Addiction Biology

SSA SOCIETY FOR THE STUDY OF ADDICTION

Acute naltrexone does not remediate fronto-striatal disturbances in alcoholic and alcoholic polysubstance-dependent populations on 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.

Dear Dr. Nestor:

Manuscript ID AB-12-2015-0322.R2 entitled "Acute naltrexone does not remediate fronto-striatal disturbances in alcoholic and alcoholic polysubstance-dependent populations on a monetary incentive delay task." which you submitted to Addiction Biology, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.

The reviewer(s) feel that you have adequately revised your submission, but recommend a few remaining minor revisions. Once these are addressed, I will be happy to accept the paper without additional outside review.

To revise your manuscript, log into http://mc.manuscriptcentral.com/adb and enter your Author Center, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision. Please DO NOT upload your revised manuscripts as a new submission.

You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript using a word processing program and save it on your computer. Please also highlight the changes to your manuscript within the document by using colored text.

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When submitting your revised manuscript, you will be able to respond to the comments made by the reviewer(s) in the space provided. You can use this space to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response to the reviewer(s).

IMPORTANT: Your original files are available to you when you upload your revised manuscript. Please delete any redundant files before completing the submission.

Because we are trying to facilitate timely publication of manuscripts submitted to Addiction Biology, your revised manuscript should be uploaded as soon as possible. If it is not possible for you to submit your revision in a reasonable amount of time, we may have to consider your paper as a new submission. If you feel that you will be unable to submit your revision within the time allowed please contact me to discuss the possibility of extending the revision time.

With the increasing popularity and impact of the journal, we have experienced a marked increase in submission of high quality papers. As a result, we now increasingly must base acceptance decisions not only on absolute merit, but also on relative priority scores. Nevertheless, the pipeline of accepted papers has increased, and with it the lag time from acceptance to publication.

As a result of this, the journal is instituting a word limit on papers of 5000 words (excluding abstract, references and figure legends). The maximum number of references is now limited to 50. If necessary, we encourage authors to provide additional material as online supporting information. Occasionally, when there is need to exceed this limit, editorial approval to do so can be sought, but page charges for additional pages will have to be carried by the authors. If, when you receive the PDF proof, your paper exceeds 8 pages you will incur a charge of £60GBP per extra page. This will

take effect for all papers accepted after 1 March 2011.

Once again, thank you for submitting your manuscript to Addiction Biology and I look forward to receiving your revision.

Sincerely,
Prof. Rainer Spanagel
Editor Addiction Biology, Addiction Biology
Rainer.Spanagel@zi-mannheim.de, Christine.Roggenkamp@zi-mannheim.de

Editor Comments to Author:

Reviewer(s)' Comments to Author:

Reviewer: 2

Comments to the Author
>>> Please find all new comments in bold below:

_____

Reviewer: 1

Comments to the Author
The authors did a good job in revising the manuscript, all my issues were sufficiently addressed.

Reviewer: 2

Comments to the Author
Please find all comment below (in line) as a reply to the different ponts raised:

Reviewer(s)' Comments to Author:

Reviewer: 1
Comments to the Author
The paper by Nestor et al. addresses the role of naltrexone on behavioral and neural correlates of monetary reward processing in alcoholic and alcoholic polysubstance-dependent populations. The authors found that naltrexone remediates slower and less accurate instrumental responding in alcoholic polysubstance-dependent individuals. Moreover, both alcoholic groups showed alterations in fronto-striatal regions during reward anticipation, that did not change after naltrexone administration. The paper is well written, the methods are sound - I have only some suggestions that could improve the present version of the manuscript.

Reviewer: Both in the Abstract and in the Introduction I miss a link between naltrexone administration and non-drug reward processing, i.e. the construct and mechanism of reward processing in the context of alcohol dependence and the opioid system is not very well presented and the authors also did not explain why they have chosen non-drug reward processing as mechanism of interest in this context. Moreover, these aspects then also needs to be included in the Discussion section.

Response: We have now done this in the abstract as follows: 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 frontostriatal reward circuitry. And in the introduction as follows:

Substance abusers, particularly alcoholics, may still be at risk for relapse in longterm 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.

> good

Reviewer: On a behavioral level, the authors assessed percentage accuracy and mean reaction time, but did not discuss the (non)findings for these two parameters differently, although they represent different indicators of reward processing. This should be included.

Response: Response accuracy and reaction time on the MID task are highly negatively correlated - the reduced latency to respond to the target increases MID accuracy. The latency torespond on win trials is measuring the appetitive incentive motivational aspects of the task, whereas accuracy is merely a by product of this motivation. We have explored this further by using a behavioural index that specifically reflects a higher relative value for reward incentives during instrumental responding (neutral reaction time / win reaction time) – where >1 reflects a higher relative motivational value of monetary incentives.

> Please deduce this perspective from the literature.

New Response: Previous reports (see references below) have used this behavioural motivational index to show a higher relative value for monetary incentives during instrumental responding, which we believe, more closely reflects the contrasts in the incentive value of these conditions computed during the functional MRI analyses at the first level.

