Acute naltrexone does not remediate fronto-striatal disturbances in alcoholic and alcoholic polysubstance-dependent populations during a monetary incentive delay task.

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Abstract

There is a concerted research effort to investigate brain mechanisms underlying addiction processes that may predicate the development of new compounds for treating addiction. One target is the brain's opioid system, due to its role in the reinforcing effects of substances of abuse. Substance-dependent populations have increased numbers of the mu opioid receptor (MOR) in fronto-striatal regions that predict drug relapse, and demonstrate disturbances in these regions during the processing of non-drug rewards. Naltrexone is currently licensed for alcohol and opiate dependence, and may remediate such disturbances through the blockade of MORs in fronto-striatal reward circuitry. Therefore, we examined the potential acute modulating effects of naltrexone on the anticipation of, and instrumental responding for, non-drug rewards in long-term abstinent alcoholics, alcoholic poly substance-dependent individuals and controls using a monetary incentive delay (MID) task during a randomized double blind placebo controlled fMRI study. We report that the alcoholic poly substance-dependent group exhibited slower and less accurate instrumental responding compared to alcoholics and controls that was less evident after acute naltrexone treatment. However, naltrexone treatment was unable to remediate disturbances within fronto-striatal regions during reward anticipation and "missed" rewards in either substance-dependent group. While we have not been able to identify the underlying neural mechanisms for improvement observed with naltrexone in the alcoholic poly-substance dependent group, we can confirm that both substance-dependent groups exhibit substantial neural deficits during an MID task, despite being in long-term abstinence.

Introduction

Substance dependence, particularly to alcohol, continues to be a major cause of harm to individuals and society (Nutt et al., 2010). Identifying the substrates of addiction in an attempt to elucidate potential neural targets for future treatment development in substance dependence remains a major challenge in neuroscience. One such neural target is the brain's opioid system, given its interactions with the dopamine (DA) system of the brain (Solinas et al., 2004), and its role in the reinforcing effects of alcohol and other substances of abuse (Colasanti et al., 2012; Mick et al., 2014; Spreckelmeyer et al., 2011).

Mu opioid receptor (MOR) numbers are reported to be significantly elevated in alcoholic patients in early abstinence (Heinz et al., 2005), particularly in the ventral striatum (VS), with increased MOR availability found to correlate with alcohol craving (Williams et al., 2009). Similarly, cocaine abusers in early abstinence have increased numbers of MORs within fronto-striatal regions (Gorelick et al., 2005), which have been found to predict relapse (Gorelick et al., 2008). A similar pattern has been reported in opiate abstinence (Williams et al., 2007; Zubieta et al., 2000). There is also good evidence that MOR blockade is effective in promoting substance abstinence (Grassi et al., 2007; Krystal et al., 2001; Srisurapanont et al., 2005). Therefore, disturbances to the brain's opioid system during early abstinence make it a viable target for protection against potential alcohol and drug relapse.

Substance abusers, particularly alcoholics, may still be at risk for relapse in long-term abstinence due to ongoing and latent disturbances in the brain's opioid system. Opioid disturbances within DA fronto-striatal reward circuitry may confer an ongoing risk for relapse to drug rewards if there is a diminished incentive value

of, and motivation to procure, non-drug rewards. Naltrexone is currently licensed for alcohol dependence, and may remediate these disturbances by restoring some balance within key fronto-striatal networks that are critical for optimizing the incentive value and attainment of non-drug rewards. The current study, therefore, investigated the effects of acute MOR blockade with naltrexone on fronto-striatal-dependent reward processing in alcoholics and polysubstance-dependent individuals who were in extended abstinence. We hypothesized that 1) alcoholic and polysubstance-dependent groups, compared to controls, would demonstrate disturbances within fronto-striatal regions in response to the prediction of potential non-drug rewards and 2) acute MOR blockade with naltrexone would have an ameliorating effect on these neural disturbances, possibly providing a credible therapeutic biomarker for treating deficiencies in non-drug reward processing that may trigger relapse to addictive behaviour.

Material and Methods

Participants

This was a randomized double blind placebo controlled multi-centre study involving three study sites in the United Kingdom (Imperial College, Cambridge and Manchester - ICCAM). For a more detailed description of the ICCAM Platform, see Paterson et al (Paterson et al., 2015). Inclusion criteria were individuals who met DSM-IV criteria for current or prior alcohol dependence ($Alcohol_{minus}$), or alcohol plus ($Alcohol_{plus}$) another substance of dependence (e.g., amphetamines, benzodiazepines, cocaine, opiates) and who would be abstinent for at least 4 weeks prior to the experimental sessions. There was no upper limit for abstinence length. All participants were aged 21 to 64. In the current study, the $Alcohol_{minus}$ group was made up of 21 abstinent alcoholics, with the $Alcohol_{plus}$ group comprised

of 25 abstinent alcoholic polysubstance-dependent individuals (having met criteria for dependence to alcohol plus one or more other substances of dependence). The $Alcohol_{plus}$ group was made up of 6 abstinent alcoholics with cocaine dependence; 6 with cocaine and opiate dependence; 4 with amphetamine, cocaine and opiate dependence; 2 with just opiate dependence; 1 with amphetamine, cocaine and solvent dependence; 1 with benzodiazepine, cocaine and opiate dependence; 1 with cocaine and GHB dependence; 1 with benzodiazepine and opiate dependence; 1 with amphetamine and cocaine dependence; 1 with benzodiazepine and cocaine dependence, and 1 with just amphetamine dependence. The healthy control group was made up of 35 participants with no previous history of substance abuse, as assessed using the ASSIST and timeline follow-back. All participants were required to provide a negative breath alcohol test and a negative urine sample for various drugs of abuse on both experimental days (screening for the presence of amphetamines, benzodiazepines, cannabinoids, cocaine and opiates).

Exclusion criteria included 1) current use of regular prescription or non-prescription medication that could not be stopped for the study duration, or would interfere with study integrity or subject safety (including but not limited to antipsychotics, anticonvulsants, antidepressants, disulfiram, acamprosate, naltrexone, varenicline); 2) current primary axis I diagnosis, past history of psychosis (unless drug-induced); 3) current or past history of enduring severe mental illness (e.g., schizophrenia, bipolar affective disorder); 4) other current or past psychiatric history that, in the opinion of a psychiatrist, contraindicated participation; 5) history or presence of a significant neurological diagnosis that may have influenced the outcome or analysis of the results (including but not limited to stroke, epilepsy, space occupying lesions, multiple sclerosis, Parkinson's

disease, vascular dementia, transient ischemic attack, clinically significant head injury); 6) claustrophobia or unable to lie still in the MRI scanner for up to 90 minutes; 7) presence of a cardiac pacemaker, other electronic device or other MRI contraindication, including pregnancy, as assessed by a standard pre-MRI questionnaire. Secondary or lifetime history of depression or anxiety was permitted in both substance abusers and healthy controls since these are very common psychiatric disorders.

Experimental visits

At the randomised placebo and naltrexone experimental visits, an eligibility check was performed. Participants' intervening drug use and concomitant medication were checked and participants completed alcohol breath, pregnancy and urine drugs of abuse screening tests. Cigarette smokers in all groups smoked *ad lib* approximately 60 minutes prior to scanning in order to avoid the potential confounds of withdrawal and/or craving during scanning.

Medications

Drug preparation, labelling and packaging was performed by UCLH Pharmacy Manufacturing Unit. Placebo was Vitamin C (100mg, supplier: Sigma, manufacturer: Norbrook) and naltrexone (50mg Nalorex® - manufacturer - Bristol-Myers Squibb) were prepared and packaged according to Investigational Medicinal Product guidelines. The maximum naltrexone plasma concentration after an acute 50 mg dose is 0.5-3 hours (Meyer et al., 1984). Therefore, participants were dosed two hours prior to each experimental scan session to ensure high MOR occupancy during testing. Naltrexone and placebo medications were supplied in

identical white opaque bottles and administered by independent nursing staff, such that both researcher and participant remained blinded.

Monetary Incentive Delay Task (MID)

We used a "monetary incentive delay task" (MID), which was based on that originally employed by Knutson (Knutson et al., 2001). While being scanned on the placebo and naltrexone experimental sessions, participants performed the MID task, during which they anticipated potential monetary gain, loss or no potential monetary outcome. During each trial, participants viewed one of three symbols (a cue) that indicated the potential to win fifty pence (square containing an ascending arrow), lose fifty pence (square containing a descending arrow) or experience no financial outcome (square containing a horizontal line - here referred to as a neutral trial). Each cue was presented for one second, with a variable duration (2-4 sec) for the subsequent anticipation period. Following the anticipation period, participants made a button press response upon the presentation of a visual target (star located within a circle). Following their response to the visual target, participants received feedback (1500 ms) as to whether they were successful or unsuccessful ("Hit" or "Miss" respectively) on each trial, and also saw a running total of their winnings up to that point in the task. Following the feedback, there was an end fixation period (3-5 sec) before the commencement of the next trial.

