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Now or *Later*? An fMRI study of the effects of endogenous opioid blockade on a decision-making network

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ABSTRACT

Previously, we found that distinct brain areas predict individual selection bias in decisions between small immediate ("*Now*") and larger delayed rewards ("*Later*"). Furthermore, such selection bias can be manipulated by endogenous opioid blockade. To test whether blocking endogenous opioids with naltrexone (NTX) alters brain activity during decision-making in areas predicting individual bias, we compared fMRI BOLD signal correlated with *Now* versus *Later* decision-making after acute administration of NTX (50 mg) or placebo. We tested abstinent alcoholics and control subjects in a double-blind two-session design. We defined regions of interest (ROIs) centered on activation peaks predicting *Now* versus *Later* selection bias. NTX administration significantly increased BOLD signal during decision-making in the right lateral orbital gyrus ROI, an area where enhanced activity during decision-making predicts *Later* bias. Exploratory analyses identified additional loci where BOLD signal during decision-making was enhanced (left orbitofrontal cortex, left inferior temporal gyrus, and cerebellum) or reduced (right superior temporal pole) by NTX. Additional analyses identified sites, including the right lateral orbital gyrus, in which NTX effects on BOLD signal predicted NTX effects on selection bias. These data agree with opioid receptor expression in human frontal and temporal cortices, and suggest possible mechanisms of NTX's therapeutic effects.

1. Introduction

The non-selective opioid antagonist naltrexone (NTX) is one of the few drugs with U.S. Food and Drug Administration (FDA) approval for the treatment of alcoholism. NTX is also used to treat other substance addictions and impulse-control disorders. NTX reduces ethanol consumption in subjects with a history of alcohol abuse (Anton et al., 1999; Davidson et al., 1999; Heidbreder, 2005; Hernandez-Avila et al., 2006; O'Brien et al., 1996), and in animals trained to self-administer ethanol (Boyle et al., 1998; Stromberg et al., 1998). Moreover, NTX reduces drug-seeking behavior triggered by drug-cue exposure in animal models (Ciccocioppo et al., 2003, 2002; Liu and Weiss, 2002) and alcohol-cue induced craving in human alcoholics (Monti et al., 1999; O'Malley et al., 2002; Rohsenow et al., 2000). However, the mechanisms of these clinically important actions of NTX are not well understood. NTX has the highest affinity for μ-opioid receptors, but substantial affinity for κ- and δ-opioid receptors (Kreek, 1996). Through its modulation of the endogenous opioids that control midbrain dopaminergic neurons, NTX alters forebrain release of dopamine (DA) (Herz, 1995; Margolis et al., 2006; Spanagel et al., 1992). Through blocking opioid disinhibition of ventral tegmental area (VTA) neurons, NTX reduces ethanol-induced release of DA in the nucleus accumbens (Altshuler et al., 1980; Harris and Erickson, 1979), which contributes to the motivation to consume alcohol. Based on this neurobiological action of NTX, candidate mechanisms for its therapeutic action in alcoholics include an attenuation of the rewarding effects of alcohol, for which there is experimental support (Sinclair, 2001; Swift et al., 1994; Volpicelli et al., 1995). There is also evidence that NTX can increase the aversive effects of ethanol (Davidson et al., 1999; de Wit et al., 1999; McCaul et al., 2000; Mitchell et al., 2009). However, NTX can also significantly reduce alcohol craving in alcoholics during abstinence (Monti et al., 1999; O'Malley et al., 2002; Rohsenow et al., 2000), and attenuate thoughts and behaviors associated with drinking (Anton et al., 1999). Thus, NTX may act, in part, via alteration of specific cognitive processes. One possibility that has received experimental support is that NTX reduces the tendency to choose impulsively (Kieres et al., 2004; Mitchell et al., 2007; O'Malley et

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al., 2002). In fact, NTX relieves a wide variety of impulse-control disorders (Grant, 2005; Kim et al., 2001; Marrazzi et al., 1995; Raymond et al., 2002; Symons et al., 2004). These data all suggest that endogenous opioids promote impulsivity, and that blocking endogenous opioid activity with NTX reduces impulsiveness.

To address whether NTX alters brain activity in regions known to predict individual bias toward immediate rewards, we used a NTX challenge in the context of functional magnetic resonance imaging (fMRI) while human subjects performed a modified delay discounting (DD) task. In a previous fMRI study of this task (Boettiger et al., 2007), we found that distinct brain areas predict individual selection bias. These areas include the dorsal prefrontal cortex, the posterior parietal cortex, the orbitofrontal cortex (OFC), the parahippocampal gyrus adjacent to the amygdala, the posterior cerebellum, and the inferior and middle temporal gyri. The task is comprised of numerous trials in which subjects are instructed to choose between two amounts of money, a smaller amount available "*Now*" (e.g. "$80 TODAY") or a larger amount available "*Later*" (e.g. "$100 in 1 Month"; Fig. 1). We have quantified individual selection bias in this task as an "Impulsive Choice Ratio" (ICR) (Mitchell et al., 2005). In a previous pharmacology study, we found that such selection bias can be manipulated by endogenous opioid blockade (Mitchell et al., 2007). To test the hypothesis that blocking endogenous opioid signaling with NTX would alter regional brain activity that predicts individual selection bias during decision-making, we compared the fMRI BOLD signal correlated with *Now* versus *Later* decision-making after acute administration of NTX (50 mg) or placebo. Subjects were either abstinent alcoholics or control subjects with no history of substance abuse, and they were tested in a double-blind randomized two-session crossover design.