Carter, R. M., Macinnes, J. J., Huettel, S. A., & Adcock, R. A. (2009). Activation in the VTA and nucleus accumbens increases in anticipation of both gains and losses. Front Behav Neurosci, 3, 21. doi: 10.3389/neuro.08.021.2009

Clithero, J. A., Reeck, C., Carter, R. M., Smith, D. V., & Huettel, S. A. (2011). Nucleus accumbens mediates relative motivation for rewards in the absence of choice. Front Hum Neurosci, 5, 87. doi: 10.3389/fnhum.2011.00087

Sescousse, G., Li, Y., & Dreher, J. C. (2015). A common currency for the computation of motivational values in the human striatum. Soc Cogn Affect Neurosci, 10(4), 467-473. doi: 10.1093/scan/nsu074

Editor >>> Please include these arguments in the manuscript.

Response to editor: We have written this under Other Statistics as follows:
We also conducted a three (Group: alcoholminus vs. alcoholplus 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.

Reviewer: The authors did not provide any information on their ROIs or are their findings based on whole brain level?

Response: The findings are based on whole brain cluster-based corrected statistics.

> Ok

Reviewer: Last, I was wondering whether the authors have also data on craving of their groups? As this is associated with for example reward processing and relapse and frontal and limbic brain regions, it might be an interesting factor, at least as control factor.

Response: Craving data was collected on each visit. Participants, however, did not report high levels of craving during these visits, quite possibly, because they were in long-term abstinence. Craving was also difficult to assess in the Alcoholplus group given the multiple substances they had been dependent on.

> Please report levels of craving as collected.

New Response: Whilst we did include a questionnaire to collect craving data from participants in the ICCAM platform, we do not believe that the craving data is clinically or scientifically meaningful for these particular participants for a number of reasons. Firstly, these participants had been abstinent for substantial period of time. Secondly, craving was only assessed at one time point - i.e. prior to drug administration at each visit. Nevertheless concerning alcohol craving, we found no significant differences between the placebo and naltrexone visits (t=0.03; df=20; p=0.98) in the alcohol minus group. In the alcoholplus group the range of substances abused makes it hard to interpret craving

data. Assessment of craving is something that would need to be carefully considered in any future uses of ICCAM platform and tailored specifically to the range of substances used.

Editor >>> Ok, please mention this under limitations.

Response to editor: We have now written this under limitations as follows:
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.

Reviewer: 2
Comments to the Author
This work presents an interesting study exploring the influence of a mu opioid receptor antagonist (naltrexone) on reward processing in the brain in two different substance-dependent samples and in healthy control subjects. The study presents its aims in a clear and brief way. The authors collected data in the framework of a big multi-center study. A total of n=21 patients with alcohol dependence, n=25 patients with alcohol and other substances dependence and n=35 healthy control subjects were analysed. The used methods are comprehensively described. The results are described in brief. Further, the authors discuss their results reflecting selected previous literature in the field. Despite the interesting topic of the study, it lacks some very important points: firstly, a-priori hypotheses in terms of anatomical regions are not totally clear. Secondary, the manuscript raised some methodological issued addressed in detail below. Since the study is based on an interesting sample, it might be of interest to combine these results with some more clinical data like symptom severity etc. in order to extend and improve the possible interpretations as well as the relevance of the results. Further, also some ideas on implications for possible future research based on these results would be of interest.
In detail, the authors should consider the following aspects:
Reviewer: The author state that the participants were long-term abstinentalcoholics and alcoholic poly-substance dependent subjects respectively. How was the mentioned "extended abstinence" defined? Since the duration and time course of abstinence might be of interest for alterations in receptor density etc. the studied samples should be as homogenous as possible regarding this factor. The authors state that participants were abstinent for at least 4 weeks. With a mean duration of abstinence of alcohol of about 13 months in both groups the range seems to be very wide. Please add the range of abstinence of alcohol to the given demographic variables, as also the duration of abstinence (Mean, SD, range) should be given for the other substances used. Did you check for the results after dividing the groups in subgroups based on duration of abstinence? And please also discuss the influence of this factor on the results.

Response: Abstinence was defined by a psychiatrist during the in-person clinical screening session as the length of time each participant had been off alcohol without relapsing.
We have now added the range of alcohol abstinence and the duration and range of abstinence for the other substances used in the alcoholplus group to the demographics table (see table 1.).
We did no attempt to split the groups based on the duration of abstinence. By including groups who had achieved a stable period in their own individual abstinence, albeit of varying durations within groups, we believed it was more likely that participants would be able to complete the entire ICCAM study. Therefore, we believe that any such split would significantly reduce our power to detect any main effects in our analyses at a group level.

> The argument regarding the inclusion of subjects is not relevant for a post-hoc analysis of the influence of duration of abstinence > please check by subgroup analyses or correlational analyses.

New Response: We do not believe that these types of analyses would be adequately powered to reveal any significant effects as different participants in the Alcoholplus group, for example, had been dependent on different substances, and therefore, abstinent from these substances for different durations.

Editor >>> Well, then put this aspect under limitations.

Response to editor: We have now written this under limitations as follows:
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.

Reviewer: Since the samples are not balanced, are possible gender effects relevant here?

Response: We have no reason to believe that there are any possible gender effects as the groups were well matched on gender distribution and did not differ significantly on this variable.

