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Naltrexone acutely enhances connectivity between the ventromedial prefrontal cortex and a left frontoparietal network

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Abstract

Background—Naltrexone, an opioid receptor antagonist that is FDA-approved for treating alcohol use disorder (AUD), reduces alcohol craving and intake. Despite known pharmacological properties, little is known regarding the effects of naltrexone on neural circuit function. Thus, a data-driven examination of the neural effects of naltrexone in human subjects may offer novel insight into its treatment mechanisms.

Methods—Twenty-one alcohol using males (22–39) participated in a double-blind, placebo-controlled crossover study of the effects of naltrexone on brain voxel-wise functional connectivity (FC) using inter-subject FC correlation (ISFCC) mapping. We first cross-correlated the time series from each gray matter voxel to produce a 6356×6356 FC matrix for each subject and session. We then subtracted the placebo FC matrix from the naltrexone FC matrix. To identify brain regions demonstrating significant reconfiguration of whole-brain FC patterns following naltrexone treatment, we statistically quantified the consistency of patterns of voxel FC changes across subjects. Permutation testing identified significant clusters of voxels undergoing significant reconfiguration. Using the identified clusters in a seed-based functional connectivity analysis, we then compared the FC patterns of affected brain areas on placebo versus naltrexone in a paired t-test. Ridge regression analyses identified self-report measures, including substance use, that significantly predicted individual differences in FC among naltrexone-modulated regions.

Results—Two clusters in the rostral anterior cingulate cortex (rACC)/ventromedial prefrontal cortex (vmPFC) demonstrated significant modulation of FC by naltrexone. Using these two proximal clusters as a single seed, specific FC changes were identified in regions associated with a left frontoparietal network (increasing), as well as visual and motor regions (decreasing). Stronger FC between the rACC/vmPFC and this set of regions on placebo was associated with more external locus of control, whereas weaker connectivity was associated with greater substance use

problems. Naltrexone strengthened these connections most among individuals who reported greater drinking to cope.

Conclusions—Enhancing connectivity between the rACC/vmPFC, implicated in alcohol craving, and components of a left frontoparietal network involved in executive control may represent an effective strategy for the treatment of AUD.

Keywords

naltrexone; medial prefrontal cortex; fMRI; resting state

INTRODUCTION

Naltrexone is an FDA-approved medication for the treatment of alcohol use disorder (AUD) that is effective in reducing alcohol craving (Helstrom et al., 2016, Maisel et al., 2013, O'Malley et al., 2002, Volpicelli et al., 1992), heavy drinking (Maisel et al., 2013, Pettinati et al., 2006, Volpicelli et al., 1992), and may reduce the risk of relapse (Maisel et al., 2013, Volpicelli et al., 1995). However, its effects on drinking are relatively small (Del Re et al., 2013, Kranzler and Van Kirk, 2001), and it may only be effective in a small subset of patients (Garbutt et al., 2014, Mann et al., 2009, Rubio et al., 2005). Elucidating how and for whom this drug exerts its therapeutic effects would improve naltrexone treatment outcomes and may help drive development of new, more effective interventions.

Naltrexone is an opioid receptor antagonist with greatest affinity for mu-opioid receptors, but at therapeutic doses it also occupies most kappa-opioid receptors, with some delta-opioid-receptor action; thus its mechanisms are multifaceted. Indeed, the original hypothesis that lead to the investigation of naltrexone as a treatment for AUD suggests that naltrexone primarily acts by interfering with alcohol-related opioid signaling at mu receptors in the midbrain (Spanagel and Weiss, 1999), blocking alcohol's rewarding effects. Support for this theory includes naltrexone's ability to block cue-induced craving responses in the nucleus accumbens (Mann et al., 2014, Myrick et al., 2008), and to reduce impulsive and cue-induced responding to rewards (Anton et al., 2008, Chamorro et al., 2012, Weber et al., 2016). However, other data suggests that naltrexone's therapeutic effects are mediated by kappa receptors, interacting with the stress-related dynorphin system, perhaps by reducing the negative affective state present among alcoholics (Ray et al., 2010), or by disrupting kappa receptor effects on dopamine circuits (Altamirano et al., 2011, Bruchas et al., 2010). Support for this view includes the fact that blocking kappa receptors reduces alcohol self-administration in alcohol-dependent rats (Walker et al., 2011). Other studies have identified effects of naltrexone on cognitive control. For example, naltrexone reverses the increased delay discounting induced by morphine administration in rats (Kieres et al., 2004). In humans, it enhances inhibitory control and has variable effects on impulsive choice that depend on individual locus of control scores (Altamirano et al., 2011, Mitchell et al., 2007), and several clinical studies have found naltrexone effective in treating impulse control disorders (Kim, 1998, Papay et al., 2014, Schreiber et al., 2011), although its effectiveness is not universal (Mouaffak et al., 2017, Toneatto et al., 2009). However, conflicting data from alcohol-dependent patients found naltrexone had no effect on several measures of impulsivity (Zorlu et al., 2016). Despite considerable research on this drug, its

neurobehavioral mechanisms of remain elusive. Thus, an examination of the neural correlates of naltrexone may identify brain functional alterations that contribute to its therapeutic effects.

Here, we investigated naltrexone-related functional brain alterations in a double-blinded, placebo-controlled crossover study of the effects of a single oral administration of 50 mg naltrexone on fMRI resting-state functional connectivity. All participants reported lifetime alcohol use, however subjects were not required to meet criteria for AUD, and consumption levels varied widely across the sample. In line with the goals of this study to identify the neural targets of naltrexone, we undertook a completely data-driven analysis of resting-state fMRI data. Naltrexone's effects are likely heterogeneous, and efforts to identify factors predicting treatment responders among large samples have yielded inconsistent results. Thus, a secondary study goal was to identify factors that predicted brain responses to naltrexone. Study findings offer new insight into the neural mechanisms of this drug for the treatment of alcohol use disorder and provide potential targets for the development of novel treatments.