For the purpose of analyzing brain activity between sessions, we defined regions of interest (ROIs) as 10 mm spheres centered on activation peaks that predict *Now* versus *Later* selection bias (Boettiger et al., 2007), comparing the BOLD signal during decision-making within these ROIs between NTX and placebo sessions. We also conducted exploratory mapwise analyses to identify other brain areas in which BOLD signal during decision-making was enhanced or reduced by NTX administration. Finally, given the widespread evidence for variability in NTX response, we conducted additional analyses to identify sites in which NTX effects on BOLD signal predicted NTX effects on selection bias.

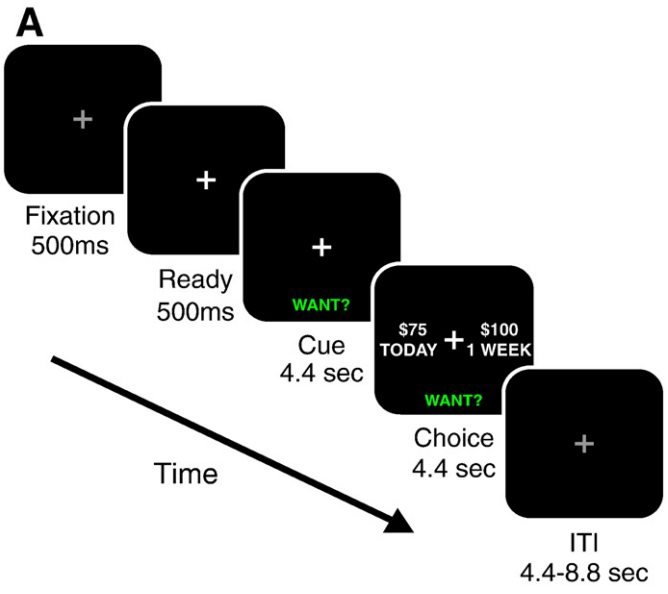

Fig. 1. Illustration of behavioral paradigm. The temporal sequence of events is depicted for an example WANT trial. Illumination of a fixation cross ("Ready") indicated the start of each trial. The instruction cue (in this case "WANT") was then displayed for 4.4 s, informing the subject as to the upcoming trial type. The two options (*Now* and *Later*) then appeared while the instruction cue remained on the screen. The specific dollar amounts (from $1 to $100) and the time of availability (from "Today" to "6 months" later) varied across trials, as did the differences between the two options. The options remained on the screen for 4.4 s, although subjects had 6.6 s to indicate their choice.

NTX is not effective in treating all alcoholics, thus, determining the moderators of NTX's effects is a critical goal of current research. Investigation of genetic moderators of NTX's effects has largely focused on the A118G (ASP40) variant of the μ-opioid receptor gene (OPRM1). This OPRM1 variant produces a μ-opioid receptor with greater affinity for β-endorphin (Bond et al., 1998), suggesting a gain-of-function effect. However, more recent data indicate that the ASP40 variant impairs transcription and thus expression of the receptor protein, resulting in a loss-of-function effect (Zhang et al., 2005). Laboratory studies demonstrate that this OPRM1 variant determines NTX's effect on physiological responses to alcohol-cue exposure (McGeary et al., 2006) and to alcohol-induced high (Ray and Hutchison, 2007). Moreover, clinical trials have yielded data demonstrating that the ASP40 variant predicts therapeutic response to NTX (Anton et al., 2008; Oslin et al., 2003); although, other studies have failed to replicate this finding (Gelernter et al., 2007). Thus, we also collected genetic material from these subjects to test whether the A118G variant of the OPRM1 gene also modulates NTX effects on immediate reward bias or underlying neural circuit activity. In addition to the A118G variant of the OPRM1 gene, we also tested several other single nucleotide polymorphisms (SNPs) that have been associated in other studies with addiction and/or impulsivity.

2. Methods

2.1. Participants

Participants were either abstinent alcoholics (AA; $n=9$) or control subjects with no history of substance abuse (CS; $n=10$). All participants were healthy, right-handed volunteers (8 females; mean age: 28.3 ± 5.8), and were paid for their participation. Participants were screened for psychoactive drug use (Biotechnostix, Inc.), including alcohol (Lifeloc Technologies, Inc.) at the start of each session. Five additional participants were tested, but were excluded from fMRI analyses due to excess head motion or equipment failure. All subjects had at least a high school education, and the two groups did not differ in terms of socioeconomic status, age, IQ, or years of education. Alcohol addiction severity (prior to sobriety for AA group) was assessed via the Alcohol Use Disorders Identification Test (AUDIT; (Saunders et al., 1993)), with mean AUDIT scores of 19 ± 7 and 5 ± 2, for the AA and CS groups, respectively (scores ≥8 indicate problem drinking). AA subjects self-reported a minimum of 2 weeks sobriety at the time of recruitment (mean sobriety = 2.5 years). All participants gave written, informed consent, in accordance with the guidelines of the University of California, Berkeley Committee for the Protection of Human Subjects. Subject payment was not dependent upon choice behavior in the task.