> Possible gender differences in the neurobiology of addiction are not well understood. Thus, unless you find your effects in both groups independently, please mention gender as a relevant factor under limitations and how you dealt with it.
New Response: The three groups were statistically balanced for gender, as reported in the results section under demographics. Therefore, we had no reason to be concerned about possible gender effects on the reported group differences in brain activation. It is our understanding that the reviewer is requesting that we conduct the same analyses in just the female participants of each group, and then separately, in the male participants. We do not believe that these types of analyses would be adequately powered with n values of 7, 4 and 6 females for the control, alcoholminus and alcoholplus groups respectively.

Editor >>> It was requested to mention gender as a possible relevant factor and I still recommend this for the limitations section.

Response to editor: We have now written this under limitations as follows:
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.

Reviewer: Since exposure to substances of abuse is highly relevant to the brain structure, did you control for brain structural differences in your brain functional analyses?

Response: We welcome the comment by the reviewer. As brain structure might be influenced by the chronic toxic effects of substances of abuse, which are in turn manifested in the form of functional

disturbances, controlling for a possible correlated covariate would not make any sense statistically. Therefore, we did not control for structural differences between the groups in our functional analyses. We did not explore differences either, as the main objective of the study was to test whether functional disturbances in response to non-drug rewards in addiction could be pharmacologically modulated/remediated. We had no a priori reason to believe that any structural differences could be differentially modulated by naltrexone or placebo when the visits were, on average, less than two weeks apart.

> The concrete relationship of brain structural and brain functional changes in the reward circuitry is not yet clear. If you have other information on a direct relationship between brain structural and functional alterations, please refer to the relevant literature. If your hypothesis is true, the results will not be changed by including brain structure as a covariate. Thus, please control for possible differences in brain structure and show the results at least in the supplements.
New Response: We have nothing to add beyond the original response - controlling for a possible correlated covariate would not make any sense statistically.

Editor >>> I have nothing to add on the previous reply.

Reviewer: How were subjects selected based on the inclusion/ exclusion criteria? By whom? That means who did the assessments to ensure that control subjects had no previous history of substance abuse etc. And also the statement on page 5/6 is very vague "... in the opinion of the investigator, contradicts participation...". If different investigators participated in the study how was ensured that a similar basis of decision was used?

Response: We welcome the question from the reviewer. Assessments related to inclusion/exclusion criteria were conducted in all participants by a psychiatrist during the in-person clinical screening session at each site. All psychiatric and substance dependence histories were subsequently reviewed by two psychiatrists to ensure uniformity of diagnostic thresholds across sites, and any discrepancies arbitrated by a third psychiatrist if required. Eligibility queries were raised at weekly teleconferences with clinical representatives from all three sites so that consensus could be reached. We have now changed this to: ...that, in the opinion of a psychiatrist, contraindicated participation...

> Ok

Reviewer: Since comorbid secondary or lifetime depression and anxiety were permitted, how did you check for a possible influence of mood and previous mood or anxiety disorders on brain activity (and since it is well known that the reward system is altered in such diseases).

Response: We welcome the question from the reviewer. Secondary or lifetime history of depression or anxiety was permitted in the substance groups since this is a very common comorbidity in substance addiction. Therefore, we have no way of confirming or rejecting the influence of previous mood or anxiety disorders on the observed activation differences between the groups as there is no statistical technique for reliably achieving this.

> Please use any scale on anxiety or mood you have to use as covariate in order to parcel out this variance. Or discuss why you did not use such a scale and add the possible influence of mood/ anxiety under limitations.

New Response: We have nothing to add beyond the original response - depression or anxiety are common comorbidities in substance addiction, and we acknowledge, may be a possible confound. There is, however, no currently known statistical technique that can adequately account for such confounds, and the use of covariates (e.g., anxiety or mood measures) that are correlated with the independent variable (i.e. group) can lead to unpredictable results. Therefore, we do not believe that the use of covariates in any of our analyses will provide greater clarity.

Editor >>> I have nothing to add on the previous reply. Please at least reflect on such confounds under limitations.

Response to editor: We have now written this under limitations as follows:
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.

Reviewer: It would also be very helpful to add information on symptom severity etc. This might also be a relevant factor explaining group differences.

Response: We did not collect measures of symptom severity that went beyond the typical screening measures (e.g., structured clinical interview, ASSIST) that were used to classify and include/exclude participants. Given that we recruited participants in extended periods of abstinence, we did not anticipate recruiting participants who displayed any symptoms of severity of dependence.

> Ok, but you collected craving severity as mentioned before which is one possible parameter…. As suggested before, please use this one.
New Response: Please refer to the new response provided to reviewer number 1 above with respect to craving.

Editor >>> see above.

Response to editor (as above):
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.

Reviewer: The total duration of the MRI session was 90 Minutes. This is a very long session leading to various questions related to data quality. Please carefully comment on that.

Response: The total duration of each scan at the placebo and naltrexone experimental visits was actually 60 minutes. This involved participants also completing a resting state scan and two other tasks. "…or unable to lie still in the MRI scanner for up to 90 minutes…" was used as an exclusion criterion as the same participants were required to undergo a longer baseline session (which also

included structural and DTI measures) to acclimatize them to the scanning environment. We have had no issues on the MID task (e.g., movement, performance) that have raised any questions across the three sites with respect to data quality.

