and dependence. Vol. 1 1992: Diane Publishing Company.
26. Lang PJ, Bradley MM, and Cuthbert BN, International affective picture system (IAPS): Technical manual and affective ratings. 1999, Gainesville: University of Florida, Center for Research in Psychophysiology.
27. Jones A, et al., Compliance with ecological momentary assessment protocols in substance users: a meta-analysis. *Addiction*, 2019 114(4): p. 609–619. [PubMed: 30461120]
28. Sobell LC and Sobell MB, Timeline Follow-back: A technique for assessing self-reported ethanol consumption., in *Measuring Alcohol Consumption: Psychosocial and Biological Methods*, Allen L and Litten R, Editors. 1992, Humana Press: Totowa, NJ p. 41–72.
29. Noone M, Dua J, and Markham R, Stress, cognitive factors, and coping resources as predictors of relapse in alcoholics. *Addictive Behaviors*, 1999 24(5): p. 687–693. [PubMed: 10574307]
30. Sinha R, Shaham Y, and Heilig M, Translational and reverse translational research on the role of stress in drug craving and relapse. *Psychopharmacology*, 2011 218(1): p. 69–82. [PubMed: 21494792]
31. Blaine SK and Sinha R, Alcohol, stress, and glucocorticoids: From risk to dependence and relapse in alcohol use disorders. *Neuropharmacology*, 2017.
32. Nikolova YS, et al., Divergent responses of the amygdala and ventral striatum predict stress-related problem drinking in young adults: possible differential markers of affective and impulsive pathways of risk for alcohol use disorder. *Molecular psychiatry*, 2016 21(3): p. 348–356. [PubMed: 26122584]
33. Martinez D, et al., Alcohol dependence is associated with blunted dopamine transmission in the ventral striatum. *Biological psychiatry*, 2005 58(10): p. 779–786. [PubMed: 16018986]
34. Yoder KK, et al., Differences in IV alcohol-induced dopamine release in the ventral striatum of social drinkers and nontreatment-seeking alcoholics. *Drug and alcohol dependence*, 2016 160: p. 163–169. [PubMed: 26832934]
35. Myrick H, et al., Differential brain activity in alcoholics and social drinkers to alcohol cues: relationship to craving. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 2004 29(2): p. 393–402. [PubMed: 14679386]
36. Townshend J and Duka T, Attentional bias associated with alcohol cues: differences between heavy and occasional social drinkers. *Psychopharmacology*, 2001 157(1): p. 67–74. [PubMed: 11512045]

37. Ravan S, et al., Molecular imaging in alcohol dependence, in Handbook of clinical neurology. 2014, Elsevier p. 293–311.
38. Urban NB, et al., Sex differences in striatal dopamine release in young adults after oral alcohol challenge: a positron emission tomography imaging study with [11C] raclopride. Biological psychiatry, 2010 68(8): p. 689–696. [PubMed: 20678752]
39. Volkow ND and Baler RD, Brain imaging biomarkers to predict relapse in alcohol addiction. JAMA psychiatry, 2013 70(7): p. 661–663. [PubMed: 23636789]
40. Zahr NM, et al., Transient CNS responses to repeated binge ethanol treatment. Addiction biology, 2016 21(6): p. 1199–1216. [PubMed: 26283309]
41. Bechara A, et al., Decision-making deficits, linked to a dysfunctional ventromedial prefrontal cortex, revealed in alcohol and stimulant abusers. Neuropsychologia, 2001 39: p. 376–389. [PubMed: 11164876]
42. Etkin A, Egner T, and Kalisch R, Emotional processing in anterior cingulate and medial prefrontal cortex. Trends in cognitive sciences, 2011 15(2): p. 85–93. [PubMed: 21167765]
43. Maier SF and Watkins LR, Role of the medial prefrontal cortex in coping and resilience. Brain research, 2010 1355: p. 52–60. [PubMed: 20727864]
44. Mon A, Durazzo TC, and Meyerhoff DJ, Glutamate, GABA, and other cortical metabolite concentrations during early abstinence from alcohol and their associations with neurocognitive changes. Drug and alcohol dependence, 2012 125(1–2): p. 27–36. [PubMed: 22503310]
45. Arnsten AF, The use of α -2A adrenergic agonists for the treatment of attention-deficit/hyperactivity disorder. Expert review of neurotherapeutics, 2010 10(10): p. 1595–1605. [PubMed: 20925474]
46. McKee SA, et al., A translational investigation targeting stress-reactivity and prefrontal cognitive control with guanfacine for smoking cessation. Journal of psychopharmacology, 2015 29(3): p. 300–311. [PubMed: 25516371]
47. Fox HC, et al., Prazosin effects on stress- and cue-induced craving and stress response in alcohol-dependent individuals: preliminary findings. Alcoholism, clinical and experimental research, 2012 36(2): p. 351–60.
48. Fox HC, et al., Guanfacine effects on stress, drug craving and prefrontal activation in cocaine dependent individuals: preliminary findings. Journal of psychopharmacology, 2012 26(7): p. 958–972. [PubMed: 22234929]
49. Shiffman S, Ecological momentary assessment (EMA) in studies of substance use. Psychological assessment, 2009 21(4): p. 486. [PubMed: 19947783]

Figure 1. Study and Task Designs with Craving and Subjective Stress Ratings by Group and Condition.