MATERIALS AND METHODS

Subjects

Twenty-four healthy males aged 22–39 years (mean: 25.7 years) participated in a double-blind, placebo-controlled crossover study of the effects of naltrexone on brain functional connectivity (FC). Due to sample size limitations, we tested only males, based on reported sex differences in naltrexone effects (Garbutt et al., 2005, Krishnan-Sarin et al., 2007, Ray et al., 2008). This study was approved by the Institutional Review Board at the University of North Carolina at Chapel Hill. Subjects were excluded if they had a known neurological or psychiatric disorder (with the exception of alcohol use disorder), cancer, cardiovascular disease, motor or visual disturbances that would impair cognitive task performance; were currently using psychoactive drugs (aside from moderate caffeine, alcohol, or nicotine), including prescription medications; were left-handed; had any MRI contraindications; or had any contraindications for naltrexone (e.g., recent opioid use). Due to possible effects on cognitive task performance, non-native English speakers, and people without a high school diploma or GED were also excluded. The absence of clinically significant psychiatric illness was confirmed using the Mini-International Neuropsychiatric Interview (MINI; (Sheehan et al., 1998)). The absence of acute alcohol intoxication or recent illicit drug use was confirmed via a Breathalyzer test and a urine drug test, respectively. Of the initial sample of 24 subjects, one withdrew from the study due to poorly-tolerated drug side effects and two additional subjects were excluded due to excessive motion and incomplete resting-state data. Therefore, the final sample consisted of 21 subjects.

Because naltrexone is effective across the spectrum of social and dependent drinkers (Davidson et al., 1996, de Wit et al., 1999, Hester, 2015, O'Malley and O'Connor, 2011), we recruited both low/moderate and heavy drinkers to power statistical analysis of the effects of alcohol use severity. All participants reported lifetime use of alcohol and recent use ranging from an average of 0–40 drinks/week (mean: 6.6). Eight out of 21 participants (38%) scored an 8 or higher on the Alcohol Use Disorders Identification Test (AUDIT), which is

considered to indicate hazardous alcohol use (Saunders et al., 1993). Subjects participated in two separate sessions lasting approximately five hours each and separated by at least five days. After providing informed consent and completing screening procedures, subjects were given a capsule containing either lactose as a placebo (PBO) or naltrexone (NTX; 50mg). They received a different pill at each visit, with order randomized and counterbalanced across subjects. Next participants completed a battery of self-report questionnaires (see below). As part of the broader goals of this study, participants also completed three computerized cognitive tasks (not reported here). Four hours after pill consumption, subjects entered the MRI scanner and underwent a series of anatomical and functional scans, including a resting-state scan completed approximately 4.5 hours after pill consumption. Prior to departing, subjects were asked to guess whether they had received the PBO or NTX pill. They were also asked to report any potential drug side effects they were experiencing.

Psychometric and Drug Use Questionnaires

All subjects completed a battery of questionnaires to assess personality, psychiatric symptoms, and drug use measures. These included the Alcohol Use Disorders Identification Test (AUDIT) (Saunders et al., 1993), the Barratt impulsivity scale (BIS) (Patton et al., 1995), the Drug Use Screening inventory (DUSI) – Domain 1 (Substance Use) (Tarter and Kirisci, 1997), the Family Tree Questionnaire (FTQ) (Mann et al., 1985) for a calculation of family history density (Cservenka et al., 2015), Rotter's Locus of Control Scale (LOC) (Rotter, 1966), Conners Adult ADHD Rating Scales-Self Report (CAARS-SR) (Conners et al., 1998), Depression Anxiety Stress Scales (DASS) (Henry and Crawford, 2005), Drinking Motives Questionnaire-Revised (DMQR) (Cooper, 1994), and the Carolina Alcohol Use Pattern Questionnaire to assess current and past binge drinking, which includes questions 10–12 of the Alcohol Use Questionnaire (Mehrabian and Russell, 1978) for the calculation of a “binge score” (Townshend and Duka, 2002).

Magnetic Resonance Imaging (MRI) data acquisition

For the resting-state fMRI scan, T2*-weighted echo-planar images (EPI) were acquired on a Siemens 3T Prisma MRI scanner with a 32-channel head coil (TR=2000 ms, TE=25 ms, FA=50°, FoV=192×192 mm; voxel size=3×3×4 mm with a 0.5 mm gap, matrix=64×64, 35 slices acquired at a 30° angle from the horizontal plane, 243 frames). During the ~8-minute scan, subjects viewed a white fixation cross on a black screen and were instructed to keep their eyes open and not to think about anything in particular.

T1-weighted high-resolution magnetization-prepared rapid gradient-echo (MPRAGE) structural images were also acquired for alignment and tissue segmentation purposes using the following parameters: TR=2530 ms TE=2.27 ms, flip angle=9°, matrix=176×512, 512 slices, final resolution=1×0.5×0.5mm³.

Resting-state MRI data preprocessing

Preprocessing of fMRI data was completed in Analysis of Functional Neuroimages (AFNI) (Cox, 1996) version 16.0.1 software and included the following procedures: slice time correction, reorienting of oblique slices to the axial plane, image realignment to correct for head motion correction, editing of outlier time points (3dDespike), nuisance variable

regression (white matter and cerebral spinal fluid signals as well as six motion covariates), linear detrending, spatial smoothing with a 5 mm full-width half-maximum Gaussian kernel, scaling to percent signal change, and bandpass filtering (0.008–0.1 Hz). Next, we analyzed head motion between each time point (framewise displacement) as calculated in the realignment step. Any time points with a framewise displacement of 0.3 mm from the previous time point, along with the time point preceding and the two following, were removed from further analysis to minimize effects of motion on functional connectivity estimates (Power et al., 2012).