> Please comment on the acting you took in order to ensure data quality, i.e. did you use movement parameters as covariates on fist level?
New Response: We did introduce movement parameters into the first level analyses in FSL FEAT. We have now stated this in the methods section under MID fMRI data analyses: as follows

The six rigid body movement parameters were also included as regressors in the model in FSL FEAT.

Editor >>> Ok

Reviewer: To improve clarity, please explicitly state the used study design.

Response: The study used a randomized double blind placebo controlled design.

> Yes, please state in the manuscript under methods.
New Response: We have inserted this into the manuscript under participants in the Materials and Methods section as follows:
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).

Editor >>> Ok

Reviewer: How much did they get paid and when? And how did they get the won money from the MID task? If relevant, please also comment on the possible influence on brain activity.
Response: Participants were paid for their time. For the first in-person clinical assessment visit, participants were immediately paid £50. They were then paid £50 for each subsequent experimental visit, which they received upon completion of the study. Participants could win up to £18 across the two runs of the MID task. Trial parameters were adjusted, however, to ensure approximately 66% success rate, leaving the chance to win approximately £12 total. This money was also paid to participants up completion of the study.

> Relevant for brain activation is the specific instruction/ money shown before the experiment etc., please add this information on the design.
New Response: The money they could win was not shown to them before they completed the MID task in the scanner.

Editor >>> Anyway, the money could have been shown to them before entering the scanner since this increases the neural response...

Response to editor: Indeed some studies have employed this procedure using the MID task, which may well have enhanced their motivation to do well on the task, and the neural response to the anticipation of winning money. Unfortunately, we did not employ this procedure on ICCAM.

Reviewer: How did the authors deal with the multi-center setting in general (quality control etc.) and with the fact that different MR scanners were used in particular?

Response: Acquisition parameters for the EPI sequences were used to create images with characteristics as similar as possible. We also used existing sequences specifically designed to reduce inter-scanner variance. For instance, at Manchester (Philips scanner) fewer EPI slices were collected, so that the data could be made as similar as possible (while remaining within SAR limits) in quality when compared with the Siemens scanners at the Cambridge and London sites. No task was observed to have significant differences between sites at the whole brain level when balanced groups of healthy controls were observed during their baseline session. Between centre issues are investigated in McGonigle et al., The ICCAM platform study: An experimentalmedicine platform for evaluating new drugs for relapse prevention in addiction. Part B: fMRI description, Journal of Psychopharmacology, in submission.

> Please refer to the publication or the relevant data in the manuscript.
New Response: The manuscript is currently still under review.

Editor >>> Ok, then the necessary information has to be available somewhere. Supplements?

Response to editor:

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. At each visit the imaging session consisted of: localiser scans to set up the positioning of those that would follow; main magnetic field mapping, and two runs of the monetary incentive delay task.

Structural Acquisition

At London and Cambridge (Siemens), high-resolution T1-weighted volumes were acquired using a magnetization-prepared rapid gradient echo (MPRAGE) sequence (TR = 2300 ms, TE = 2.98 ms, TI = 900 ms, flip angle = 9°, field of view = 256 mm, image matrix = 240 x 256) with a resolution of 1 mm isotropic. For the volume, 160 abutting straight sagittal slices were collected in an interleaved right to left manner, resulting in whole head coverage. Parallel imaging using Generalized Autocalibrating Partially Parallel Acquisition (GRAPPA) with an acceleration factor of 2 was performed.

At Manchester (Philips), high-resolution T1-weighted volumes were also acquired using an MPRAGE sequence (TR = 6.8 ms, TE = 3.1 ms, TI = 900 ms, flip angle = 9°, field of view = 270 mm, image matrix = 256 x 256) with an in-plane resolution of 1.055 x 1.055 mm and a slice thickness of 1.200 mm. For the volume, 126 abutting straight sagittal slices were collected in an interleaved right to left manner, resulting in whole head coverage. Parallel imaging using Sensitivity Encoding (SENSE) with an S reduction of 1.8 was performed.

These T1-weighted volumes followed ADNI protocols to minimise inter-centre differences.

Functional Acquisition

At London and Cambridge (Siemens), functional imaging was performed using a multi-echo gradient echo echoplanar imaging (EPI) sequence (TR = 2000 ms, TE = 13 & 31 ms, flip angle = 80°, field of view = 225 mm, image matrix = 64 x 64) with an in-plane resolution of 3.516 x 3.516 mm and a slice thickness of 3.000 mm. The phase encoding direction was anterior to posterior. Echo spacing was 0.52 ms.

For each volume, 36 abutting oblique axial slices were collected in an ascending manner at an angle of around 30° to the anterior (AC) and posterior commissure (PC) line. This results in slightly less than whole brain coverage, with the most superior 9 mm not being imaged in most participants.

To achieve the desired resolution and repetition time, parallel imaging using GRAPPA with an acceleration factor of 2 was performed. The first three volumes of each functional run were automatically discarded to allow for T1 saturation effects and are not included in any number of volumes reported here.

At Manchester (Philips) identical parameters were used for EPI acquisition, but with 34 slices being collected and with acceleration achieved using SENSE.

Reviewer: Although loss processing might also be altered in these samples and might therefore be relevant for interpreting the results, the authors decided to focus on gain. Please base this decision on the literature including latest publications. And please comment on this under limitations.
Response: While exploring the neural substrates of loss processing we believe is certainly relevant to addiction, the primary objective to examine the neural correlates of reward (gain) processing, was largely driven by the need to keep scanning time during experimental visits to 60 minutes. We have now discussed this as a limitation in the discussion as follows:
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.