Note: AUD=Alcohol Dependent HC= Healthy Controls. (a) In study 1, HC and treatment seeking AUD were recruited for a single fMRI scan. In study 2, treatment entering AUD participants with varying days of abstinence completed an initial single fMRI scan and then followed using intensive longitudinal measurement for two weeks. (b) Study 1 and 2 fMRI paradigm for provocation of stress- and alcohol cue provoked craving uses sustained exposures to six 1-minute consecutive runs of visual images for alcohol, stress, or neutral cue conditions presented in a block design. Plasma Cortisol levels from blood was measured prior to, immediately following and at 5 minute recovery timepoint post provocation runs (indicated by Δ). Subjective stress and craving were measured after all baseline and provocation runs (indicated by black lines) in each condition block. Heart rate was measured continuously (indicated by red line). (c) Study 1: Alcohol craving at baseline and in response to alcohol cue, neutral, and stressful visual images are plotted for the AUD and HC groups, and showed a baseline difference between groups AUD>HC ($p < .000001$). After correction for baseline levels of craving, AUD also showed significantly higher craving responses relative to HC group, to alcohol cues ($F(1,595)=286.2$, $p < 0.0001$) and stress cues ($F(1,595)=209.3$, $p < 0.0001$), but not to neutral stimuli. Within the AUD group, craving was significantly higher in response to alcohol cues than the neutral cues at each time point (p 's<0.05) and during later runs in the stress condition relative to neutral (p 's<0.05). Note: * denotes significantly different from neutral condition. (d) The subjective stress response to alcohol cue, neutral, and stressful visual images are plotted in AUD individuals and HC. AUD show greater baseline subjective stress relative to HC group, ($F(1,255)=41.92$, $p <$

0.0001). Both AUD and HC show significantly greater subjective response to stress cues than to neutral and to alcohol cues, ($F(2,903)=160.1$, $p<0.0001$) and ($F(2,882)=206.8$, $p<0.0001$). In AUD, at the beginning and end of the alcohol cue runs, stress was higher than in the neutral condition (p 's <0.05). Note: * denotes significantly different from neutral condition.

Figure 2. Study 1 sagittal slices from whole brain voxel-based analyses of fMRI contrasts for AUD and HC group focused on hypothesized regions of the Ventromedial Prefrontal Cortex (VmPFC), Rostral Anterior Cingulate (rACC), and Ventral Striatum (VS) or Dorsal Striatum (DS) functional cue reactivity.

In each column, first sagittal slices showing AUD average group activation are presented, which are then followed by HC sagittal slices showing average HC group activation. The third and fourth columns show the average change in ROI beta weights for the VmPFC/rACC and VS/DS ROIs. *All differences are significant after whole brain correction at $p < 0.001$, with an alphaSim cluster correction at 0.05.* Row 1: In the stress- neutral condition, AUD show VmPFC and VS hypoactivation relative to HC (peak differences MNI coordinates: 17,52,-15 and 15,14,-9, respectively). Row 2: In the alcohol cue- neutral condition, AUD show VmPFC and VS hypoactivation relative to HC (peak differences MNI coordinates: 15,24,-21 and 18,8,-12, respectively). Row 3: In the neutral-baseline contrast, AUD show VmPFC and VS hyperactivation, relative to HC (peak differences MNI coordinates: 18,51,-12 and 18,17,-9, respectively). Row 4: In the stress- alcohol cue condition, AUD show rostral Anterior Cingulate hypoactivation and DS hyperactivation relative to HC (peak differences MNI coordinates: 15,37,10 and 15,7,-11, respectively). (Also see Supplemental Table 1.1 for full list of group differences).

Figure 3. Study 2: Prefrontal and Ventral Striatal Associations with Abstinence Days from Whole-Brain Voxelf-based Analyses.

a1-a3: In the stress condition (stress-neutral contrast), lower the Days Abstinent from alcohol in AUD participants on scan day and treatment entry was associated with greater hypoactivation of the VmPFC/rACC and VS regions in response to stress relative to neutral contrast (peak activation association MNI coordinates: 2,36,12 and 9,19,-2, respectively). b1-b3: In the alcohol cue condition (alcohol cue- neutral contrast), lower Days Abstinent from alcohol in AUD participants on scan day and treatment entry was associated with greater hypoactivation of the VmPFC/rACC and VS regions in response to alcohol cues relative to neutral contrast (peak activation association MNI coordinates: 2,34,-3 and 11,8,-11, respectively). c1-c3: In the neutral condition (neutral-baseline contrast), lower Days Abstinent from alcohol in AUD participants on scan day and treatment entry was associated with greater hyperactivation of the VmPFC/rACC and VS regions in the neutral condition (peak activation association MNI coordinates: 14,41,7 and 14,9,-10, respectively). All differences are significant after whole brain correction at $p<0.001$, with an alpha of 0.05. (Also see Supplemental Table 1.2 for full list of associations). Note: Activation of rACC in a1 and b1 extends into the lateral ventricle.