Because the primary objective of this study was to examine the neural correlates of reward processing, we chose to use a smaller number of loss trials in an attempt to increase the incentive salience of win trials during the task. Consequently, there were a total of 18 "win", 6 "lose" and 18 "neutral" trials on

each run of the task. The MID task was additionally tailored to adapt to the visual target reaction time of each participant by using a staircase algorithm, such that the presentation of the visual target became shorter as performance improved during the experiment. This staircase algorithm enabled us to set a limit on the success rate of each participant (~66%), which additionally served to incentivize participants to engage in the task. Participants were instructed to maximize their winnings and were told they would receive them at the end of the study. Dependent measures were percentage accuracy and mean reaction time (milliseconds) to the visual target on each of the MID trials. There were two functional MRI runs of the task (432 seconds each). The task was programmed using E-Prime version 2.0 (Psychology Software Tools, Pittsburgh, USA).

Functional MRI (fMRI) Data Acquisition

All centres operated MRI machines with a main magnetic field of 3 tesla (T). Centres in London and Cambridge operated nominally identical 3T Siemens Tim Trio systems running the syngo MR B17 software with a Siemens 32 channel receive-only phased-array head coil. The Manchester centre operated a 3T Philips Achieva running version 2.6.3.5 software and an 8 element SENSE head coil. For anatomical images, 160 high-resolution T1-weighted anatomic MPRAGE axial images (FOV 256 mm, thickness 1.0 mm, voxel size 1.0×1.0×1.0) were acquired (total duration 303 s). Functional data were acquired using a T2* weighted echo-planar imaging sequence collecting 36 non-contiguous (0% gap) 3.0 mm axial slices covering the entire brain (TE=31 ms, TR=2000 ms, FOV 225 mm, 64×64 mm matrix size in Fourier space). The two runs of the MID task produced a total of 432 volumes of functional MRI data.

MID fMRI data analyses

Data pre-processing and statistical analysis were conducted using FEAT (fMRI Expert Analysis Tool) from the FMRIB Software Library (www.fmrib.ox.ac.uk/fsl). Pre-statistical processing was as follows: motion correction utilizing FMRIB's Linear Image Registration Tool (MCFLIRT; non-brain matter removal using Brain Extraction Tool (BET); spatial smoothing with a 5-mm full-width half maximum Gaussian kernel; mean-based intensity normalization; nonlinear high-pass temporal filtering (Gaussian-weighted least squares straight line fit, with sigma = 25.0 sec). The six rigid body movement parameters were also included as regressors in the model in FSL FEAT.

For each participant, first level whole-brain mixed-effects analyses were performed by modelling the MID anticipation periods (i.e. *Neutral, Win*) as explanatory variables within the context of the general linear model on a voxel-by-voxel basis (variable boxcar functions for the cue + variable anticipation period regressors were convolved with the haemodynamic response function). The win and neutral outcome periods ("Hit" and "Miss") were also modelled (stick functions for "hit" and "miss" trial period regressors were convolved with the haemodynamic response function). During these first level analyses, the *win anticipation>neutral anticipation*, *win hit>neutral hit* and *win miss>neutral miss* contrasts was formulated. Owing to the small number of loss trials in the current task, the loss cue + anticipation and outcome periods were regressed out of the functional time series as conditions of no interest. The end fixation period of the task served as the implicit baseline. Registration was conducted through a two-step procedure, whereby EPI images were first registered to the high-resolution T1 structural

image, then into standard (Montreal Neurological Institute, MNI avg152 template) space, with 12-parameter affine transformations.

Two (Group: $alcohol_{minus}$ & $alcohol_{plus}$ combined vs. control) by two (Drug: placebo vs. naltrexone) whole brain cluster-based repeated measures ANOVA analyses were performed as part of a higher-level mixed-effects analysis on the *win anticipation>neutral anticipation*, *win hit>neutral hit* and *win miss>neutral miss* contrasts. These higher-level analyses were conducted using FLAME (FMRIB's Local Analysis of Mixed Effects). Cluster (Gaussianised F) statistical images were determined by $Z>2.3$ with a corrected cluster significance threshold of $p<0.05$. This ANOVA analysis produced a total of three (i.e. drug effect, group effect, drug x group interaction) zF statistical images.

Other Statistics

Between groups demographics (see Table 1.) were examined using Kruskal–Wallis (gender distribution and drug order) or one-way ANOVA analyses. For analyses conducted on the MID behavioural data, we used a three (Group: $alcohol_{minus}$ vs. $alcohol_{plus}$ vs. control) by two (Drug: placebo vs. naltrexone) by two (Condition: neutral vs. win) repeated measures ANOVA analyses. We also conducted a three (Group: $alcohol_{minus}$ vs. $alcohol_{plus}$ vs. control) by two (Drug: placebo vs. naltrexone) repeated measures ANOVA on an index of the relative motivational value (RMV). This value is based on the ratio of mean reaction times to the target on neutral trials compared to that on win trials - i.e. $RT_{neutral}/RT_{win}$. Here a value >1 reflects a higher relative value of monetary incentives (Sescousse et al., 2015), and which more closely reflects the contrasts in the incentive value of these conditions computed during the functional MRI analyses. We extracted

the mean BOLD signal change from the group zF-statistic ANOVA clusters and conducted three (Group: $alcohol_{minus}$ vs. $alcohol_{plus}$ vs. control) by two (Drug: placebo vs. naltrexone) repeated measures ANOVA analyses to explore the direction of the effects observed in the cluster-based analyses. All analyses were conducted using the Statistical Package for the Social Sciences (SPSS Inc., Chicago).

Results

Demographics

Table 1 shows the between group demographics for the control, $alcohol_{minus}$ and $alcohol_{plus}$ groups. The groups significantly differed on most of the measures reported herein, including age ($alcohol_{minus}$>$alcohol_{plus}$ & control), years of education ($alcohol_{plus}$<control), IQ ($alcohol_{plus}$<control), alcohol exposure (control & $alcohol_{plus}$<$alcohol_{minus}$), and cigarette ($alcohol_{plus}$>control) and cannabis ($alcohol_{plus}$>$alcohol_{minus}$ & control) use history. The groups did not differ on handedness score or gender distribution. We further report that the groups did not differ significantly on drug treatment order (χ^2 = 0.48, df=2, p> 0.7) during the study.

-Insert Table 1 about here-

MID Performance

Figure 1A below shows the mean MID accuracy (%) for the two conditions in the $alcohol_{minus}$, $alcohol_{plus}$ and control groups during the placebo and naltrexone sessions. A three (Group: $alcohol_{minus}$ vs. $alcohol_{plus}$ vs. control) by two (Drug: placebo vs. naltrexone) by two (Condition: neutral vs. win) repeated measures ANOVA showed a significant effect of condition (F=46.3; df=1, 78; p<0.001 - win>neutral) and a significant drug x group interaction (F=4.04; df=2, 78; p<0.05). Follow-up analyses revealed that, across MID conditions, the $alcohol_{plus}$ group was significantly less accurate than both the $alcohol_{minus}$ (p<0.001) and control (p<0.01) groups during the placebo session only. Figure 1B below shows the mean MID reaction time (milliseconds) for the two conditions. The same ANOVA demonstrated a significant effect of condition (F=63.6; df=1, 78; p<0.001 - win<neutral) and a significant drug x group interaction (F=4.07; df=2, 78; p<0.05). Follow-up analyses revealed that, across MID conditions, the $alcohol_{plus}$ group was significantly slower than both the $alcohol_{minus}$ and controls groups (p<0.05) during the placebo session only. Finally, figure 1C shows the computed index of the RMV. A three (Group: $alcohol_{minus}$ vs. $alcohol_{plus}$ vs. control) by two (Drug: placebo vs. naltrexone) repeated measures ANOVA showed no effect of drug (F=0.61; df=1, 78; p=0.43), group (F=0.45; df=2, 78; p=0.63) or a drug x group interaction (F=0.62; df=2, 78; p=0.53) on this index, however.