> Ok

Reviewer: I strongly recommend integrating the results on outcome processing in the manuscript, since the results of a cohesive experiment should be analyses and discussed as a whole and not be published in a separated way (see statement on page 9).
Response: We have now included the results of these analyses in the manuscript in the results section as follows: 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 - alcoholminus and alcoholplusneutral hit contrast.

We have also discussed these findings as follows: We also observed that the alcoholminus and alcoholplus 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.

> Ok

Reviewer: Why were the two patients groups combined for the analysis of the functional data (see page 10)?
Response: We welcome the question from the reviewer. We initially conducted three (Group: alcoholminus vs. alcoholplus vs. control) by two (Drug: placebo vs. naltrexone) whole brain cluster-based repeated measures ANOVA analyses on the win anticipation>neutral anticipation, win hit>neutral hit and win miss>neutral miss contrasts in FSL. We did not observe any group x drug interactions, however, leading us to collapse across the two substance groups in order to increase the power to find clusters related to a main effect of group in FSL. Following the definition of group effect clusters in FSL, we then performed three (Group: alcoholminus vs. alcoholplus 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 in SPSS. This was done in order to reveal whether the alcoholminus andalcoholplus groups independently contributed to the main ANOVA group effect.

> Please state this clearly in the manuscript.
New Response: This is written in the Functional MRI results section of the manuscript as follows:
As we did not observe any significant group x drug interactions for a three (Group: alcoholminus vs. alcoholplus 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.

>>> Ok

Reviewer: What kind of matching was used to parallelize the study groups? Since the authors state that the groups differed with regard to various variables including age, years of education, IQ etc. these are variables which should be controlled statistically. At least, the influence of these systematic differences between groups has to be discussed.

Response: We did not use any specific matching to parallelize study groups, which may have led to the observed demographic differences reported in the manuscript. The observed group differences in performance and BOLD signal, we acknowledge, may have been influenced by certain demographic variables (e.g., smoking and cannabis use). While these variables are likely to be correlated with the independent variable of group, we have briefly discussed their potential

influence as a limitation to interpreting the results. This has been written in the discussion as follows:

Limitations of the current study include a lack of complete matching of groups with respect to cannabis and cigarette use which means we cannot unequivocally dismiss their potential influence on altered reward processing in fronto-striatal circuitry of the alcoholminus, and particularly, the alcoholplus groups.

> And what is about the other variables? Please include them in the limitations or argue, why i.e. age has no influence on reward processing.
New Response: Although the alcoholplus were the youngest group, and showed the greatest disturbances in frontostriatal activation, we have amended this under the Limitations text as follows: Limitations of the current study include a lack of complete matching of groups with respect to age, cannabis and cigarette use which means we cannot unequivocally dismiss their potential influence on altered reward processing in fronto-striatal circuitry of both the alcoholminus and alcoholplus groups.

Editor >>> Ok

Reviewer: Please also state if any covariates of no interest were used.

Response: Strong correlations between covariates and independent variables, we believe, should be avoided. There is currently no known statistical technique that can adequately account for such confounds, and the use of covariates (e.g., smoking, cannabis use) correlated with the independent variable (in this case, group) can lead to unpredictable results. Therefore, we did not use covariates in any of our analyses.

> If this is your perspective you should deduce it from the literature and comment on it under limitations. AND, you should best as possible match the groups!

New Response: We have nothing to add beyond the original response - strong correlations between covariates and independent variables, we believe, should be avoided.

Editor >>> Nothing to add to my previous comment.

Reviewer: It does not become clear, why the RMV was used, since no specific hypotheses were mentioned in the introduction.

Response: The RMV index was used as it more closely reflects, on a behavioural level, the contrast in the incentive value of the win and neutral conditions, which is similarly computed during the functional analyses with the win>neutral contrasts.

> Ok, please clearly state this in the manuscript.

New Response: We have written this under Other Statistics as follows:

We also conducted a three (Group: alcoholminus vs. alcoholplus 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.

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

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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

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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)

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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

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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-

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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-

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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

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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.

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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

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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

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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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Declaration of Conflicting Interests

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article:

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.

Trevor Robbins has research grants with Eli Lilly and Lundbeck, has received royalties from Cambridge Cognition (CANTAB), has received editorial honoraria from Springer Verlag, Elsevier, Society for Neuroscience; has performed educational lectures for Merck, Sharpe and Dohme and does consultancy work for Cambridge Cognition, Eli Lilly, Lundbeck, Teva and Shire Pharmaceuticals.

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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.