Figure 4. Study 2 Whole Brain Relationship between Abstinence Days-Covaried Task (Stress, Cue, Neutral Condition) X Heavy Drinking Days During Early AUD Treatment.

(a) Estimated survival function for time to heavy drinking day (HDD) lapse, shown by length of abstinence in the first 2 weeks of treatment initiation. Participants with longer abstinence were less likely overall to relapse to HDD, and to relapse to HDD at a slower rate than those with shorter abstinence days. Solid line indicates survival curve for mean days of abstinence prior to treatment initiation, dotted lines indicate survival curves for one standard deviation in length of Days Abstinent above and below the mean. b1-b3: Participants with AUD who showed greater VmPFC and VS hypoactivation in response to stress (stress-neutral contrast; peak activation association MNI coordinates: 14,38,3 and 12,8-12, respectively) relapse to heavy drinking faster than those with increased activation of these regions in response to stress. c1-c3: Participants with AUD who showed greater hypoactivation in the VmPFC and VS in response to alcohol cues (alcohol cues-neutral contrast; peak activation association MNI coordinates: 12,38,2 and 14,10,12, respectively) relapse to heavy drinking faster than those with increased activation of these regions in responses to alcohol cues. All differences are significant after whole brain correction at $p<0.001$, with an alpha of 0.05. (Also see Supplemental Table 1.3 for full list of associations).

Table 1.

Participant Demographics.

Subject Variable	Study I				Study II			
	Healthy Controls (HC) (N=43)		AUD patients (N=44)		AUD patients (N=69)		Mean	SD
	Mean	SD	Mean	SD	Mean	SD		
Age	32	10	33	11	36	11		
Years of Education	16.4	2.3	14.7	1.92	13.8	2.1		
Shipley IQ	113	7	112	7	106	7		
Average Years of Alcohol Use	12.5	2.2	13.3	1.7	14.22	1.3		
Average Days of Alcohol Use/Month *	6	0.9	19.1	1.2	23.6	4.7		
Average Amount of Alcohol Use/Month *	11.5	2	119.3	15.1	139	26.2		
Days Abstinent at Scan	11.75	10.7	5.5	1.2	4.7	0.9		
	N	%	N	%	N	%		
Gender (% male)	23	53.5	28	63.6	42	61		
Race (% Caucasian)	25	58.1	26	59	28	41		
Smokers (%) *	0	0	21	47.7	36	52		
Lifetime Mood Disorder *	6	13.9	16	36.4	16	23.2		
Lifetime Anxiety Disorder *	1	2.3	28	65.1	26	37.7		
Lifetime PTSD	1	2.3	1	2.3	1	1.5		
Lifetime other SUD *	3	6.9	12	27.2	12	17.4		

Note: Study 1: The Alcohol Use Disorder (AUD) and Healthy Control (HC) groups in Study 1 are matched for age, sex, race, IQ, years of education, and years of regular alcohol consumption (i.e. no significant differences, $p > 0.05$), but were significantly different as expected in frequency of drinking alcohol ($t(85) = 57.5$, $p < 0.0001$), usual amount of alcohol consumed ($t(85) = 46.4$, $p < 0.0001$), in number of nicotine smokers ($\chi^2(1, N=85) = 22$, $p < 0.0001$), in lifetime diagnoses of mood disorders ($\chi^2(1, N=85) = 5.78$, $p < 0.02$), anxiety disorders ($\chi^2(1, N=85) = 36.79$, $p < 0.01$), and other substance use disorders (SUDs; $\chi^2(1, N=85) = 6.28$, $p < 0.0001$). There was no difference in lifetime diagnoses of Post-Traumatic Stress Disorder (PTSD) between the HC and AUD in Study 1.

* $p < 0.05$ indicating significant group difference. Please note as Study 2 pertains to prediction of drinking outcome, the Study 2 sample was not compared to the HC group in Study 1 on demographic and clinical measures. There were no differences in demographic, drinking and smoking characteristics, or lifetime diagnoses of mood disorders, PTSD, or SUDs of the AUD samples in Study 1 and 2, but the Study 1 AUD sample included significantly more lifetime anxiety disorders diagnoses ($\chi^2(1, N=113) = 7.99$, $p < 0.005$) compared to the Study 2 sample. Also note, Study 2 sample includes 42 AUD patients from Study 1 (also see Figure 1a).