2.2. Experimental paradigm

Each subject completed two sessions separated by a minimum of 48 h. At the start of each session, subjects gave written, informed consent and after completing screening for contraindications for NTX, subjects were administered either a 50 mg NTX capsule or an identical placebo capsule. Experimenter and subject were blind to capsule content; capsule order was counter-balanced across subjects. Three hours after pill ingestion, subjects performed a delay discounting task in the context of an fMRI scanning session. For the task, subjects made a series of choices between *Now* and *Later* hypothetical monetary rewards. Later amounts were $2, $5, $10, $20, or $100, at 1 of 5 future delays: 1 WEEK, 2 WEEKS, 1 MONTH, 3 MONTHS, or 6 MONTHS. The *Now* option was always a lesser amount available TODAY. *Now* and *Later* options randomly appeared on the right or left side. Subjects were given task instructions and a brief practice

session to familiarize them with the DD task. Subjects performed the DD task within an fMRI scanner, while we collected continuous whole-brain bold oxygen level dependent (BOLD) signal. Scanning comprised 8 functional scans of 37 trials each (32 choice trials, and 5 "null" trials); trial type order was pseudorandom and unique for each subject (total scanning duration ~1 h). For each trial, subjects were shown a cue that indicated how to select between the two subsequently displayed hypothetical reward options, each option was a dollar amount available at a point in time (Fig. 1); these two alternatives are considered as "*Now*" and "*Later*". The *Now* option was available "TODAY" and was the lesser of the two amounts by a percentage randomly selected from the set: {30%, 15%, 10%, 5%}. Right-left position of each option was randomized across trials. Subjects indicated their choice by pressing a button on an MRI-compatible keypad. Instruction cues included: WANT (W), DON'T WANT (DW), SOONER, and LARGER, the latter two cues are considered together as "CONTROL" (CON). In the W condition, subjects chose their preferred option. In the DW condition, subjects were asked to make the same evaluation and press the button corresponding to the opposite choice. On CON trials, subjects selected the option reflecting either the sooner time point or larger amount of money, depending on the instruction cue. CON trial performance verified subject comprehension and compliance with task instructions. The DW condition provides a gross assessment of motor impulsiveness, by comparing inferred choices in the DW trials as a function of time with choices in the W condition as a function of time. This comparison rules out spurious impulsive choice as a result of unintended motor responses. Trial types frequencies were weighted with ratios of 1/2 for the W condition and 1/6 each for each of the other conditions. The order of inter-trial intervals was fixed across subjects. As the choice condition followed the cue condition at a fixed delay for each trial, "null" trials were included in each run (5 trials per run); in these trials, the instruction cue appeared and no options followed. Subjects saw each of the 120 possible choices two or three times, although not necessarily in the same trial type context. All choices made in the task were based on hypothetical rewards, although subjects were instructed to choose as if they would actually receive their choices.

2.3. Analysis of behavioral data

Single factor between-group comparisons were made via unpaired *t*-tests. Within group comparisons of condition were made via paired *t*-tests. All *t*-tests were 2-tailed and were conducted using Excel or SPSS. Multifactorial comparisons were made using mixed repeated-measures ANOVA in SPSS, with group as a between subjects factor. Where sphericity assumptions were violated, a Greenhouse–Geisser non-sphericity correction was applied. For each subject, we calculated an Impulsive Choice Ratio (ICR), which is the proportion of *Now* choices relative to all W condition choices made. Reward preference in DW trials was inferred to be the unselected option. From these DW responses, we calculated an inferred ICR (*i*ICR) for each delay time and took the sum of |ICR − *i*ICR| across all delays as a gross index of motor error. To ensure the validity of parametric statistical tests, we used an arcsine-root transformation of ICR data prior to statistical comparisons.

2.4. Imaging

T2*-weighted images (EPI) were acquired on a 4T Varian Inova whole body magnetic resonance scanner (Palo Alto, CA) equipped with a TEM send-receive radio frequency (RF) head coil, using a 1-shot gradient echo EPI sequence (TR = 2200 ms, TE = 28 ms, flip angle = 20°) to detect BOLD contrast. The sequence employed phase map correction to reduce Nyquist ghosts. To facilitate coverage of the ventral/orbital PFC, sampling of k-space was in the order positive to negative (De Panfilis and Schwarzbauer, 2005). 40 coronal slices (3.5 mm thick with 0.5 mm gap) per volume were obtained (FOV = 22.4 × 22.4, in-plane resolution = 3.5 mm). Each fMRI acquisition was preceded by 22 s of dummy gradient RF pulses to achieve steady-state tissue magnetization and minimize startle-induced motion. Low-resolution T1-weighted co-planar images were acquired for each participant. In addition, a magnetization-prepared fast low-angle shot high-resolution (MPFLASH) T1-weighted image was acquired for the purposes of normalizing data to standardized space. E-Prime software (PST, Inc., Pittsburg, PA) synchronized the stimulus display with the fMRI acquisition and collected subject responses via an MRI-compatible keypad. An LCD projector (Epson, Long Beach, CA) projected stimuli onto a rear projection screen (Stewart, Torrance, CA), which the subjects viewed via a mirror mounted within the head coil.