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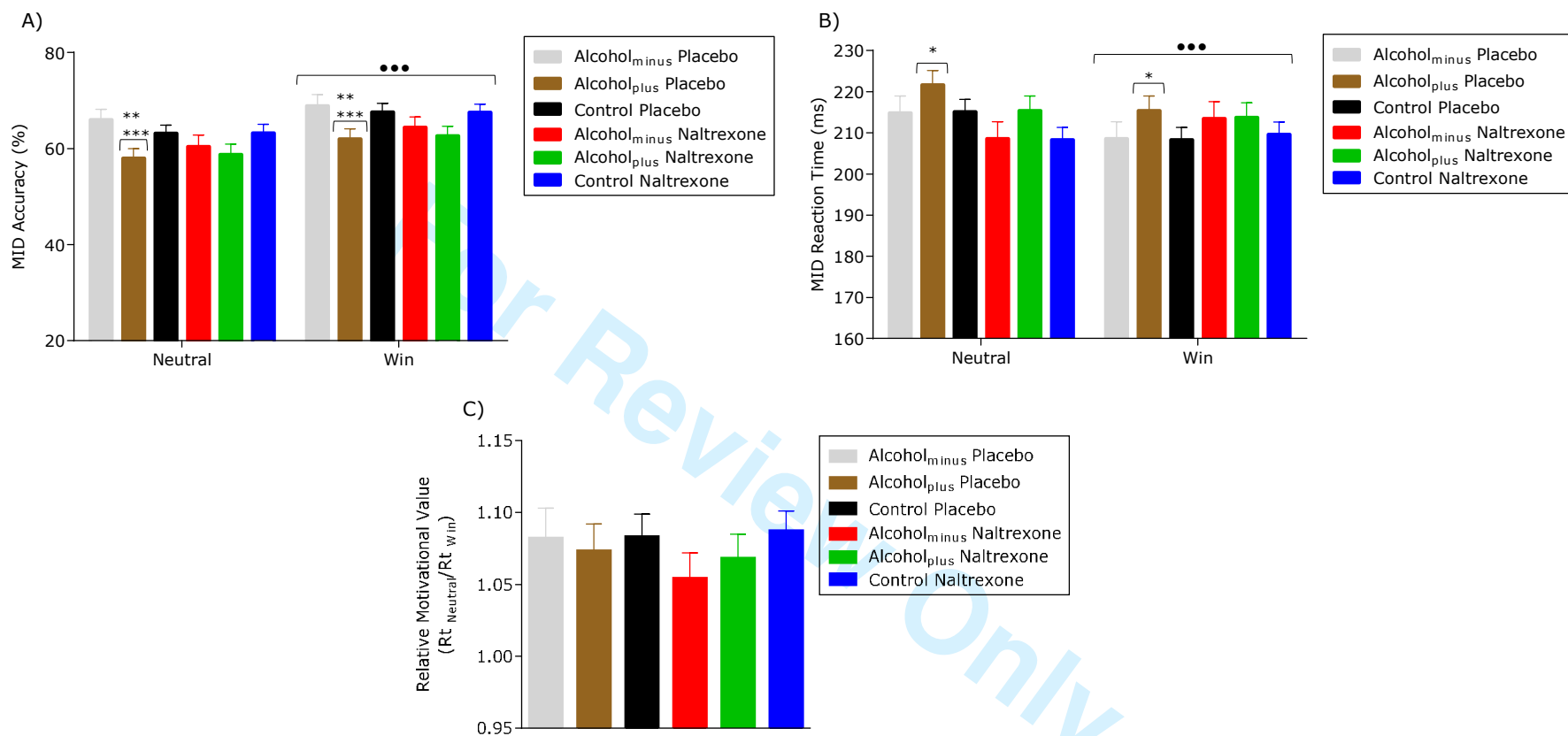