-Insert Figure 1 about here-

Functional MRI

All three groups demonstrated statistically significant activation patterns across fronto-striatal regions during the placebo and naltrexone challenges for the *win anticipation>neutral anticipation* contrast at a whole brain level (see Supplementary Figs 1 & 2). As we did not observe any significant group x drug interactions for a three (Group: $alcohol_{minus}$ vs. $alcohol_{plus}$ vs. control) by two (Drug: placebo vs. naltrexone) whole brain cluster-based repeated measures ANOVA, we decided to collapse across the two substance groups in order to increase the power to detect clusters related to a main effect of group. The two (Group: $alcohol_{minus}$ & $alcohol_{plus}$ combined vs. control) by two (Drug: placebo vs. naltrexone) whole brain cluster-based repeated measures ANOVA analyses showed a significant main effect for group (see Supplementary Fig 3), but did not reveal a significant main effect for drug or a drug x group interaction. Table 2 shows the cluster-based statistics from this ANOVA group effect, which comprised 12 separate clusters covering cerebellar, occipital, temporal, frontal and striatal regions.

-Insert Table 2 about here-

In order to assess the direction of the observed group effect, we performed three (Group: $alcohol_{minus}$ vs. $alcohol_{plus}$ vs. control) by two (Drug: placebo vs. naltrexone) repeated measures ANOVA analyses on the mean BOLD signal change within each of the group ANOVA zF-statistic clusters. These were performed in order to reveal whether the $alcohol_{minus}$ and $alcohol_{plus}$ groups independently contributed to the main ANOVA group effect.

In the left orbitofrontal cortex (OFC) cluster, there was a main effect of group (F=5.25; df=2, 78; p<0.01), which revealed that only the alcohol$_{plus}$ group was significantly lower than the control group (p<0.01- Fig 2A) in this region. Within the right inferior frontal gyrus (IFG)/insula cluster, however, a main effect of group (F=4.25; df=2, 78; p<0.05) showed that there was a significant BOLD signal reduction in both the alcohol$_{minus}$ and alcohol$_{plus}$ groups (p<0.05 - Fig 2B) compared to the control group. There was also a main effect of group in the left (F=4.17; df=2, 78; p<0.05) and right (F=4.12; df=2, 78; p<0.05) ventral caudate/nucleus accumbens (NAcc) showing that the alcohol$_{minus}$ group (p<0.05), and to a greater degree, the alcohol$_{plus}$ group (p<0.01) exhibited a significantly lower BOLD signal change than the control group across these striatal regions (Fig 3A & 3B).

-Insert Figure 2 about here-

-Insert Figure 3 about here-

Additionally, there was a significant effect of group in the right frontal pole cluster (F=6.23; df=2, 78; p<0.05 - alcohol$_{minus}$<control, p<0.05; alcohol$_{plus}$<control, p<0.01); right cerebellum cluster (F=3.5; df=2, 78; p<0.05 - alcohol$_{plus}$<control, p<0.05); right parahippocampal gyrus cluster (F=6.40; df=2, 78; p<0.01 - alcohol$_{minus}$<control, p<0.05; alcohol$_{plus}$<control, p<0.01); right supramarginal gyrus cluster (F=4.10; df=2, 78; p<0.05 - alcohol$_{minus}$ and alcohol$_{plus}$<control, p<0.05); left middle temporal gyrus/parahippocampal gyrus cluster (F=7.73; df=2, 78; p<0.01 - alcohol$_{minus}$<control, p<0.05; alcohol$_{plus}$<control, p<0.001) and the left occipital fusiform gyrus cluster (F=3.32; df=2, 78; p<0.05 - alcohol$_{plus}$<control, p<0.05). We did not, however, observe a significant effect of group in either the left (F=2.21; df=2, 78; p<0.1) or right

(F=2.25; df=2, 78; p<0.09) anterior cingulate cortex (ACC) clusters, suggesting that the original observed group effect in this region was due to a conflation of the $alcohol_{minus}$ and $alcohol_{plus}$ groups. In order to confirm this, we collapsed across the two groups and conducted a Two (Group: $alcohol_{minus}$ & $alcohol_{plus}$ combined vs. control) by two (Drug: placebo vs. naltrexone) repeated measures ANOVA to verify a significant effect of group in the left (F=4.88; df=1, 79; p<0.5 - $alcohol_{minus}$ & $alcohol_{plus}$ combined<control - Fig 4A), and right (F=5.06; df=1, 79; p<0.5 - $alcohol_{minus}$ & $alcohol_{plus}$ combined<control - Fig 4B) ACC clusters.

-Insert Figure 4 about here-

The same whole brain cluster-based repeated measures ANOVA analysis also revealed a significant main effect of group for the *win miss>neutral miss* contrast in the left insula (140 voxels; x=-42; y=14; z=-12; zF=3.72; df=1, 79; p<0.001) and the right ACC (415 voxels; x=4; y=44; z=4; zF=3.51; df=1, 79; p<0.001) only. As with the anticipation contrast, we additionally conducted the same three by two repeated measures ANOVA on the mean BOLD signal change within these two clusters. There was a significant effect of group in the left insula (F=4.51; df=2, 78; p<0.05 - $alcohol_{minus}$ and $alcohol_{plus}$<control, p<0.05 - Fig 5A) and in the right ACC (F=4.21; df=2, 78; p<0.05 - $alcohol_{minus}$ and $alcohol_{plus}$<control, p<0.05 - Fig 5B), showing that the $alcohol_{minus}$ and $alcohol_{plus}$ groups independently contributed main ANOVA group effect. This same analysis also showed a trend towards a drug effect in both the insula (F=2.87; df=1, 78; p=0.09) and ACC (F=3.13; df=1, 78; p=0.08) clusters, likely driven by the direction of signal change on the naltrexone session in the $alcohol_{minus}$ and $alcohol_{plus}$ groups. Therefore, we additionally performed post hoc within group paired t-test analyses and showed that in the $alcohol_{plus}$ group only, there was a

attenuation of the BOLD signal change during the naltrexone compared to the placebo session in both the insula (-t=2.12; df=24, p<0.05) and the ACC (-t=2.26; df=24, p<0.05) clusters. There were no significant main effects for the *win hit>neutral hit* contrast.

-Insert Figure 5 about here-

Discussion

This study set out to examine fronto-striatal activation during reward anticipation and instrumental responding in long-term abstinent alcoholic and alcoholic polysubstance-dependent individuals in order to evaluate the acute modulating effects of MOR blockade on these processes. The study showed that the $alcohol_{plus}$ group exhibited slower and less accurate instrumental responding across MID conditions compared to both the $alcohol_{minus}$ and control groups during the placebo session, an effect that was less evident after naltrexone but with no absolute improvement in speed and accuracy of responding as a result of drug treatment. The study additionally showed, however, that while there were no effects on the relative motivational value (RMV) for rewards, there were disturbances within fronto-striatal regions during reward anticipation and "missed" rewards in both substance dependent groups that were not reliably remediated by acute naltrexone treatment.

The observed slower and less accurate responding of the $alcohol_{plus}$ group may suggest a low degree of motivation during the sustained cognitive demands of general instrumental effort. Using a behavioural motivational index that specifically reflects a higher relative value for reward incentives during instrumental responding, however, we observed no difference between groups or any effects of naltrexone. The apparent remediation produced by acute naltrexone

in the alcohol$_{plus}$ group seems most likely to be a consequence of changes in response to naltrexone in the comparison groups as there was little evidence of absolute improvements in behavioural functioning produced by naltrexone in the alcohol$_{plus}$ group.

Reduced BOLD activation changes in the alcohol$_{plus}$ group

Under conditions of reward anticipation, the alcohol$_{plus}$ group exhibited significantly lower activation change in the OFC compared with that of the control group across drug treatments. There is previous evidence of hypofunctioning in the OFC (London et al., 2000), particularly during abstinence (Volkow et al., 1992). The OFC has important functional connections with the striatum (Volkow et al., 2000), and is known to code the motivational value of stimuli (Koeneke et al., 2008). The OFC also contains a high number of MOR (Gorelick et al., 2005), suggesting that any disturbance to the brain's opioid system might be modulated by naltrexone. The current results, however, provide no evidence for an acute modulatory effect in the OFC, instead suggesting that disturbances within striato-orbitofrontal circuitry that subserves reward prediction and motivational processes, are sustained in long-term polysubstance, but not alcohol, abstinence.