2.5. Image analysis

fMRI data were processed offline with a combination of in-house software, SPM2 (Wellcome Dept. of Imaging Neuroscience, London, UK), and the Artifact Detection Tools package (http://web.mit.edu/swg/software.htm). Images were first reconstructed into Cartesian space, and sinc interpolated in time to correct for timing differences in slice acquisition. Data were next motion corrected using a six-parameter, rigid-body, least-squares algorithm (Friston et al., 1995), realigning all volumes to the first EPI volume, then smoothed with a 7 mm full-width half-maximum (FWHM) Gaussian smoothing kernel. Each subject's co-planar and MPFLASH anatomical images were co-registered to the first EPI volume, and then normalized to Montreal Neurological Institute (MNI) space and resampled to 2 mm^3 isotropic voxels. The functional data were analyzed in an event-related manner based on the modified general linear model as implemented in the statistical package SPM2. Each subject's model included a design matrix of regressors of interest, which were δ functions positioned at the onset time of each experimental event type (ready; instruction cue; choice pair) convolved with a canonical hemodynamic response function (HRF). Noise spikes were rarely detected in the data (ArtRepair2 Toolbox); these were modeled as covariates of no interest with duration of 2.2 s and were not convolved with an HRF. Data were high-pass filtered using a cut-off of 128 s, and serial autocorrelations were corrected with a restricted maximum likelihood algorithm using a second-order autoregressive model (AR2 + white noise).

For each subject, linear contrast images were calculated from the regressor parameter estimates; contrast images were then normalized into MNI space using the parameters derived from the co-registered MPFLASH. Contrast maps were smoothed resulting in a total smoothing kernal of 10 mm FWHM. To test whether naltrexone (NTX) would alter brain activity during subjective decision-making, we compared the mean contrast values of (WANT–CON) from the NTX and placebo (PBO) sessions within predefined ROIs from both NTX and PBO sessions. ROIs were 10 mm^3 spheres centered on coordinates identified in a previous study (Boettiger et al., 2007). We also subtracted NTX and PBO session contrasts to assess mapwise drug effects; the subtracted contrasts were then subject to both simple regression and one-sample *t*-test analyses, as implemented in the SnPM3 analysis package (http://www.sph.umich.edu/ni-stat/SnPM/). To control for multiple comparisons, statistical non-parametric maps are corrected for Type I error ($\alpha < 0.001$; whole-brain volume), determined by random permutation of the NTX-PBO subjective decision-making contrast parameter estimates (with either ICR change or subject) in 5000 new analyses (Nichols and Hayasaka, 2003; Nichols and Holmes, 2002). Activation peaks were localized within defined anatomical regions, identified from the T1-weighted anatomical images obtained from each subject, with reference to standard brain atlases (Duvernoy et al., 1991; Schmahmann et al., 1999; Tzourio-Mazoyer et al., 2002).

2.6. Genetic analysis

DNA extraction and analysis was conducted according to standard methods on samples obtained from subjects providing informed consent. The Ernest Gallo Clinic and Research Center Genomics Core carried out genotyping of the following polymorphisms with polymerase chain

Table 1
Subject demographics.

Participants	AA ($n=9$)	CS ($n=10$)	t_{17}	p value
General				
Age (years)	30 ± 8	27 ± 3	1.49	ns
Education (Hollingshead subscore)	6 ± 1	6 ± 1	1.77	ns
Hollingshead SES	51 ± 9	50 ± 2	0.34	ns
Shipley Institute of Living Scale (raw)	65 ± 8	72 ± 8	1.70	ns
Gender (# female)	5	3		ns†
Ethnicity (# non-white)	2	1		ns†
Alcohol-related				
AUDIT	19 ± 7	5 ± 2	6.30	<0.001
DUSI-I (%)	75 ± 15	13 ± 18	8.06	<0.001
FTQ (#)	3 ± 2	1 ± 1	2.99	<0.01

Values are reported as mean ± standard deviation. Reported p values reflect the results of unpaired two-tailed comparison between groups. AA, abstinent alcoholic; CS, control subject; SES, socioeconomic status; AUDIT, Alcohol Use Disorders Identification Test; DUSI-I, Drug Use Screening Inventory, Domain I; FTQ, Family Tree Questionnaire; †p value represents results of χ^2 test. ns: $p>0.05$.

reaction using TaqMan® technology (Applied Biosystems): adrenergic alpha-2A-receptor (ADRA2A; rs1800544), ankyrin repeat and kinase domain containing 1 (ANKK1/DRD2 Taq I; rs1800497), brain-derived neurotrophic factor (BDNF; rs6265), cathechol-*O*-methyltransferase (COMT; rs4680), dopamine D2 receptor (DRD2; rs6277), dopamine D4 receptor (DRD4; rs1800955), kappa-opioid receptor (OPRK1; rs1051660), mu-opioid receptor (OPRM1; rs1799971), and tryptophan hydroxylase 1 (TPH1; rs1800532).

To estimate which genotypes had the greatest predictive value for the NTX effects on ICR and ROI activity, linear multiple regression analyses were carried out using SPSS. For each multiple regression analysis, we entered genotypes stepwise, divided into five blocks. The blocks were as follows: block 1 — OPRM1; block 2 — OPRK1; block 3 — COMT, DRD2 TaqI, DRD2_7a, DRD4; block 4 — ADRA2, TPH1; block 5 — BDNF.

3. Results

3.1. Sample characteristics

The AA and CS groups were matched in terms of demographic variables. Specifically, the groups did not differ in terms of education level, age, SES, IQ, gender composition, or ethnic composition (Table 1). However, as expected, the AA subjects reported significantly higher levels of alcohol abuse and alcohol-related problems (Table 1). A family history of alcohol abuse was also more common among the AA group (Table 1).

3.2. NTX effects on behavior

Replicating our previous finding (Mitchell et al., 2007), a mixed design repeated-measures ANOVA found no significant main effect of drug ($F_{1,17}=1.07$, $p=0.341$) or group ($F_{1,17}=0.01$, $p=0.941$) on RTs, although, as expected, we did observe a significant effect of trial type ($F_{1,24}=49.1$, $p<0.001$) (Table 2). This latter effect reflected increasing RTs from the CON to WANT to DW trial types. We also found no significant drug × group interaction effect on RT ($F_{1,17}=2.14$, $p=0.161$).