Whole-brain inter-subject FC correlation (ISFCC) analysis

The goal of this functional connectivity analysis was to identify, from the whole brain, regions demonstrating significant alterations in functional connectivity (FC) following naltrexone administration. Functional connectivity analyses were conducted using a combination of AFNI and MATLAB software following a series of steps diagrammed in Figure 1. This inter-subject FC correlation (ISFCC) approach represents an extension of the inter-subject correlation method (Kauppi et al., 2010) for the analysis of data across time (i.e. time series) to that of data across space (i.e. seed-to-voxel FC patterns). Following preprocessing to clean the fMRI data, resting-state scans were warped to an MNI template and resampled to $6 \times 6 \times 6$ mm³. Downsampling the spatial resolution enabled us to efficiently carry out whole-brain functional connectivity analysis and can also improve the signal-to-noise ratio due to averaging of the time series. Next, time series from all voxels within a gray matter mask underwent a Pearson correlation analysis with Fisher z-transformation to produce a 6356×6356 FC matrix for each subject and session (i.e., placebo, naltrexone). Next, FC changes were calculated by subtracting the placebo FC matrix from the naltrexone FC matrix, producing a 6356×6356 FC change matrix. This process was replicated for each subject.

The next part of the ISFCC analysis sought to identify those voxels for which the patterns of FC change were consistent across individuals, which would indicate reliable and statistically meaningful drug effects. For each voxel – represented by a single column in the FC change matrix – the vector of changes in FC with every other voxel was correlated between subjects, producing a series of $6356 \times 21 \times 21$ similarity matrices. These Pearson correlations underwent Fisher z-transformation. For each subject, the mean and t-statistic of inter-subject correlations at every voxel was calculated. Finally, a one-sample mixed-effects meta-analysis was conducted using AFNI's 3dMEMA which utilized the mean and t-statistic maps for each subject to quantify the consistency of inter-subject correlations across all brain voxels in a group statistical test. This mixed model, which covaried for session order and age, was designed to account for variability both within and across subjects. The threshold for significance (cluster size 5 voxels given $p < 0.001$, one-tailed) was determined based on permutation testing of the global maximum cluster size over 1000 iterations, which was conducted using AFNI's 3dclust function and in-house Matlab code.

Seed-based functional connectivity analysis

Although we designed the whole-brain ISFCC analysis (above) to identify the region(s) demonstrating significant FC changes on naltrexone, we also sought to identify the specific

patterns of those FC changes. Therefore, to investigate the particular sets of neural connections affected by naltrexone administration, we also conducted a voxel-wise seed-based FC analysis. First, we extracted the average time series from a “seed” defined as voxels within significant clusters identified in the previous whole-brain analysis. Next, whole brain, voxel-wise FC of the seed was calculated for each subject and each session (i.e., placebo, naltrexone) separately. Next, a paired t-test, covarying for session order and age, identified regions in which FC with the seed differed between the placebo and naltrexone sessions. Significant clusters were identified based on permutation testing of the global maximum cluster size ($p < 0.001$, two-tailed; 7 voxels).

Network Analysis

To explore whether connectivity changes identified in the voxel-wise analyses (both ISFCC and seed-based) occurred within large-scale networks, we examined the relationship between the voxel-wise FC maps and canonical resting-state networks previously identified in an independent sample (Smith et al., 2009). Ten group independent component analysis (ICA) spatial maps were downloaded for this purpose (<http://www.fmrib.ox.ac.uk/datasets/brainmap+rsns/>). For each subject, the mean inter-subject correlation value at every gray matter voxel was correlated with those same voxel values of each ICA spatial map. Larger values in this analysis indicated that voxels for which FC effects were highly consistent across subjects were strongly associated with a given component; We Z-transformed the resulting Pearson correlations and conducted one-sample *t*-tests for each ICA network to identify networks associated with naltrexone-related changes in FC as calculated with ISFCC. The same process was repeated for the seed-based connectivity results: Voxel-wise differences in naltrexone and placebo connectivity maps were correlated with ICA spatial maps for each subject, and one-sample *t*-tests on correlation values identified networks with significant seed-based connectivity changes.

Behavioral analysis

To explore whether certain participant characteristics are associated with the identified patterns of functional connections, ridge regression analyses identified questionnaire measures that significantly predicted 1) individual differences in placebo session FC and 2) FC changes (i.e., naltrexone minus placebo), among regions where FC was significantly altered by naltrexone. First, the average FC value of the seed region with those clusters detected in the seed-based FC analysis was calculated for each subject and each session (i.e., placebo, naltrexone); because functional connections can be either positive or negative, voxels in regions with negative FC values were inversely weighted for this calculation. For the first behavioral analysis, the FC values during placebo were predicted so as to determine whether the identified set of functional connections is associated with particular characteristics, in general. A second analysis predicted naltrexone-related changes in FC (naltrexone-placebo) as calculated with ISFCC in order to identify if particular behaviors were associated with the direction and magnitude of drug effects on FC. For both analyses, features (i.e., questionnaire measures) were first sorted according to their correlation with the dependent variable (functional connectivity). The first measure added to the model was that with the highest correlation value. In a leave-one-out cross-validation analysis, the model was estimated for a set of 20 subjects and then applied to predict the left out subject;

this process was repeated to produce a predicted value for each subject. Fit between actual and predicted values evaluated based on R^2 value. Additional features were then added or removed to the model in a step-wise manner based on improvement to the R^2 value between actual and predicted FC values, adjusted for the number of features in the model (i.e., R^2 -adjusted value), until no other features could be added or removed.