Independent BOLD activation reductions in the alcohol$_{minus}$ and alcohol$_{plus}$ groups

Compared to controls, the alcohol$_{minus}$, and to a greater degree, the alcohol$_{plus}$ group, exhibited reduced bilateral ventral caudate/NAcc activation in response to the anticipation of potential monetary rewards. The current result

concurs with previous research findings of altered striatal activity for non-drug rewards in substance dependence (Buhler et al., 2010; Bustamante et al., 2014; Diekhof et al., 2008; Gradin et al., 2014; Peters et al., 2011; Wrase et al., 2007) and may be consistent with a sustained striatal reward deficiency syndrome (Blum et al., 2000; Koob et al., 2004) in long-term substance abstinence. There are also high levels of MORs in the caudate (Arvidsson et al., 1995), making this region a credible target for modulation with naltrexone. The current findings, however, do not appear to support a remediating effect of naltrexone in this particular behavioural context.

The current study also found that both the $alcohol_{minus}$ and $alcohol_{plus}$ groups demonstrated reduced activation changes compared with controls in the frontal pole and IFG/insula regions during reward anticipation. The PFC represents both cognitive and reward-related information processing (Watanabe et al., 2007), whereas the insula is implicated in reward and risk prediction (Preuschoff et al., 2008) and addiction relapse (Paulus et al., 2005; Seo et al., 2013), possibly due to its role in awareness of interoceptive (i.e. bodily) states (Critchley et al., 2004). The current findings may, therefore, suggest that in long-term alcohol and polysubstance abstinence, there are sustained disturbances within a network of regions that function to integrate the cognitive interpretation of motivational drives (Goldstein et al., 2007) and other emotional and interoceptive states.

We also observed that the $alcohol_{minus}$ and $alcohol_{plus}$ groups exhibited reduced activation changes compared with controls in the anterior insula, and notably, the rostral ACC (rACC) during "missed" rewards. The rACC has been labelled as the "affective division" of the cingulate (Bush et al., 2000; Devinsky et al., 1995), through processing the emotional components of errors (Luu et al.,

2003; van Veen et al., 2002). The observed decrease in error-related rACC and insula activation may have resulted from decreases in arousal during misses, an effect that was apparently exacerbated by acute opioid blockade with naltrexone. This blunting of error-related signalling by naltrexone in substance abusers may have clinical implications, where arousal and conflict monitoring are necessary responses to violations in prediction that require adjustments to ongoing behaviour during treatment. The effects of naltrexone in the insula and ACC, however, may encourage further investigations regarding the effects of opioid blockade on error-related neural responses in addiction populations.

Interdependent BOLD activation reductions in the alcohol$_{minus}$ and alcohol$_{plus}$ groups

When combined, the alcohol$_{minus}$ and alcohol$_{plus}$ groups exhibited reduced activations in the ACC during the anticipation of monetary reward compared to controls that were not modulated by naltrexone. The ACC has been implicated in addiction and its cognitive sequelae (Goldstein et al., 2002; Peoples, 2002; Volkow et al., 2002), with disturbances in this region reported in a number of abstinent substance abusing populations (Bolla et al., 2004; Eldreth et al., 2004; Nestor et al., 2011; Salloum et al., 2007). One of these differences was observed for the caudal dorsal ACC (cdACC), a region involved in processing the value of actions, motivation and expected outcomes under conditions of reward (Kouneiher et al., 2009). This may suggest that neural processing within a motivational and reward prediction cognitive network remains compromised in long-term substance abstinence.

Limitations of the current study include a lack of complete matching of groups with respect to age, cannabis and cigarette use, anxiety and mood

measures, which means we cannot unequivocally dismiss their potential influence on altered reward processing in fronto-striatal circuitry of both the $alcohol_{minus}$ and $alcohol_{plus}$ groups. Furthermore, we did not thoroughly assess alcohol and drug craving at each session across the groups, which may have had a possible influence on our metrics of motivation and reward processing. Moreover, dependence on (and abstinent from) multiple and varying substances of abuse in the $alcohol_{plus}$ group underpowered us to statistically examine the influence of these measures on indices of motivation and reward processing. While our groups were well matched on the distribution of gender, the small number of females in each group did not permit us to examine the influence of gender effects on the neurobiology of reward and motivational processes in the two substance-dependent groups. The reduced number of loss trials in our MID task also meant we were unable to examine the neural correlates of loss anticipation and outcome, where sensitivity to punishment may well have implications for treatment and drug relapse.

In summary, the current study set out to map the impact of MOR blockade upon neural networks disrupted in substance dependence and has demonstrated evidence of sustained disturbances within fronto-striatal regions of long-term abstinent alcoholics and polysubstance-dependent individuals. It has also shown that acute naltrexone treatment produced a relative minor amelioration of behavioural performance on a monetary delayed incentive task in an alcoholic, polydrug abuser group ($alcohol_{plus}$), but not in a group of patients with "pure" alcoholic abuse ($alcohol_{minus}$). Moreover, naltrexone was unable to reverse neural changes in fronto-striatal systems associated with the MID task, possibly

suggesting the potential insensitivity of this task for elucidating possible therapeutic effects on neural biomarkers in future experimental medicine studies.

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David Nutt is an advisor to British National Formulary, MRC, General Medical Council, Department of Health, is President of the European Brain Council, past President of the British Neuroscience Association and European College of Neuropsychopharmacology, chair of the Independent Scientific Committee on Drugs (UK), is a member of the International Centre for Science in Drug Policy, advisor to Swedish government on drug, alcohol and tobacco research, editor of the Journal of Psychopharmacology, sits on advisory Boards at Lundbeck, MSD, Nalpharm, Orexigen, Shire, has received speaking honoraria (in addition to above)

from BMS/Otsuka, GSK, Lilly, Janssen, Servier, is a member of the Lundbeck International Neuroscience Foundation, has received grants or clinical trial payments from P1vital, MRC, NHS, Lundbeck, has share options with P1vital, has been expert witness in a number of legal cases relating to psychotropic drugs, and has edited/written 27 books, some purchased by pharmaceutical companies.

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Ed Bullmore is employed half-time by the University of Cambridge and half-time by GSK and is a shareholder in GSK.

Liam Nestor was employed by GSK during some of this work.

Eugenii Rabiner worked for GSK until 2011 and is a shareholder in GSK. He is a consultant to GSK, TEVA, Lightlake Therapeutics, AbbVie, and Roche.

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Table 1. Demographic variables for the control, alcohol$_{minus}$ and alcohol$_{plus}$ groups. *Age* *p<0.05 - alcohol$_{minus}$>alcohol$_{plus}$ & control; *Edu* **p<0.01 - alcohol$_{plus}$<control; *IQ* *p<0.05 - alcohol$_{plus}$<control; *Alcohol Exposure* ***p<0.001 control<alcohol$_{minus}$ & *p<0.05 - alcohol$_{plus}$<alcohol$_{minus}$; *Cigarette Use* **p<0.01 - alcohol$_{plus}$>control; *Cannabis Use* ***p<0.001 - alcohol$_{plus}$>alcohol$_{minus}$ & control. Also shown are the months of abstinence from alcohol in all three groups and additional substances of dependence in the alcohol$_{plus}$ group. Data are expressed as means ± SEM. Ranges of substance asbtinence are also provided in parentheses.

	Control (n=35)	**Alcohol$_{Minus}$ (n=21)**	**Alcohol$_{Plus}$ (n=25)**
Gender (Female/Male)	7/28	4/17	6/19
Age	41.11 ± 1.54	46.23 ± 1.96*	39.60 ± 1.52
Edu	13.45 ± 0.45	12.66 ± 0.65	11.32 ± 0.42**
IQ	105.91 ± 1.71	105.28 ± 1.82	99.36 ± 2.39*
Handedness	46.08 ± 9.75	55.74 ± 14.12	62.91 ± 11.22
Alcohol Exposure (yrs)	0.80 ± 0.44***	18.71 ± 1.88	13.42 ± 1.94*
Cigarette Use (pack yrs)	9.99 ± 2.11	17.44 ± 4.45	22.27 ± 3.31**
Cannabis Use (yrs)	0.34 ± 0.34	2.80 ± 1.05	8.64 ± 1.78***
Alcohol Abstinence (mths)	0.34 ± 0.2 (5.0)	14.08 ± 4.23 (78.5)	13.69 ± 2.50 (34.5)
Cocaine Abstinence (mths)	-	-	24.10 ± 4.86 (82.5)
Opiate Abstinence (mths)	-	-	39.47 ± 14.75 (274)
Amphetamine Abstinence (mths)	-	-	156.85 ± 51.48 (306)
Benzodiazepine Abstinence (mths)	-	-	64.50 ± 51.87 (161.5)
GHB Abstinence (mths)	-	-	36.0 ± 0.00 (0)
Solvent Abstinence (mths)	-	-	396.0 ± 0.00 (0)

Table 2. ANOVA group effect clusters from a two (Group: $alcohol_{minus}$ & $alcohol_{plus}$ combined vs. control) by two (Drug: placebo vs. naltrexone) whole-brain cluster-based repeated measures ANOVA for the win anticipation>neutral anticipation contrast. Statistical images were first thresholded using clusters determined by $Z>2.3$ with a corrected cluster significance level of $p<0.05$. The *P* value corresponding to the maximum zF-statistic within each cluster is shown. Co-ordinates are represented in Montreal Neurological Institute (MNI) space.