A mixed design repeated-measures ANOVA considering ICR as the dependent variable found a significant main effect of group ($F_{1,17}=10.11$, $p<0.001$), but not of NTX ($F_{1,17}=1.22$, $p=0.286$) on ICR. Replicating our previous results (Boettiger et al., 2007; Mitchell et al., 2005, 2007), the group effect was due to significantly higher ICRs among the AA group relative to the CS group. We also found no significant drug × group interaction effect on ICR ($F_{1,17}=0.48$, $p=0.496$).

Table 2
NTX effects on behavior.

Participants	AA ($n=9$)	CS ($n=10$)	All ($n=19$)
Reaction time changes (%)			
CON	−6 ± 9	8 ± 10	1 ± 12
W	−5 ± 15	3 ± 14	−1 ± 15
DW	−4 ± 16	3 ± 17	0 ± 16
ICR changes (%)	5 ± 14	3 ± 14	4 ± 14

Changes in reaction times and ICR in the delay discounting task following 50 mg naltrexone (NTX), relative to the placebo session. AA, abstinent alcoholic; CS, control subject; W, WANT trials; DW, DON'T WANT trials; CON, control trials (SOONER and LARGER); ICR, Impulsive Choice Ratio. All data given as mean ± standard deviation.

3.3. NTX effects on brain activity during subjective preference decisions

In a previous fMRI study, we found that during decisions between small, immediate ("*Now*") versus larger, delayed ("*Later*") rewards the magnitude of the fMRI BOLD signal scales with individual immediate reward bias in several brain areas (Boettiger et al., 2007). Here we first tested whether acute NTX administration alters brain activity during subjective decision-making in these areas. We then conducted an exploratory analysis to identify other areas in the brain in which activity during decision-making is significantly altered. Finally, across the whole brain, we tested for correlations between NTX's effect on brain activity during *Now* versus *Later* decision-making and its effect on choice behavior.

3.3.1. Effects within choice-predicting ROIs

For the ROI analyses, we defined the ROIs as 10 mm^3 spheres centered on the coordinates identified in our previous study (Boettiger et al., 2007). These areas each predict individual immediate reward selection bias during *Now* versus *Later* decision-making. These areas can be divided according to those in which activity during such decisions is directly correlated with *Now* bias ("*Now* areas") and one in which activity during such decisions is inversely correlated with *Now* bias ("*Later*" area). For each ROI, the mean parameter estimates (β values) for a subjective decision-making contrast (WANT–CON) were extracted from both the NTX and the placebo sessions and then subject to further statistical analyses.

3.3.1.1. "Now" areas. To test for significant effects of NTX on BOLD activity during decision-making in *Now* ROIs, we conducted paired *t*-tests with ROI β values as dependent variables. For the three ROIs that were the focus of our previous study (posterior parietal cortex, superior frontal gyrus, and parahippocampal gyrus), we did not observe a significant effect of NTX on brain activity during subjective decision-making (t_{18}, minimum p value = 0.548). We observed similar effects in additional areas in the temporal lobe and cerebellum (see Table 3), in

Table 3
Peak coordinates for ROI analyses.

Brain region	Hemisphere	MNI coordinates [*x*, *y*, *z*] (mm)
Now ROI centers		
Supramarginal gyrus (parietal lobe)	Right	66, −42, 44
Cerebellum, posterior lobe (lobule VIII)	Right	36, −52, −62
Middle temporal gyrus	Right	56, −52, −2
Inferior temporal gyrus	Right	68, −50, −12
Parahippocampal gyrus (gyrus ambiens, entorhinal area, semilunar gyrus)	Right	18, −4, −28
Superior frontal gyrus	Left	−22, 50, 44
Later ROI center		
Lateral orbital gyrus	Right	58, 36, −10

ROI anatomical region, hemisphere, and coordinates of ROI center based on the Montreal Neurological Institute (MNI) coordinate system value are given.

which activity during *Now* versus *Later* decisions is also known to correlate with *Now* bias (t_{18}, minimum p value = 0.489). These results indicate that any effect that NTX may have on temporal discounting behavior is not mediated by actions in any of these brain areas.

3.3.1.2. "Later" area. We next tested for a significant effect of NTX on BOLD activity during decision-making in the *Later* ROI in the OFC, again using a paired t-test with ROI β values as dependent variables. Here we observed that during NTX sessions, brain activity during subjective decision-making was significantly elevated (Fig. 2; t_{18}, $p = 0.045$). Together with our previous finding that activity in the OFC during *Now* versus *Later* decisions inversely correlates with *Now* bias, this result suggests that NTX may reduce impulsive decision-making via effects on the OFC.

3.3.1.3. Group interaction with NTX effects on ROI. A two-way factorial ANOVA with ROI β values as dependent variables failed to identify a significant group × drug interaction for any of the *Now* or *Later* ROIs (maximum $F_{1,17} = 1.44$, $p = 0.256$). These findings suggest that NTX does not exert differential brain effects based on alcohol use history in areas that predict *Now* versus *Later* bias.