RESULTS

Psychometric and substance use summary data are presented in Table 1. A table of self-reported side effects during the two study sessions is reported in Table 2. Participants accurately guessed the pill they had received in 74% cases (chance accuracy=50%): 16 of 21 participants accurately guessed when they had received placebo (76%), and 15 of 21 accurately guessed when they had received naltrexone (71%).

Whole-brain inter-subject functional connectivity correlation (ISFCC) analysis

After permutation correction, two clusters spanning portions of the rostral anterior cingulate cortex (rACC) and ventromedial prefrontal cortex (vmPFC) ($x=5$, $y=46$, $z=4$, 9 voxels and $x=-9$, $y=52$, $z=-6$, 6 voxels) were significantly associated with naltrexone-related FC changes based on ISFCC analysis (Figure 2).

Seed-based functional connectivity analysis

Due to the spatial proximity of the two significant clusters identified in the whole-brain connectivity analysis, we compared their separate seed-based FC maps and deemed them highly similar according to a Pearson correlation across all voxels ($r=0.81$, $p<0.001$). Therefore, in subsequent analyses we combined all voxels within the two clusters to form a single seed.

The seed-based FC analysis indicated that in the placebo condition, activity in the rACC/vmPFC was positively correlated with activity in the medial prefrontal cortex, posterior cingulate cortex, and angular gyrus - regions associated with the default mode network (Raichle et al., 2001) - as well as with activity in the striatum and anterior insula/inferior frontal gyrus (Figure 3a-b). Following acute naltrexone administration, the rACC/vmPFC demonstrated both increases and decreases in connectivity throughout the brain (Figure 3c). Clusters of significantly increased connectivity comprised a left frontoparietal network of regions, whereas clusters of significantly reduced connectivity were found in sensory regions (Figure 3d, Table 3). Confirming our seed-to-voxel findings, our network-level analysis found significantly increased connectivity between the rACC/vmPFC seed and the left and right frontoparietal networks, and significant decreases in connectivity between the seed and the medial visual and sensorimotor networks (Figure 3e). Mean rACC/vmPFC FC values within all clusters demonstrating either naltrexone-related increases (Figure 4A) or decreases (Figure 4B) are plotted in Figure 4 for both placebo and naltrexone sessions. Lines represent each participant, demonstrating individual variability in rACC/vmPFC FC changes after acute naltrexone administration.

Network Analysis

Significant resting-state FC changes associated with naltrexone versus placebo according to whole-brain ISFCC results were identified within two resting-state networks: the executive control network and the left frontoparietal network (Figure 2c). The executive control network is focused in the medial prefrontal cortex and is associated with the performance of cognitive tasks (Smith et al., 2009). The left frontoparietal network includes the left dorsolateral prefrontal cortex and left parietal cortex and is also associated with cognitive functions as well as language. Significant seed-based FC changes with resting-state networks were also identified (Figure 3e). The rACC/vmPFC seed demonstrated increased FC with the left and right frontoparietal networks and decreased FC with the medial visual and sensorimotor networks.

Exploration of a role for side effects on functional connectivity

We undertook several exploratory analyses to ensure that the detected brain changes related to naltrexone administration did not simply reflect the presence of drug side effects in this sample. By far the most prevalent side effect reported following naltrexone administration in this sample was fatigue (1½1 subjects), which was significantly more frequent than reports of fatigue following placebo (4/21 subjects; Table 2). The mean ISFCC value across all voxels within the two rACC/vmPFC clusters (corresponding to regions displayed in Figure 2b) was calculated for each subject. A two-sample *t*-test compared the mean rACC/vmPFC value in subjects with and without fatigue during the naltrexone session. We found no significant difference in FC changes between individuals who did versus those who did not experience fatigue. In fact, the change in FC (mean ISFCC values) was actually more prominent, though non-significantly, among those not reporting fatigue (fatigue group mean: 0.023, no fatigue group mean: 0.041, $t=-1.76$, $p=0.09$). We also calculated the mean functional connectivity value of all voxels demonstrating significant positive connectivity changes as well as negative connectivity changes with the rACC/vmPFC in the seed based analysis (corresponding to regions displayed in Figure 3d) for each subject. There were no significant effects of self-reported fatigue on rACC/vmPFC connectivity changes with “positive” regions (fatigue group mean: 0.11, no fatigue group mean: 0.14, $t=-0.71$, $p=0.49$) or “negative” regions (fatigue group mean: -0.22 , no fatigue group mean: -0.22 , $t=-0.14$, $p=0.89$). No other side effects were reported by more than two subjects, and thus did not likely contribute to detected changes in brain FC following naltrexone.

Exploratory brain-behavior analyses

To evaluate the functional significance of the network of regions targeted by naltrexone, we identified questionnaire variables that predicted the placebo session FC between rACC/vmPFC and the set of regions altered by naltrexone according to the seed-based FC analysis. A combination of several questionnaire measures predicted placebo session FC among these regions: a more external “locus of control” associated with stronger connectivity, whereas multiple substance use measures associated with reduced connectivity ($R^2=0.91$, $p<0.001$; Figure 5a; Table 4).

Naltrexone shows variable therapeutic effectiveness, and the source of such variability remains incompletely understood. Individual differences in personality, behavioral, or

psychiatric factors represent possible proxies for variations in neural responses to naltrexone. Indeed, a combination of several questionnaire measures predicted naltrexone effects on rACC/vmPFC FC. The strongest predictor was self-reported tendency towards “drinking to cope;” greater naltrexone induced changes in rACC/vmPFC FC associated with more self-reported drinking to cope. Other predictors included a hyperactivity measure and density of familial problem drinking ($R^2=0.66$, $p<0.001$; Table 4).