Cluster Region	Voxels	p value	HS	x(mm)	y(mm)	z(mm)	Voxel zF-Stat
Occipital Fusiform Gyrus	798	<0.0001	L	-46	-66	-20	6.41
Inferior Frontal Gyrus/Insula	351	<0.0001	R	52	16	-2	4.56
Middle Temporal/Parahippocampal Gyrus	324	<0.0001	L	-60	-14	-16	3.72
Supramarginal Gyrus	319	<0.0001	R	68	-34	36	3.47
Parahippocampal Gyrus	228	<0.001	R	36	-28	-14	4.37
Caudate/Nucleus Accumbens	214	<0.01	L	-8	14	-2	3.65
Cerebellum	194	<0.01	R	22	-46	-24	3.36
Anterior Cingulate Cortex	192	<0.01	L	-1	-8	32	4.08
Anterior Cingulate Cortex	182	<0.01	R	6	18	28	4.22
Caudate/Nucleus Accumbens	162	<0.01	R	10	10	4	3.45
Frontal Pole	155	<0.05	R	20	58	-8	4.10
Orbitofrontal Cortex	147	<0.05	L	-30	32	-14	5.04

Figure 1. MID task performance in the alcohol$_{minus}$, alcohol$_{plus}$ and control groups during the placebo and naltrexone sessions for A) mean percentage accuracy; B) mean reaction time and C) relative motivational value (RMV). Accuracy and reaction time data were analyzed using a three (Group: alcohol$_{minus}$ vs. alcohol$_{plus}$ vs. control) by two (Drug: placebo vs. naltrexone) by two (Condition: neutral vs. win) repeated measures ANOVA. RMV was analysed using a three (Group: alcohol$_{minus}$ vs. alcohol$_{plus}$ vs. control) by two (Drug: placebo vs. naltrexone) repeated measures ANOVA. MID accuracy: •••p<0.001 - Win>Neutral; **p<0.01 - alcohol$_{plus}$<control on placebo; ***p<0.001 - alcohol$_{plus}$<alcohol$_{minus}$ on placebo. MID reaction time: •••p<0.001 - Win<Neutral; *p<0.05 - alcohol$_{plus}$<alcohol$_{minus}$ & control on placebo. Data are expressed as means ± SEM.

Figure 2. Three (Group: alcohol$_{minus}$ vs. alcohol$_{plus}$ vs. control) by two (Drug: placebo vs. naltrexone) repeated measures ANOVA on the mean BOLD signal change scores within the group ANOVA zF-statistic clusters for the win anticipation>neutral anticipation contrast. Results showed that the alcohol$_{plus}$ group had significantly less activation change in A) the left OFC compared to the control group (**p<0.01) and that the control group had significantly greater activation change in B) the right IFG/insula compared to both the alcohol$_{minus}$ and alcohol$_{plus}$ groups (*p<0.05). Data are expressed as means ± SEM. Co-ordinates are represented in Montreal Neurological Institute (MNI) space. OFC: orbitofrontal cortex; IFG: inferior frontal gyrus.

Figure 3. Three (Group: alcohol$_{minus}$ vs. alcohol$_{plus}$ vs. control) by two (Drug: placebo vs. naltrexone) repeated measures ANOVA on the mean BOLD signal change scores within the group ANOVA zF-statistic clusters for the win anticipation>neutral anticipation contrast. Results showed that the control group had significantly greater activation change in A) the right caudate/NAcc compared to both the alcohol$_{minus}$ (*p<0.05) and alcohol$_{plus}$ (**p<0.01) groups and in B) the left caudate/NAcc compared to both the alcohol$_{minus}$ (*p<0.05) and alcohol$_{plus}$ (**p<0.01) groups. Data are expressed as means ± SEM. Co-ordinates are represented in Montreal Neurological Institute (MNI) space. NAcc: nucleus accumbens.

Figure 4. Two (Group: alcohol$_{minus}$ & alcohol$_{plus}$ combined vs. control) by two (Drug: placebo vs. naltrexone) repeated measures ANOVA on the mean BOLD signal change scores within the group ANOVA zF-statistic clusters for the win anticipation>neutral anticipation contrast. Results showed that the control group had significantly greater activation change in A) the left anterior cingulate cortex (*p<0.05) and in B) the right anterior cingulate cortex (*p<0.05) compared to alcohol$_{minus}$ & alcohol$_{plus}$ combined. Data are expressed as means ± SEM. Co-ordinates are represented in Montreal Neurological Institute (MNI) space.

Figure 5. Three (Group: $alcohol_{minus}$ vs. $alcohol_{plus}$ vs. control) by two (Drug: placebo vs. naltrexone) repeated measures ANOVA on the mean BOLD signal change scores within the two group ANOVA zF-statistic clusters for the win miss>neutral miss contrast. Results showed that the control group had significantly greater activation change in A) the left insula (*p<0.05) and in B) the right anterior cingulate cortex (*p<0.05) compared to both the $alcohol_{minus}$ and $alcohol_{plus}$ groups. Within group analyses also revealed that the $alcohol_{plus}$ group had a greater BOLD signal reduction on naltrexone compared to placebo in both these regions (•p<0.05). Data are expressed as means ± SEM. Co-ordinates are represented in Montreal Neurological Institute (MNI) space.