3.3.1.4. Genotype interaction with NTX effects

3.3.1.4.1. Effects on choice behavior. Multiple linear regression analysis was used to determine whether any of the SNPs we assayed had significant predictive value in terms of the NTX effect on ICR. We found that a single SNP had significant predictive power: the Val66Met locus of BDNF ($r = 0.52$, $t_{15} = -2.27$, $p = 0.04$). That is, electing *Now* more often during the NTX session was associated with fewer Val alleles at position 66 in the BDNF protein. No other genotype factor added into the model added significantly more predictive power than BDNF genotype alone for the NTX effect on ICR. A two-way factorial ANOVA with ICR as dependent variable confirmed a significant interaction between drug condition and BDNF genotype ($F_{1,13} = 3.99$, $p = 0.045$; Fig. 3). There was no significant main effect of BDNF genotype on choice behavior ($p > 0.8$).

3.3.1.4.2. Effects on brain activity during subjective choice. We performed an identical multiple linear regression analysis using the OFC ROI β values as the dependent variable to test whether any of the tested genotypes held significant predictive value for NTX's effect. This procedure found that none of the SNPs tested significantly predicted the NTX effect on OFC activity during *Now* versus *Later* choices.

3.3.2. Mapwise analysis of NTX effects

3.3.2.1. Sites in which NTX raised or lowered activity. In addition to our ROI-based analyses, we conducted an exploratory mapwise analysis of NTX's effect on BOLD signal during subjective decision-making. We found that NTX elevated activity during *Now* versus *Later* decisions in the left OFC, the left inferotemporal cortex, and bilateral sites in the cerebellum (Fig. 4A). In addition, we found that NTX depressed activity during *Now* versus *Later* decision-making in the right superior temporal gyrus (Fig. 4B). See Table 4 for peak coordinates.

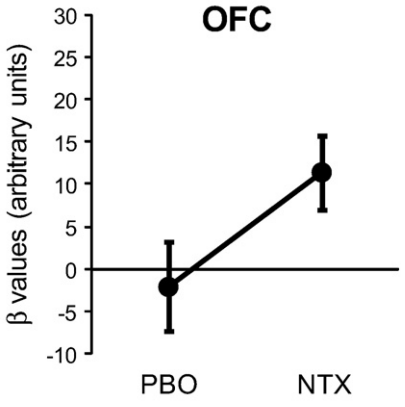

Fig. 2. NTX significantly elevates activity during decision-making in the OFC. Plot of the mean *Now* versus *Later* subjective decision-making parameter estimates for the OFC ROI as a function of drug condition. Plots reflect mean ± S.D. NTX, naltrexone; PBO, placebo.

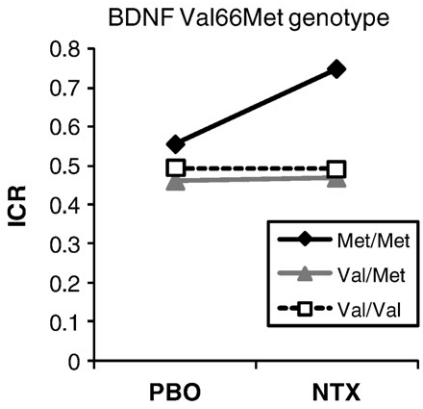

Fig. 3. Genotype at the BDNF 66 locus predicts NTX effect on ICR. Ratio of impulsive choices as a function of genotype and drug condition. There was a significant interaction effect between drug condition and genotype at the BDNF 66 locus on impulsive choice probability. The $66^{Met/Met}$ genotype was associated with greater *Now* bias on NTX. Met/Met, $66^{Met/Met}$; Val/Met, $66^{Val/Met}$; Val/Val, $66^{Val/Val}$; BDNF, brain-derived neurotrophic factor.

3.3.2.2. Correlation between NTX effects on brain and on behavior. Our final analysis employed simple regression analysis to identify brain areas in which NTX's effects on decision-making activity predicted NTX's effect on behavior. To accomplish this, we entered the choice condition drug contrasts (NTX–PBO) from each subject into a mapwise simple regression analysis. This correlation analysis exploited the intersubject variability in NTX behavioral effects to identify differences in NTX effects on the BOLD signal during *Now* versus *Later* choices. Using the ICR change of each subject as a between subjects statistical regressor, we found a significant positive correlation between ICR change and session difference in activity during preference-based decision-making in a constellation of areas (Table 5). Notably, one such site was in the right lateral OFC (Fig. 5A; Table 5), falling within the OFC ROI investigated above. As can be seen in the regression plot of Fig. 5B, the NTX effect on OFC activity during *Now* versus *Later* decisions was inversely correlated with the NTX effect on ICR. In other words, elevated OFC activity on NTX was associated with an increased likelihood of selecting the larger, delayed reward (Fig. 5B).

4. Discussion

4.1. NTX modulation of OFC function

Determining the functional effects of NTX on the human brain is an important step in understanding the bases for variability in its efficacy. This information may not only help predict whether an individual will respond to NTX, it may also help identify which of NTX's actions are critical to its therapeutic effect. This latter information may prove especially valuable in driving the development of new treatments for alcoholism and related disorders, as it could suggest which brain regions are critical therapeutic targets and thus help guide or validate animal experiments. To date, this study is only the second published report about the effects of NTX in the brains of people with a history of alcohol dependence. Here we found that among areas that predict individual bias towards immediate rewards, a single area was modulated by NTX during decision-making: the OFC. Interestingly, the other recent study found that NTX modulates OFC responses to alcohol cues in alcohol dependent subjects (Myrick et al., 2008). Together these findings highlight the OFC as a key brain area affected by NTX.