DISCUSSION

This whole-brain analysis of the effects of naltrexone in alcohol drinkers revealed altered intrinsic FC in portions of the rostral anterior cingulate and medial prefrontal cortex across subjects. Follow-up analyses suggest that naltrexone enhances FC between these medial prefrontal regions and regions of a left frontoparietal network. A network-based analysis confirmed that patterns of FC changes closely matched two canonical resting state networks: an executive control network consisting of medial prefrontal, anterior cingulate, superior frontal, anterior insula, and striatal regions, and a left frontoparietal network. While the medial prefrontal executive control network has been associated with emotion, cognition, action-inhibition, and pain based on an analysis of tasks in which this set of regions is activated, left frontoparietal network functions include cognitive, memory, and language processes (Smith et al., 2009). Interactions among the regions targeted by naltrexone may serve to exert control over automated responses to stressors, previously reinforced cues, or the rewarding effects of the drug itself. We consider mechanisms supported by the current findings that may contribute to the effects of naltrexone on alcohol consumption.

The role of rACC/vmPFC in addiction

A vast literature implicates the rACC/vmPFC in emotional regulation (Etkin et al., 2011, Hänsel and von Känel, 2008, Motzkin et al., 2015), depressive symptoms (Hänsel and von Känel, 2008, Koenigs and Grafman, 2009), stress and coping (Hänsel and von Känel, 2008, Maier and Watkins, 2010, Sinha et al., 2016), the placebo effect (Zubieta et al., 2005), physiological responses (Bechara et al., 1996, Damasio et al., 1990, Motzkin et al., 2014), and reward processing and valuation (Hare et al., 2009, Xue et al., 2008). Interestingly, the rACC/vmPFC is also a key region involved in drug craving responses. Activity in this region correlates with cue-induced alcohol craving (Heinz et al., 2009, Heinz et al., 2004, Kuhn and Gallinat, 2011, Seo et al., 2013) and predicts subsequent relapse (Grusser et al., 2004, Seo et al., 2013). Furthermore, vmPFC activity is associated with the stress response (Sinha et al., 2016), correlates with stress-induced craving responses (Seo et al., 2013), and may relate to coping abilities (Sinha et al., 2016). These relationships are particularly notable given the identification of the rACC/vmPFC as a target of naltrexone in the current study, since naltrexone reduces alcohol craving (Helstrom et al., 2016, Maisel et al., 2013, O'Malley et al., 2002, Volpicelli et al., 1992). In fact, a study of the effects of naltrexone on alcohol cue-related neural activation identified attenuation of signals in this region (Lukas et al., 2013), which offers a potential neural mechanism of reduced cue-induced alcohol craving.

Left dorsolateral prefrontal cortex and craving

We detected specific increases in rACC/vmPFC FC with a set of regions consistent with a left frontoparietal network, with the largest cluster in the left dorsolateral prefrontal cortex (DLPFC), specifically spanning the left middle frontal gyrus. This network association was confirmed by our network analysis, which demonstrated increased FC between the rACC/vmPFC and both the left and right frontoparietal networks. Possible effects of naltrexone on the left DLPFC and its impact on craving was recently demonstrated by a study of women given a naltrexone and bupropion combination therapy or placebo (Wang et al., 2018); that study reported an association between reduced FC within the left DLPFC in the treatment condition with reductions in food craving. Interactions between the DLPFC and rACC/vmPFC, also have the potential to influence drug craving (Hayashi et al., 2013). Consistent with this idea, experimental manipulation of these connections via repetitive transcranial magnetic stimulation (rTMS) of the left DLPFC increases dopamine release in the vmPFC (Cho and Strafella, 2009) and attenuates craving-related activation in the vmPFC region (Hayashi et al., 2013). Indeed, several studies report that stimulation of the right or left DLPFC significantly reduces self-reported alcohol craving among individuals with an AUD (Boggio et al., 2008, Mishra et al., 2010). Modulation of medial prefrontal cortex activity by the left DLPFC also predicts value-based decision-making under demands for self-control (Hare et al., 2009, Hare et al., 2014), suggesting a more general role of the left DLPFC in promoting executive control over medial prefrontal responses to rewards. Taken together, these studies highlight a brain FC mechanism by which naltrexone may regulate craving.

Kappa opioid receptor system: theoretical mechanism of naltrexone effects

Although naltrexone likely exerts its actions through some combination of actions on opioid receptor systems, the neural effects observed in the current study may particularly implicate the kappa opioid system. The opioid receptor kappa 1 gene (OPRK1), which mediates stress induced craving and relapse risk (Xu et al., 2013), is most highly expressed in the rACC/vmPFC relative to other adult neocortical regions (<http://hbatlas.org/>, <http://www.brainspan.org>). The rACC/vmPFC also receives dopaminergic projections from the ventral tegmental area (VTA). Of particular note, dopamine release from the VTA is regulated by kappa opioid receptors present on specific VTA neurons. Binding of dynorphin, and endogenous ligand induced by stress, reduces dopamine release specifically from neurons projecting to the medial prefrontal cortex (Margolis et al., 2006). Thus, it is reasonable that naltrexone's action on kappa receptors could modulate the function of the rACC/vmPFC through this circuit, possibly attenuating craving signals in that region. It is also worth noting the effects of naltrexone show remarkable overlap with that of mu-opioid system activation in the rACC and left dorsolateral prefrontal cortex during placebo administration (Zubieta et al., 2005), indicating that mu-opioid receptors are also well situated to mediate the effects observed in the current study.