Figure 1

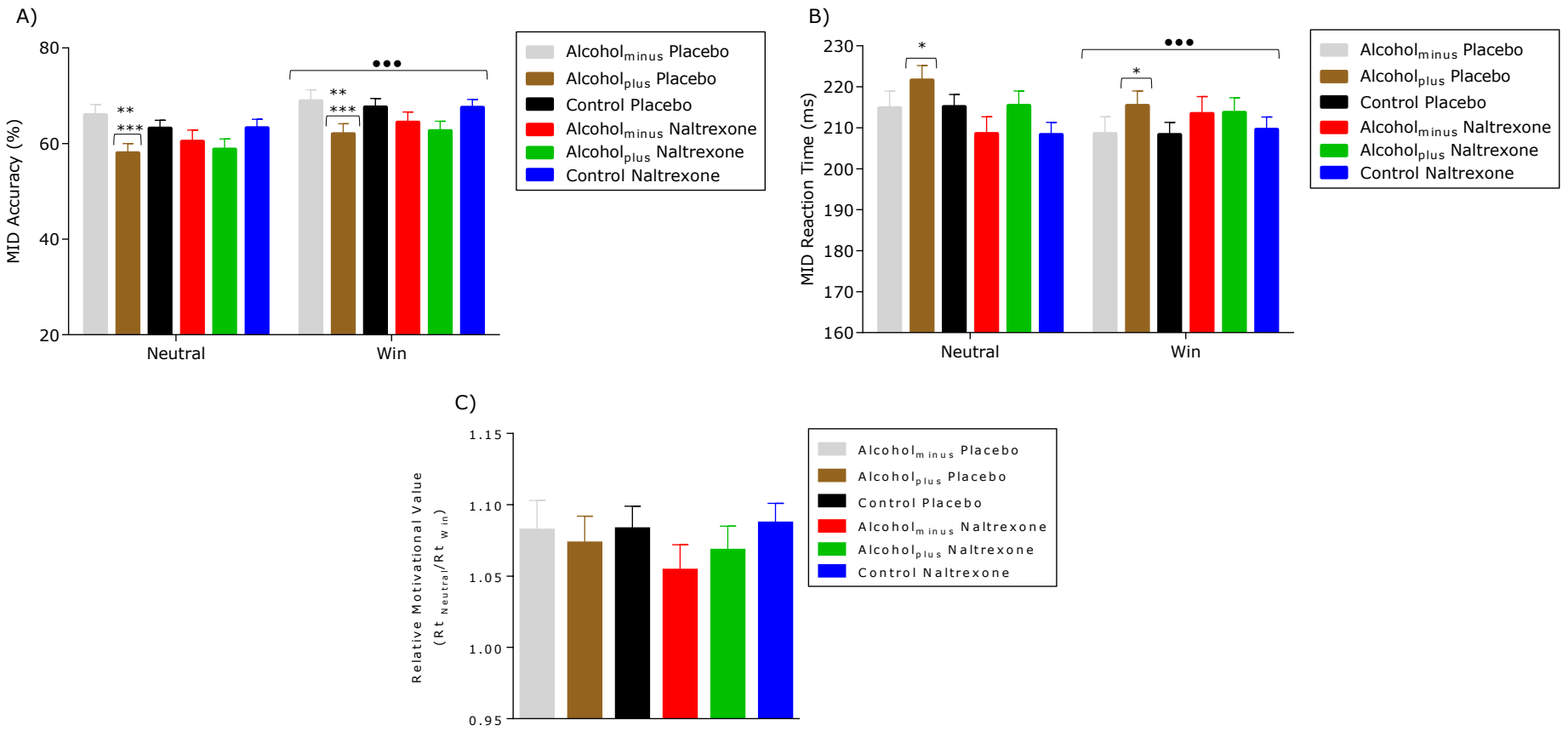

A)
MID Accuracy (%)
80
60
40
20
**
***
**
***
●●●
Neutral
Win
Alcohol minus Placebo
Alcohol plus Placebo
Control Placebo
Alcohol minus Naltrexone
Alcohol plus Naltrexone
Control Naltrexone
B)
MID Reaction Time (ms)
230
220
210
200
190
180
170
160
*
*
●●●
Neutral
Win
Alcohol minus Placebo
Alcohol plus Placebo
Control Placebo
Alcohol minus Naltrexone
Alcohol plus Naltrexone
Control Naltrexone
C)
Relative Motivational Value
(Rt Neutral/Rt Win)
1.15
1.10
1.05
1.00
0.95
Alcohol minus Placebo
Alcohol plus Placebo
Control Placebo
Alcohol minus Naltrexone
Alcohol plus Naltrexone
Control Naltrexone

Figure 2

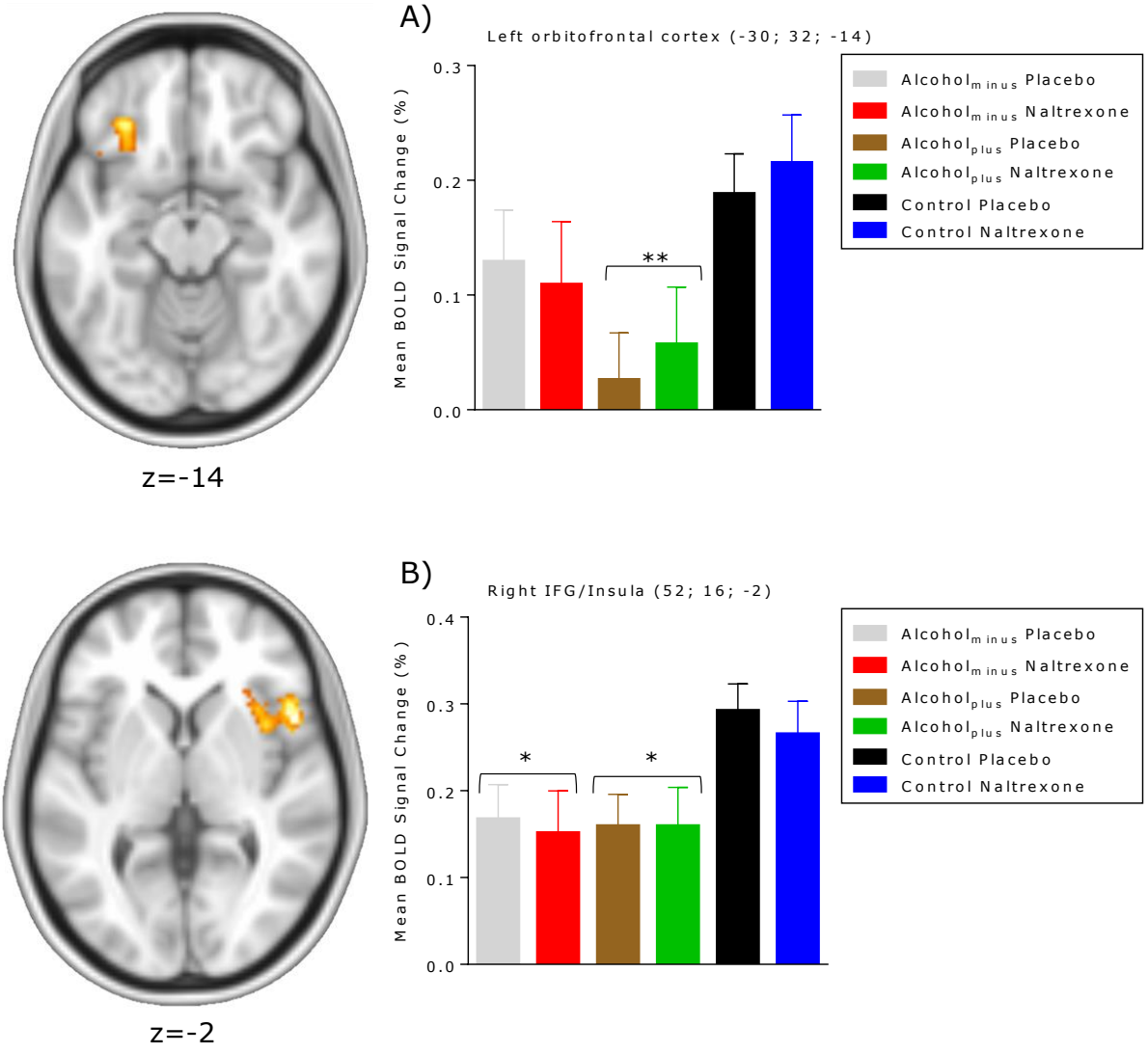

A)
Left orbitofrontal cortex (-30; 32; -14)
Mean BOLD Signal Change (%)
0.3
0.2
0.1
0.0
**
Alcohol minus Placebo
Alcohol minus Naltrexone
Alcohol plus Placebo
Alcohol plus Naltrexone
Control Placebo
Control Naltrexone
z=-14
B)
Right IFG/Insula (52; 16; -2)
Mean BOLD Signal Change (%)
0.4
0.3
0.2
0.1
0.0
*
*
Alcohol minus Placebo
Alcohol minus Naltrexone
Alcohol plus Placebo
Alcohol plus Naltrexone
Control Placebo
Control Naltrexone
z=-2

Figure 3

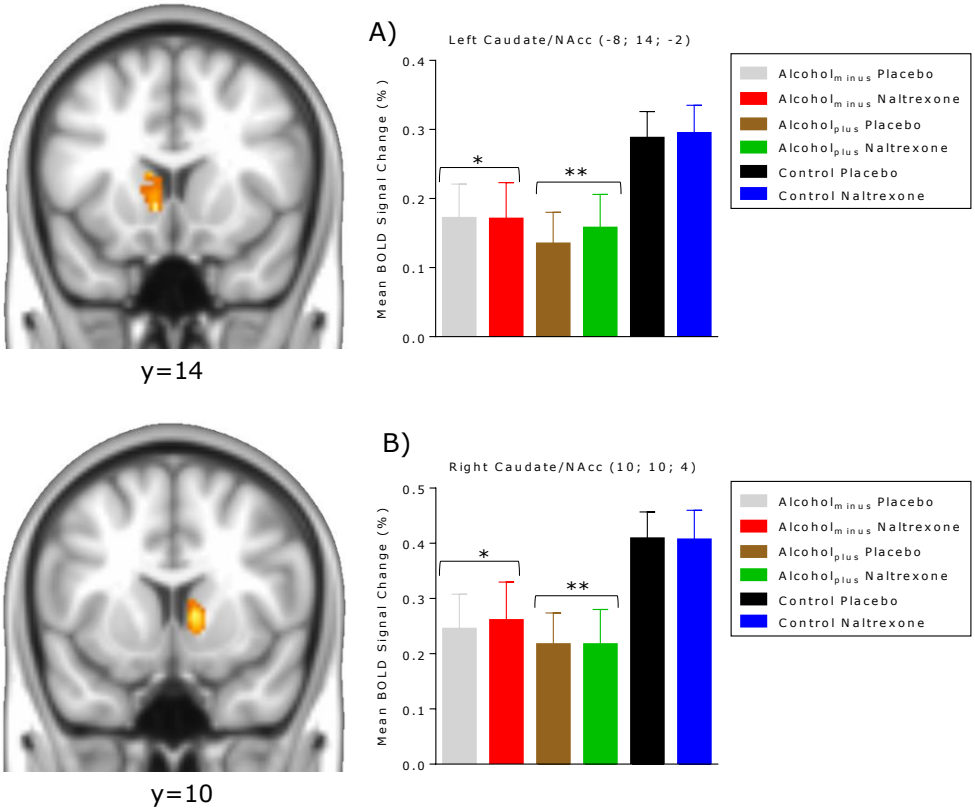

A)
Left Caudate/NAcc (-8; 14; -2)
Mean BOLD Signal Change (%)
0.4
0.3
0.2
0.1
0.0
*
**
Alcohol minus Placebo
Alcohol minus Naltrexone
Alcohol plus Placebo
Alcohol plus Naltrexone
Control Placebo
Control Naltrexone
y=14
B)
Right Caudate/NAcc (10; 10; 4)
Mean BOLD Signal Change (%)
0.5
0.4
0.3
0.2
0.1
0.0
*
**
Alcohol minus Placebo
Alcohol minus Naltrexone
Alcohol plus Placebo
Alcohol plus Naltrexone
Control Placebo
Control Naltrexone
y=10