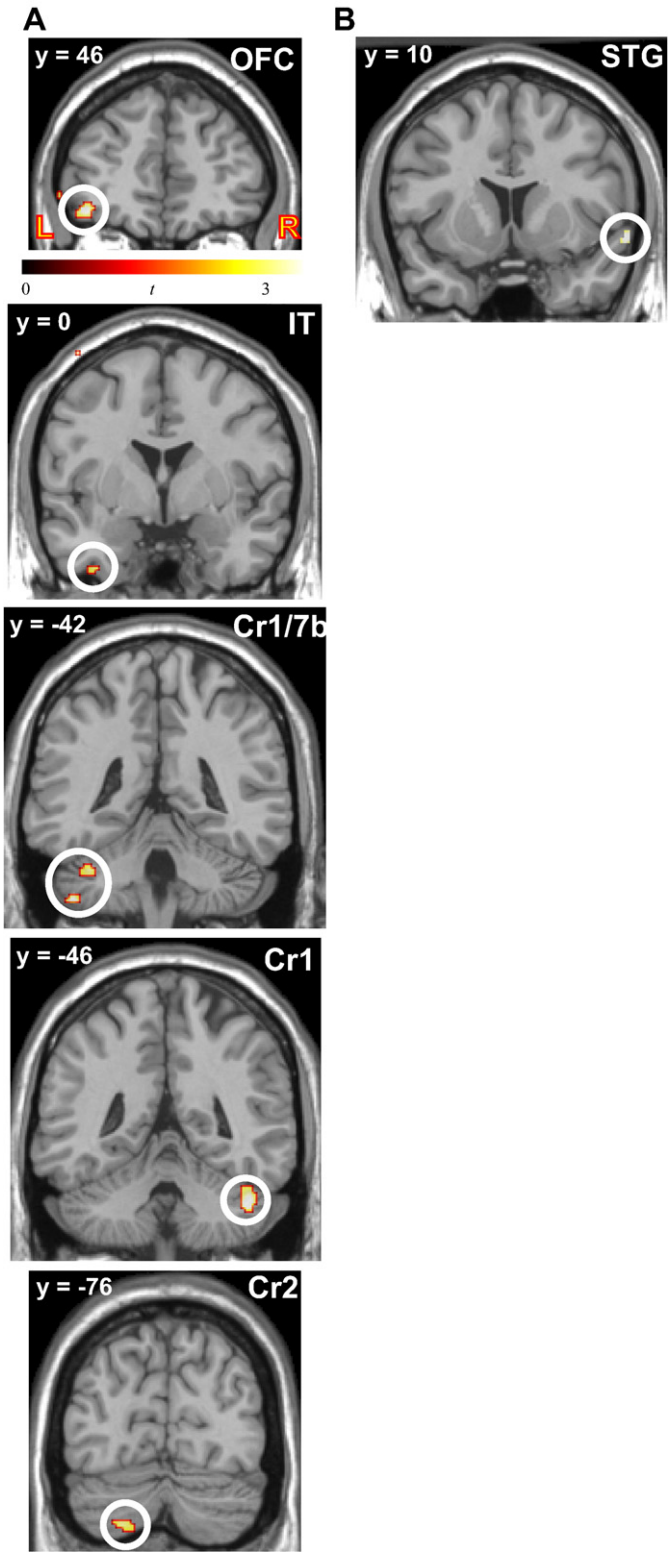

Fig. 4. Mapwise analyses of NTX effects. Statistical non-parametric *t*-maps contrasting [WANT–CON] activity in NTX and placebo sessions, overlaid on a standard T1-weighted anatomical image. Criterion threshold was based on permutation-based voxelwise correction of FWE ($p<0.001$), with a minimum cluster size of 5 contiguous voxels. A) Areas more active during subjective decision-making on NTX. B) The indicated area in the superior temporal gyrus was less active during subjective decision-making on NTX. Cr1, cerebellum crus 1; Cr2, cerebellum crus 2; 7b, cerebellum lobule 7b; IT, inferotemporal cortex; OFC, orbitofrontal cortex; STG, superior temporal gyrus.

Table 4
Peak coordinates for sites of activity modulation by NTX.

Brain region	Hemisphere	MNI coordinates [*x*, *y*, *z*] (mm)
NTX *increased* activity during decision-making		
Lateral orbital gyrus	Left	−40, 46, −11
Inferior temporal gyrus	Left	−37, 0, −41
Cerebellum, crus I	Left	−37, −42, −35
Cerebellum, lobule VIIb	Left	−44, −42, −49
Cerebellum, crus I	Right	45, −46, −35
Cerebellum, crus II	Left	−20, −76, −45
NTX *decreased* activity during decision-making		
Superior temporal gyrus	Right	63, 10, −8

Anatomical region, hemisphere, and peak coordinates based on the Montreal Neurological Institute (MNI) coordinate system value are given.

The OFC is thought to play a critical role in moderating impulsive choice (Mobini et al., 2002; Rudebeck et al., 2006), and in representing subjective value during choice tasks (Izquierdo et al., 2004; Padoa-Schioppa and Assad, 2006; Roesch and Olson, 2004; Schoenbaum and Roesch, 2005). OFC damage impairs the ability of animals to change their responses to stimuli that are no longer reinforced (Dias et al., 1996; McAlonan and Brown, 2003; Mishkin, 1964; Ostlund and Balleine, 2007; Schoenbaum and Roesch, 2005; Schoenbaum et al., 2007; Tait and Brown, 2007). Likewise, patients with damage to the OFC, due either to focal lesions (Rolls et al., 1994) or to degenerative frontotemporal dementia (Rahman et al., 1999), also show impairment in changing their responses when the "rules" for correct responding change. Human neuroimaging studies also support a role for the OFC in overcoming learned response contingencies (Boettiger et al., 2004; Cools et al., 2002; Elliott et al., 2000; O'Doherty et al., 2004; Remijnse et al., 2006).