Prior naltrexone fMRI studies

Several previous fMRI studies have examined the effects of naltrexone in the context of cue-induced craving responses. One reported neural mechanism of naltrexone's effects is on reduction of presumed reward responses in the ventral striatum following drug cue

presentation (Mann et al., 2014, Myrick et al., 2008, Ray et al., 2015, Schacht et al., 2017, Shi et al., 2018), but see also (Schacht et al., 2013, Spagnolo et al., 2014); however this conclusion is largely driven by a priori-defined regions-of-interest defined by anticipated results. Although the present study focused on resting state FC rather than task-related activation, it is noteworthy that we did not identify significant FC effects of naltrexone in the ventral striatum. It is possible that naltrexone's effects there are only revealed in the presence of drug cues or other rewards. However, another resting-state fMRI study of naltrexone effects used a predefined ventral striatal seed to detect reduced connectivity with the amygdala and hippocampus, which was interpreted as reflecting dopamine reward system changes (Kohn et al., 2018). Consistent with this interpretation, the naltrexone effects in the rACC/vmPFC found here parallel some prior fMRI studies of naltrexone effects on cue-induced activation in this prefrontal component of the dopamine reward pathway (Ray et al., 2015, Shi et al., 2018), supporting the possibility that our primary finding of altered rACC/vmPFC FC represents an effect on reward circuitry.

Additional whole-brain analyses (as opposed to regions-of-interest analyses) of naltrexone's effects indicate robust reductions in cue reactivity in visual and sensorimotor areas following naltrexone treatment (Courtney et al., 2016, Lukas et al., 2013, Ray et al., 2015). The effects of naltrexone on these regions echo findings in the current FC-based study. Specifically, we detected decreased FC between the rACC/vmPFC and the medial visual and sensorimotor networks using a network analysis, suggesting a possible brain connectivity mechanism for the observed attenuation of these signals during drug cue presentation.

Another recent fMRI study reported reduced resting-state FC within the left DLPFC among obese women given a naltrexone (32 mg) and bupropion (360 mg) combination therapy for four weeks (Wang et al., 2018). The major pharmacological differences between our study and that of Wang et al. should be noted. However, both studies suggest a key role for the left DLPFC in naltrexone effects, although they employed fundamentally different FC methods and present qualitatively different findings. Whereas Wang and colleagues found reduced FC *within* a local cluster of voxels in the left DLPFC, we detected an increase in FC *between* the left DLPFC and the rACC/vmPFC. A second whole-brain connectivity measure employed by Wang et al. only tested for either a global increase or a global decrease in FC, whereas our analysis was designed to capture global *changes* in FC, in recognition of the fact that, with changes in brain states, brain regions tend to increase FC with some parts of the brain while also decreasing FC with other parts (Di and Biswal, 2015, Elton and Gao, 2014, Elton and Gao, 2015). Although these methodological differences (as well as other differences including dosing duration, sex, and clinical characteristics of the samples) make integration across these two resting-state studies tenuous, evidence of reduced local FC in the left DLPFC coupled with our finding of enhanced FC between the left DLPFC and rACC/vmPFC suggest that naltrexone reduces within-network FC in favor of between-network connections for the left frontoparietal and medial prefrontal/executive control networks. Consistent with this view, Morris and colleagues (Morris et al., 2017) recently tested the effects of naltrexone on resting-state FC using graph theory measures of local and global efficiency and found that naltrexone reduced local efficiency, which is thought to reflect greater cross-network integration (Wig, 2017).

Behavioral predictors of brain connectivity

Variation in the brain connections altered by naltrexone in this study, particularly the rACC/vmPFC, associated with differences in attributional style and with self-reported substance use. These data harmonize with other evidence linking variation in the rACC/vmPFC with individual differences in locus of control (Harnett et al., 2015, Hashimoto et al., 2015). Moreover, a more external attribution correlates with higher neuroticism (Hashimoto et al., 2015). Thus, given the vmPFC's reported role in negative emotion regulation (Etkin et al., 2011, Hänsel and von Känel, 2008, Motzkin et al., 2015), we speculate that the association between locus of control scores and enhanced rACC/vmPFC-left frontoparietal FC reflects an adaptive coping mechanism to manage negative affective states.

In addition to a more internal attribution style, individuals with weaker FC between the vmPFC/rACC and regions identified in the seed-based analysis reported greater density of familial alcoholism, and higher scores on several alcohol-related measures. This finding comports with recent evidence of reduced frontal FC in people with AUD relative to healthy controls in a placebo session (Morris et al., 2017). One inconsistency in our study was the association of "number of drinks per week" with greater vmPFC/rACC FC, likely driven by two participants whose reported drinking was among the greatest in terms of quantity, but who, conversely, exhibited limited alcohol-related problems. This dissociation of drinking quantity from familial alcoholism risk and drinking-related problems therefore suggests that deficits in this set of neural connections targeted by naltrexone may represent a vulnerability to alcohol use problems, rather than a consequence of alcohol exposure.

We also identified a combination of predictors of naltrexone-mediated brain changes. Consistent with many prior studies (Garbutt et al., 2014, Krishnan-Sarin et al., 2007, Rubio et al., 2005), a family history of alcoholism was associated with greater naltrexone-related effects; however, our findings uniquely indicated that the strongest predictor of naltrexone-related effects was a strong tendency to "drink to cope," based on responses to Cooper's Drinking Motives Questionnaire. Contrary to prevailing theories, this association suggests that naltrexone may be particularly effective in alcoholics whose drinking is driven by negative reinforcement, in which individuals seek to alleviate negative affective states. Although this study sample largely consisted of drinkers who did not meet criteria for AUD, drinking to cope is believed to a pre-existing risk factor for AUD (Carpenter and Hasin, 1999, Holahan et al., 2001), and its association with naltrexone effects in this study is this likely to be relevant across the spectrum of alcohol drinking. Such a mechanism would also implicate the kappa/dynorphin system, since dysphoric states generated by stress are produced by this system (Land et al., 2008). Hypothetically, naltrexone may be more effective in individuals with heightened kappa/dynorphin, either as a result of heavy drinking, early adverse experiences, or genetics. Indeed, a polymorphism in the kappa opioid receptor gene (OPRK1) has been associated with differential responses to naltrexone (Pechtel and Pizzagalli, 2011). Adverse childhood experiences, associated with increased negative affective symptoms (Shapero et al., 2014), have also been shown to influence naltrexone effects on medial prefrontal cortex activation during the presentation of negative emotional stimuli (Savulich et al., 2017). Indeed, alterations in drug craving responses among individuals with adverse childhood experiences have noted in both the rACC and

dorsolateral prefrontal cortex during exposure to drug cues (Elton et al., 2015). Future studies should consider these additional factors in analyses of individual differences in naltrexone treatment response.