Figure 4

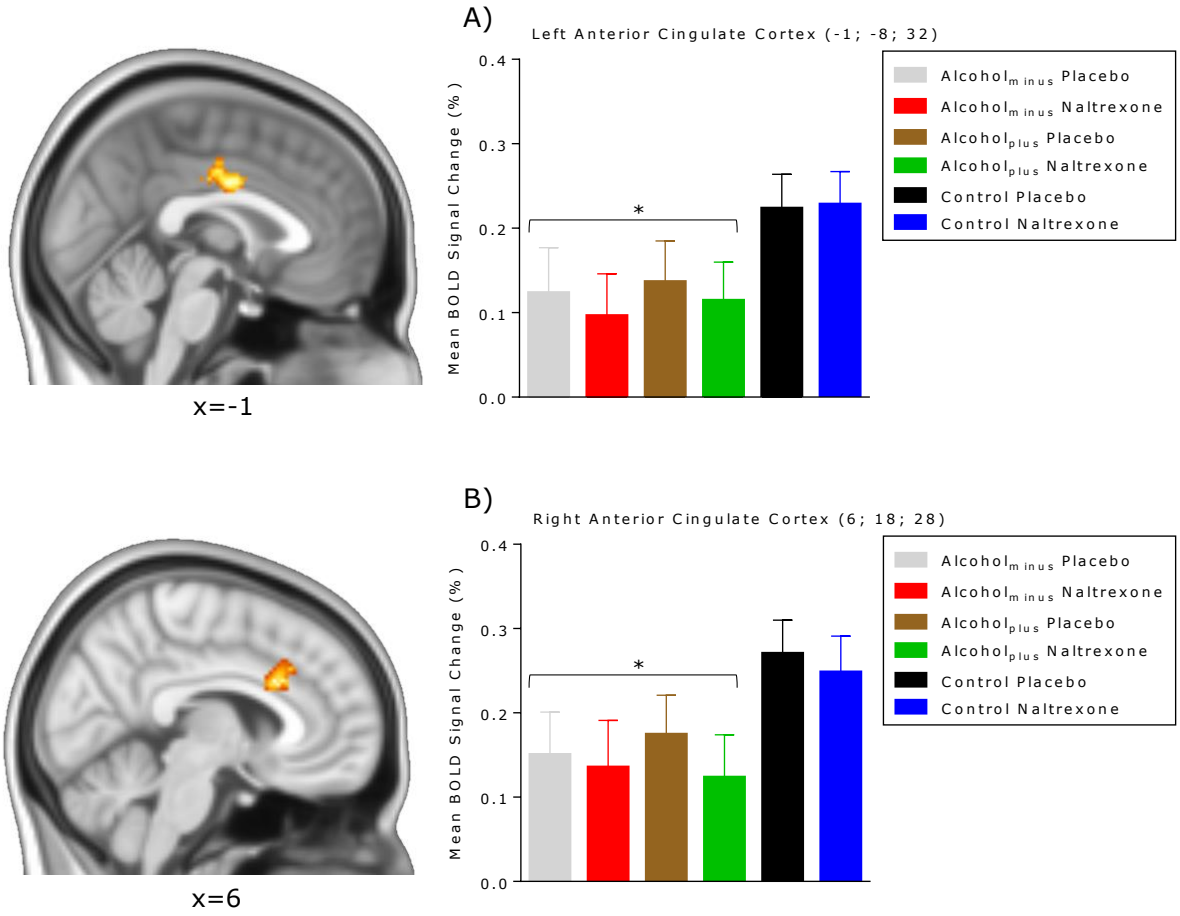
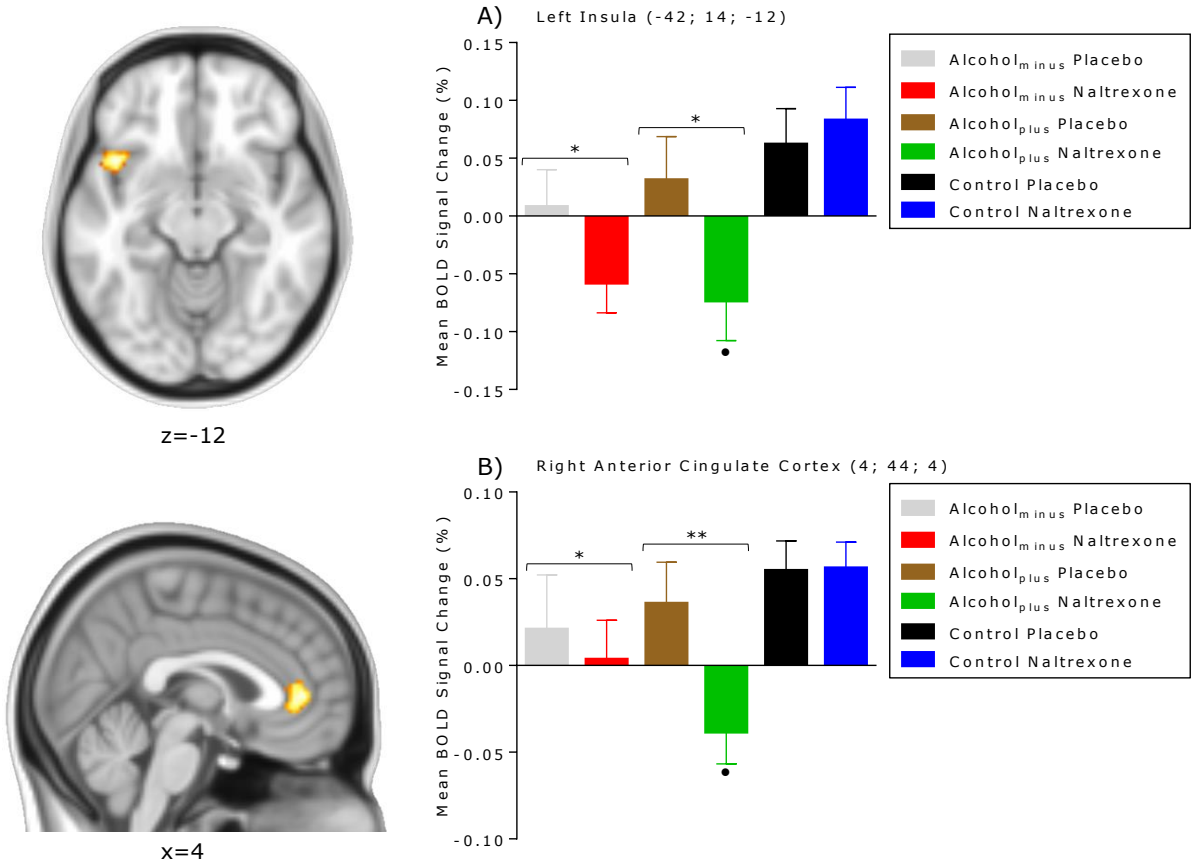

A)
Left Anterior Cingulate Cortex (-1; -8; 32)
Mean BOLD Signal Change (%)
0.4
0.3
0.2
0.1
0.0
*
Alcohol minus Placebo
Alcohol minus Naltrexone
Alcohol plus Placebo
Alcohol plus Naltrexone
Control Placebo
Control Naltrexone
x=-1
B)
Right Anterior Cingulate Cortex (6; 18; 28)
Mean BOLD Signal Change (%)
0.4
0.3
0.2
0.1
0.0
*
Alcohol minus Placebo
Alcohol minus Naltrexone
Alcohol plus Placebo
Alcohol plus Naltrexone
Control Placebo
Control Naltrexone
x=6