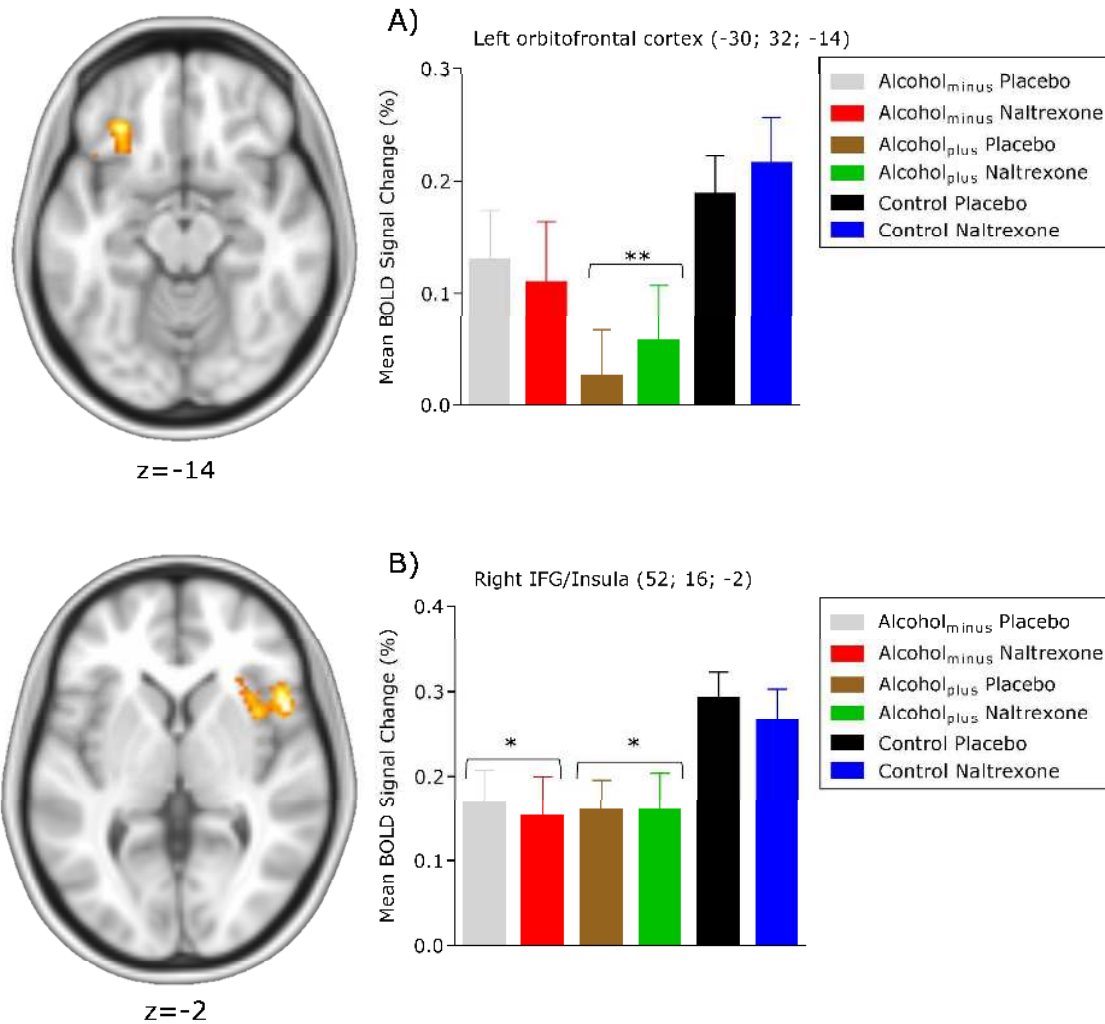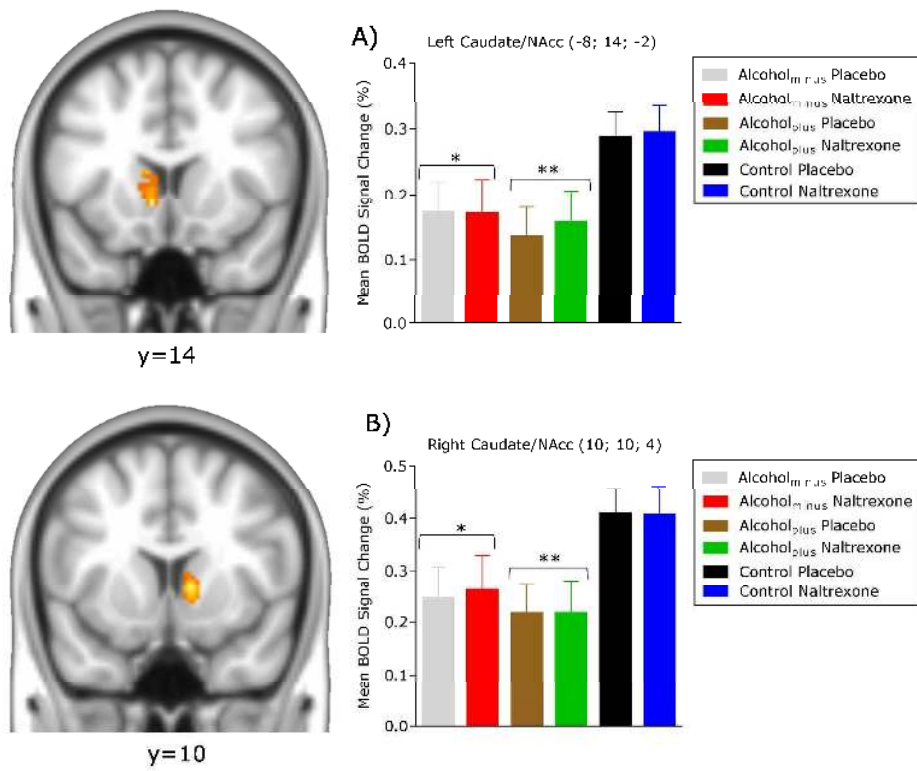study. *Neuropsychopharmacology, 23*(3), 326-334.