Clinical Implications

Enhancing connectivity between the rACC/vmPFC and regions associated with a left frontoparietal network implicated in executive control may represent an effective strategy for the treatment of AUD. As one possible strategy to manipulate these connections, brain stimulation studies have begun to test transcranial magnetic stimulation to the left dorsolateral prefrontal cortex or ventromedial prefrontal cortex for the treatment of addictions. Moreover, our findings suggest that treatments targeting the ventromedial prefrontal cortex and left dorsolateral prefrontal cortex may be most effective in individuals whose alcohol use is motivated by negative reinforcement. Other strategies to increase cognitive-based coping strategies may boost naltrexone effectiveness.

Limitations

The results of this study should be interpreted with consideration of several limitations. First, this sample was relatively small, particularly for the individual differences analyses. Although the ridge regression analyses demonstrated good predictive ability, those results should be considered preliminary and requiring further exploration and replication. Additionally, our analyses were conducted in a voxel-wise manner, and although we correlated our findings with previously-defined networks, future work should further consider the large-scale network effects of naltrexone. Furthermore, we recruited a single-sex sample that consisted of both heavy and moderate alcohol drinkers and the findings may not necessarily translate to females, or to more severe AUD samples. The latter concern is somewhat mitigated by evidence showing qualitatively similar effects of naltrexone across the drinking spectrum included here, from social drinkers to non-treatment seeking AUD (Davidson et al., 1996, O'Malley and O'Connor, 2011, Setiawan et al., 2011, Tidey et al., 2008). It should also be noted that our study was conducted in a sample of sober individuals, but naltrexone's neural mechanisms may differ under the influence of alcohol. In addition, while behavioral effects and pharmacokinetics (Ferrari et al., 1998) of naltrexone are largely comparable with acute or chronic dosing, an extended administration period could produce different findings than those reported here. Finally, this resting-state analysis tested the neural effects of naltrexone, but the implications of the detected brain changes for alcohol use remains to be tested. However, it is noteworthy that the FC changes related to naltrexone did not associate with reported naltrexone side effects, suggesting that the observed brain changes likely relate to its clinical efficacy.

Conclusions

Using an innovative FC approach (ISFCC), this study revealed new evidence that naltrexone alters functional brain systems implicated in executive functions. The results suggest that naltrexone primarily targets connections between prefrontal regions implicated in craving and self-control, offering a potential neural mechanism through which this pharmacological treatment effectively reduces alcohol use. Under placebo conditions, these neural connections were weaker among those with greater substance use problems, and naltrexone-

induced functional connectivity changes were most pronounced in individuals who drink to cope. The findings have implications for adjuvant therapies and for the development of improved therapies that target these systems.

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Figure 1. Functional connectivity analysis schematic.

Preprocessed data (1) were warped to an MNI template and resampled to $6 \times 6 \times 6 \text{ mm}^3$ (2). Time series (3) from all voxels (v) within a gray matter mask underwent a Pearson correlation analysis with Fisher z -transformation to produce a 6356×6356 FC matrix for each subject and session (i.e., PBO, NTX) (4). Next, FC changes were calculated by subtracting the PBO FC matrix from the NTX FC matrix (5a) for each subject (5b). Reliable changes in FC patterns were examined by correlating the vector of FC values at each voxel between each subject (s) with every other subject (6). To quantify the consistency of inter-subject correlations across all brain voxels (7), a mixed-effects meta-analysis (AFNI 3dMEMA) of inter-subject correlations was conducted independently at every voxel, covarying for session order and age.

Figure 2. Acute naltrexone alters functional connectivity in the rostral anterior cingulate cortex (rACC)/ventomedial prefrontal cortex (vmPFC) and prefrontal networks.

Both cluster-level and network-level analyses of whole-brain, voxel-wise functional connectivity demonstrate acute naltrexone effects. a) Unthresholded results of the analysis of the effects of naltrexone on whole-brain, voxel-wise functional connectivity. b) Significant clusters from the voxel-wise analysis after correction for multiple comparisons ($\alpha=0.05$). c) Bar graph depicting the association of ten previously-published resting-state networks (Smith et al., 2009) with naltrexone effects on whole-brain, voxel-wise functional connectivity. Error bars represent standard errors of the mean. Asterisks (*) denote a significant effect of naltrexone on network functional connectivity.

Figure 3. Seed-based analysis of rACC/vmPFC functional connectivity.

Whole-brain seed-based functional maps are displayed for the group mean values during a) the placebo (PBO) visit and b) the naltrexone (NTX) visit. c) A paired t-test revealed naltrexone-related differences in rACC/vmPFC functional connectivity. d) Several clusters demonstrated significant naltrexone-related functional connectivity changes with the rACC/vmPFC after a correction for multiple comparisons. e) Bar graph depicting the association of ten previously-published resting-state networks (Smith et al., 2009) with naltrexone effects on rACC/vmPFC functional connectivity. Bar colors for each network correspond with those

in Figure 2. Error bars represent standard errors of the mean. Asterisks (*) denote a significant effect of naltrexone on network functional connectivity.