Figure 5

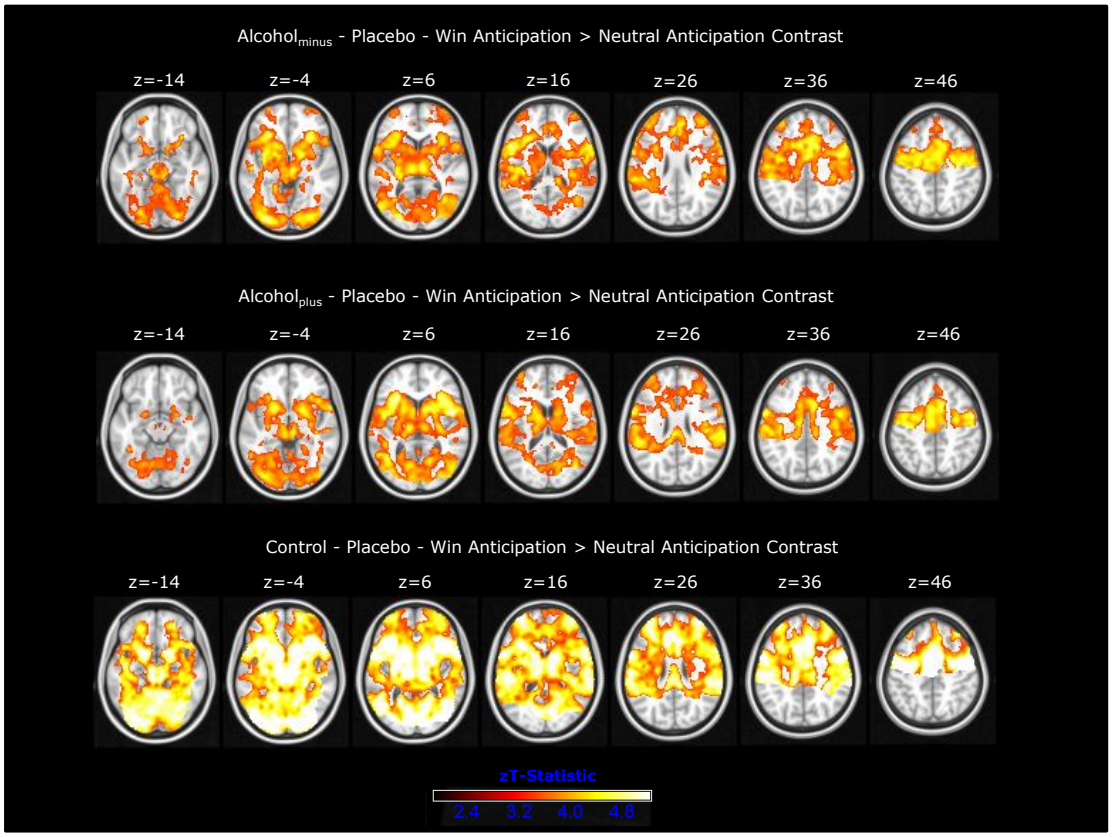

z=-12
x=4
A)
Left Insula (-42; 14; -12)
Mean BOLD Signal Change (%)
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
*
*
Alcohol minus Placebo
Alcohol minus Naltrexone
Alcohol plus Placebo
Alcohol plus Naltrexone
Control Placebo
Control Naltrexone
B)
Right Anterior Cingulate Cortex (4; 44; 4)
Mean BOLD Signal Change (%)
0.10
0.05
0.00
-0.05
-0.10
*
**
Alcohol minus Placebo
Alcohol minus Naltrexone
Alcohol plus Placebo
Alcohol plus Naltrexone
Control Placebo
Control Naltrexone

Supplementary Figure 1. Showing average BOLD activation changes across the whole brain for the win anticipation > neutral anticipation contrast during the placebo session in the $alcohol_{minus}$, $alcohol_{plus}$ and control groups. *Z* (Gaussianized T) statistic images were thresholded using clusters determined by $Z>2.3$ and corrected cluster significance level of $p<0.05$. The scale represents the colour (from dark to light yellow) of the cluster corresponding to the increasing *Z*-statistic. The structural image represents the MNI152 average normal brain with corresponding horizontal coordinates (inferior-superior).

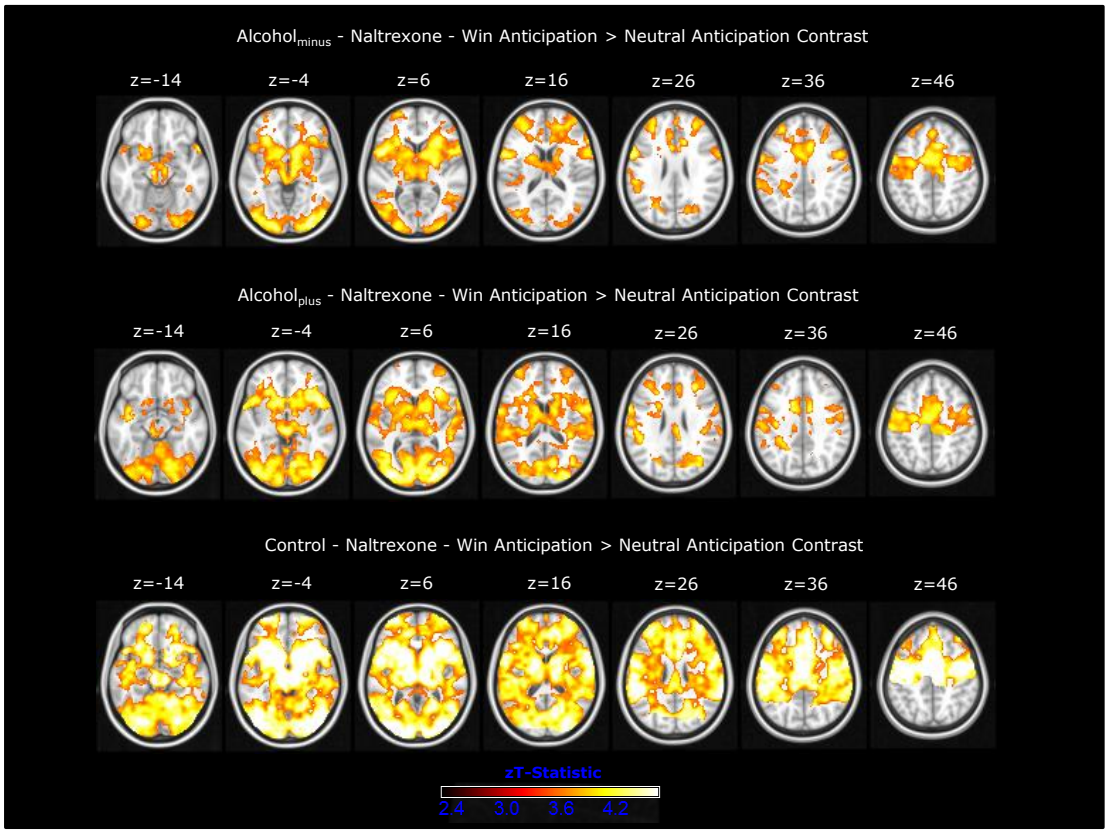
Supplementary Figure 2. Showing average BOLD activation across the whole brain for the win anticipation > neutral anticipation contrast during the naltrexone session in the $alcohol_{minus}$, $alcohol_{plus}$ and control groups. *Z* (Gaussianized T) statistic images were thresholded using clusters determined by $Z>2.3$ and corrected cluster significance level of $p<0.05$. The scale represents the colour (from dark to light yellow) of the cluster corresponding to the increasing *Z*-statistic. The structural image represents the MNI152 average normal brain with corresponding horizontal coordinates (inferior-superior).

Supplementary Figure 3. ANOVA group effect zF-Statistical map from a two (Group: $alcohol_{minus}$ & $alcohol_{plus}$ combined vs. control) by two (Drug: placebo vs. naltrexone) whole-brain cluster-based repeated measures ANOVA for the win anticipation>neutral anticipation contrast. Statistical images were first thresholded using clusters determined by $Z>2.3$ with a corrected cluster significance level of $p<0.05$. The scale represents the colour (from dark to light yellow) of the cluster voxels corresponding to the increasing zF-statistic. Co-ordinates are represented in Montreal Neurological Institute (MNI) space.

Supplementary Figure 1.



Supplementary Figure 2.



Supplementary Figure 3.