Figure 1.

Figure 2.

A) Left orbitofrontal cortex (-30; 32; -14)

z=-14

B) Right IFG/Insula (52; 16; -2)

z=-2

Figure 3.

Figure 4.

A) Left Anterior Cingulate Cortex (-1; -8; 32)

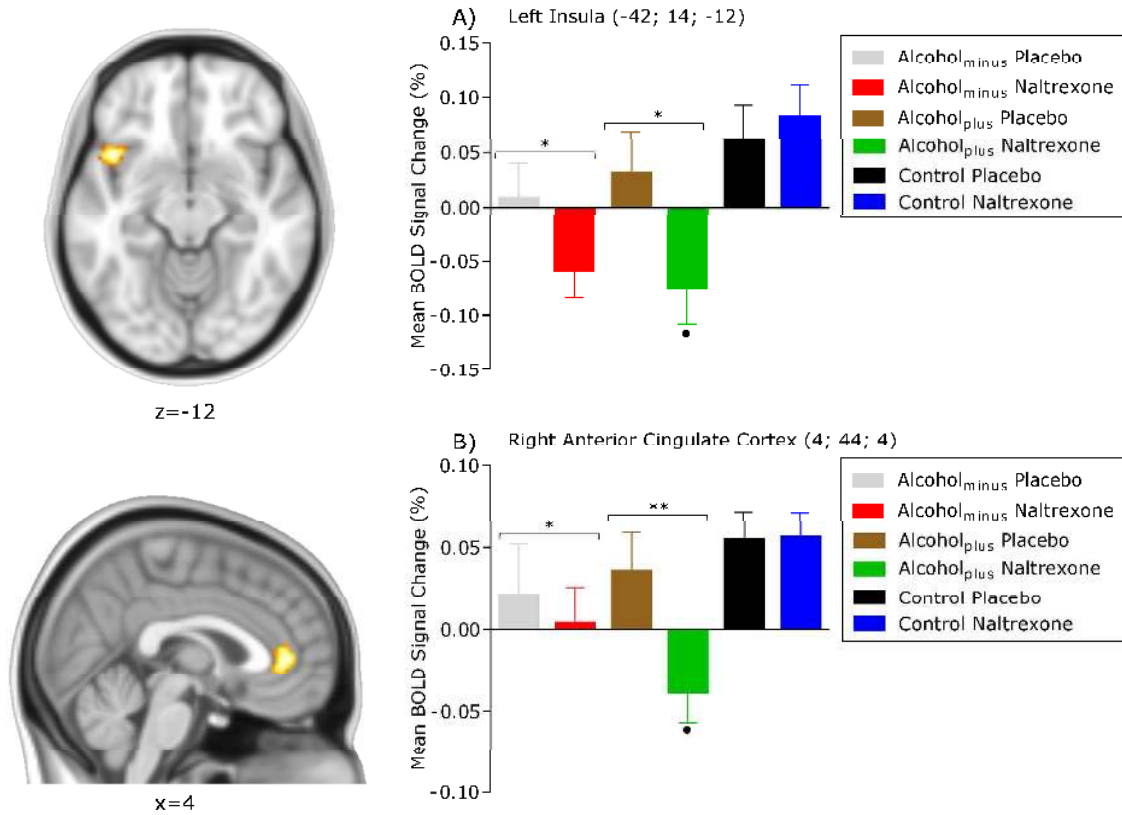B) Right Anterior Cingulate Cortex (6; 18; 28)
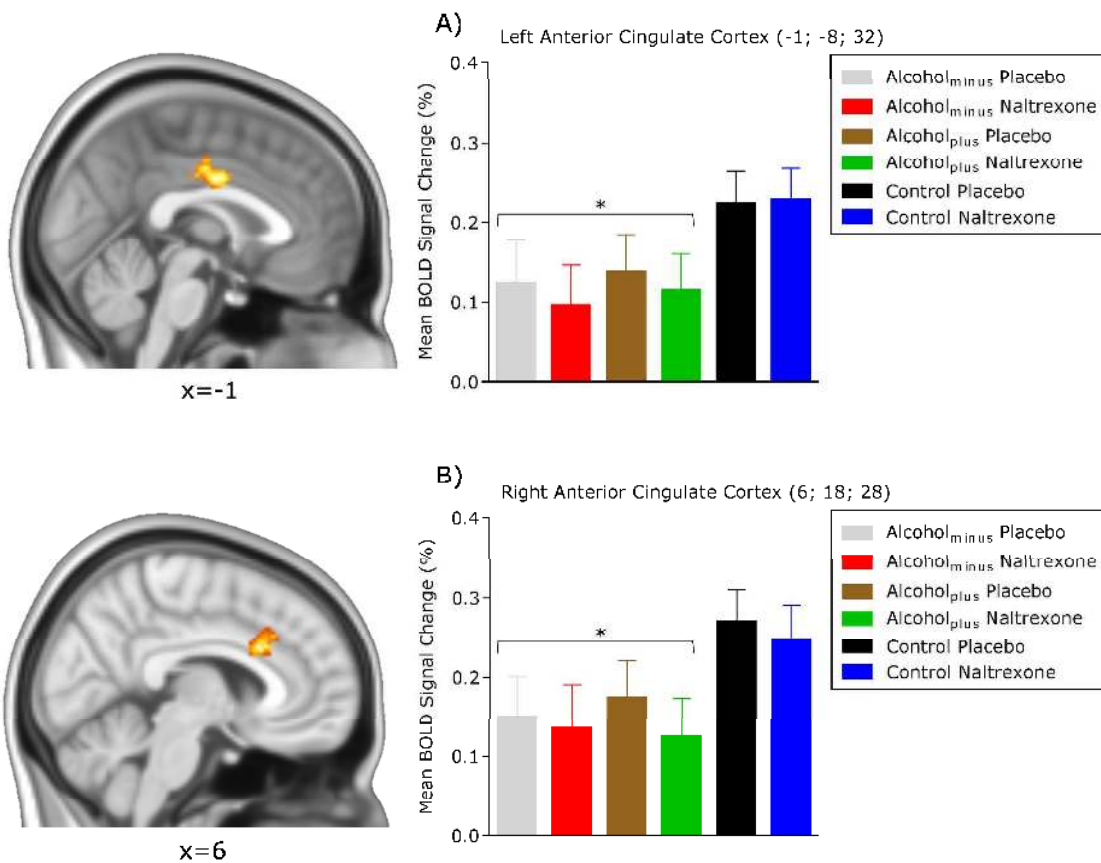
Figure 5.

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.

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