Figure 4. Individual differences in naltrexone effects on resting-state functional connectivity. The mean functional connectivity value between the rACC/vmPFC and all voxels with which it significantly increased (left panel) or decreased (right panel) in functional connectivity on naltrexone are plotted for each subject on placebo (PBO) and naltrexone (NTX). Each line color represents a different subject, and the slope of the line represents the degree of change in functional connectivity for that individual.

a. Prediction of rACC/mPFC FC on Placebo **b. Prediction of rACC/mPFC FC change on Naltrexone versus Placebo**

Figure 5. Prediction of functional connectivity among regions affected by naltrexone.

Scatter plots of the predicted versus actual values of functional connectivity based on questionnaire measures identified in ridge regression analysis. Relationships are displayed for a) rACC/vmPFC functional connectivity on placebo and b) rACC/vmPFC functional connectivity changes on naltrexone versus placebo.

Table 1.

Psychometric and Substance Use Questionnaire Data

(N=21)	Mean ± SD	Range		Mean ± SD	Range
<i>Psychometric</i>			<i>Substance Use</i>		
BIS Total	57.5 ± 11.3	39–83	AUDIT Total	7.1 ± 4.8	1–19
<i>Attention</i>	14.3 ± 3.5	10–21	<i>Consumption</i>	5.1 ± 2.8	1–11
<i>Motor</i>	22.9 ± 4.7	15–33	<i>Dependence</i>	0.29 ± 0.64	0–2
<i>Non-planning</i>	20.3 ± 4.6	13–30	<i>Harm</i>	1.7 ± 2.0	0–7
Locus of Control	9.9 ± 3.5	5–21	Drinks per Week	6.8 ± 9.0	0–40
CAARS			DUSI – Domain 1	2.7 ± 2.3	0–7
<i>Inattention</i>	8.6 ± 4.5	0–15	Drinking Motives Questionnaire		
<i>Hyperactivity</i>	12.3 ± 4.9	4–24	<i>Social</i>	15.6 ± 5.4	5–25
<i>Impulsivity</i>	6.2 ± 3.9	1–16	<i>Coping</i>	7.7 ± 2.0	5–11
<i>ADHD</i>	8.5 ± 2.9	3–13	<i>Enhancement</i>	12.4 ± 4.7	5–21
<i>DSM-Inattention</i>	5.4 ± 3.0	0–13	<i>Conformity</i>	6.4 ± 1.7	5–10
<i>DSM-Hyperactivity</i>	7.0 ± 3.6	2–13	CAUP-Q		
<i>DSM-Total ADHD</i>	12.3 ± 5.5	2–23	<i>Binge Score</i>	16.5 ± 19.0	1.2–86.2
DASS			<i>Under 18 Binge Frequency</i>	0.62 ± 0.86	0–3
<i>Depression</i>	2.6 ± 3.6	0–15	<i>18–21 Binge Frequency</i>	2.5 ± 2.0	0–6
<i>Anxiety</i>	1.2 ± 1.6	0–5	<i>Past 12 mo. Binge Frequency</i>	1.9 ± 2.0	0–5
<i>Stress</i>	4.8 ± 4.7	0–16	Family History Density	0.15 ± 0.21	0–0.83

Table 2.

Self-Reported Side Effects

Side effects	Naltrexone (n=21)	Placebo (n=21)	Odds Ratio	p-value
Fatigue	11 (52%)	4 (19%)	4.68 (95% CI 1.17–18.7)	0.029
light-headedness	2 (10%)	1 (5%)	2.11 (95% CI 0.18–25.2)	0.56
Nausea	2 (10%)	0	5.51 (95% CI 0.25–122)	0.28
thirst/dry mouth	2 (10%)	0	5.51 (95% CI 0.25–122)	0.28
Vomiting	1 (5%)	0	3.15 (95% CI 0.12–81.7)	0.49
clammy hands	1 (5%)	0	3.15 (95% CI 0.12–81.7)	0.49
elevated heart rate	1 (5%)	0	3.15 (95% CI 0.12–81.7)	0.49
eye-strain	1 (5%)	0	3.15 (95% CI 0.12–81.7)	0.49
irritability	1 (5%)	0	3.15 (95% CI 0.12–81.7)	0.49
difficulty focusing	1 (5%)	0	3.15 (95% CI 0.12–81.7)	0.49
shakiness	1 (5%)	0	3.15 (95% CI 0.12–81.7)	0.49
headache	1 (5%)	0	3.15 (95% CI 0.12–81.7)	0.49
“fuzziness”	1 (5%)	0	3.15 (95% CI 0.12–81.7)	0.49
altered taste perception	0	1 (5%)	0.32 (95% CI 0.012–6.26)	0.49

Table 3.

Regions demonstrating significant connectivity changes with the rACC/mPFC following naltrexone versus placebo administration

Region	# Voxels	x	y	z
<i>Increasing connectivity on naltrexone</i>				
Left Middle Frontal Gyrus	46	-41	21	39
Left Inferior Parietal Lobule	18	-40	-67	46
Left Middle Temporal Gyrus	10	-60	-43	-9
<i>Decreasing connectivity on naltrexone</i>				
Right Cuneus	11	22	-76	31
Left Middle Occipital Gyrus	10	-14	-92	17
Right SMA/Superior Frontal Gyrus	9	15	-4	65

Table 4.

Predictors of functional connectivity

a. rACC/mPFC Connectivity on Placebo	
<i>Predictors</i>	<i>standardized coefficients</i>
Locus of Control	0.0337
Drinks/week	0.0336
AUDIT Dependence	−0.0372
Drug Use Screening Inventory	−0.0344
Drinking Motives - Conformity	−0.0326
Drinking Motives - Enhancement	−0.0207
Family History Density	−0.0168
b. rACC/mPFC Connectivity: Naltrexone-Placebo	
<i>predictors</i>	<i>standardized coefficients</i>
Drinking Motives - Coping	0.0405
CAARS Hyperactivity	0.0288
Family History Density	0.0271
Depression	−0.0199
CAARS SelfConcept	−0.0197