Such inability to refrain from previously preferred actions in response to formerly rewarding cues that are no longer reinforced is highly reminiscent of the transition from flexible to habitual responding that is characteristic of drug addiction. It should come as no surprise then, that neuroimaging studies have repeatedly found abnormal OFC functioning in substance abusers (Boettiger et al., 2007; Dom et al., 2005; Ersche et al., 2005; London et al., 2000; Volkow and Fowler, 2000). The present data suggest that NTX may support the long-term decision-making critical to recovery from alcoholism by increasing activity in the OFC. Determining whether this biomarker is an addiction vulnerability or relapse risk factor is an important future question.

4.2. Immediate reward bias and endogenous opioids

These data are consistent with our previous finding that blockade of endogenous opioids with NTX can alter immediate reward bias and

Table 5
Peak coordinates for sites in which NTX co-modulated BOLD and ICR.

Brain region	Hemisphere	MNI coordinates [*x*, *y*, *z*] (mm)
NTX BOLD effect inversely related to ICR effect		
Middle frontal gyrus	Right	40, 46, 6
Lateral orbital gyrus	Right	51, 38, −16
Superior temporal pole	Left	−47, 12, −14
Middle temporal pole	Left	−27, 9, −37
Inferior frontal junction	Right	45, 8, 28
Inferior temporal gyrus	Right	50, 4, −38
Middle temporal gyrus	Left	−54, 2, −22
Supplementary motor area	Right	15, 1, 72
Cerebellum, lobule VI	Right	36, −43, −32
	Right	32, −64, −21
NTX BOLD effect directly related to ICR effect		
Middle orbital gyrus	Left	−20, 57, −14
Inferior temporal gyrus	Left	−59, −9, −29
Middle temporal gyrus	Left	−68, −38, 5
Intraparietal sulcus	Left	−44, −45, 61

Anatomical region, hemisphere, and peak coordinates based on the Montreal Neurological Institute (MNI) coordinate system value are given.

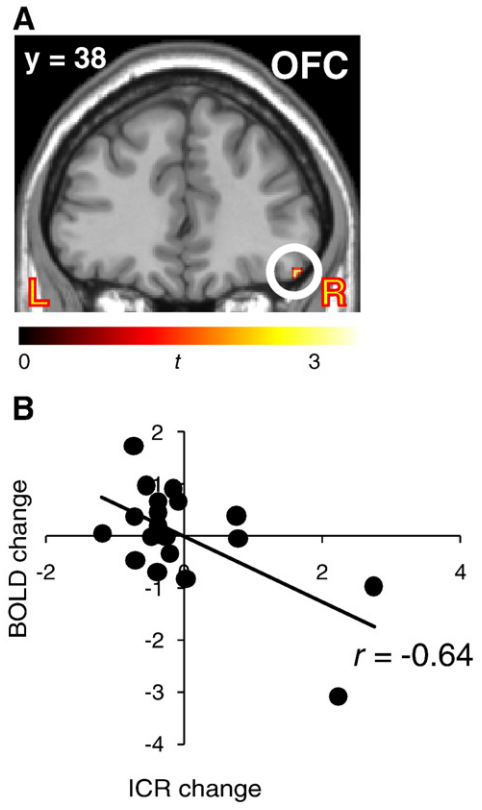

Fig. 5. NTX effect on the OFC predicts NTX effect on choice behavior. Statistical non-parametric *t*-maps depicting results of a mapwise simple regression analysis. A) A site in the OFC is shown in which NTX's effect on activity during decision-making significantly correlated with NTX's effect on choice behavior. B) Plot of each subject's NTX session change in *Now* versus *Later* subjective decision-making parameter estimate as a function of change in ICR for the peak of the region displayed in B; peak regression statistic displayed in plot. Units are in standard deviations, mean = 0.

that this effect is highly variable across individuals (Mitchell et al., 2007). The present results provide new data regarding possible neurobiological sources for this variability. First, we found that a common polymorphism in the BDNF gene predicted individual NTX effect on immediate reward bias. Second, we identified brain regions in which the difference in activity during *Now* versus *Later* decisions between NTX and placebo sessions predicted changes in choice behavior. These areas included the right lateral OFC, an area in which high activity during *Now* versus *Later* decisions predicts *Later* reward selection bias. Consistent with this finding, here we show an association of NTX elevated OFC activity with reduced selection of immediate rewards. The two primary targets of NTX, μ-opioid receptors and κ-opioid receptors, are both expressed at high levels in the OFC (Gorelick et al., 2005; Staley et al., 1997; Zubieta et al., 1999, 1996), thus NTX may be directly mediating its effects on OFC activity via local receptors. However, many of the OFC's inputs, including the dorsal prefrontal cortex, temporal cortex, the amygdala, and the VTA, also show rich expression of opioid receptors (Gorelick et al., 2005; Maurer et al., 1983; Staley et al., 1997; Zubieta et al., 1999, 1996), thus NTX's effect on OFC activity may alternatively be mediated indirectly through one or more of these areas. For example, opioid agonists directly inhibit cortically projecting VTA dopamine neurons in rats (Margolis et al., 2006), thus NTX's actions at opioid receptors in the VTA could increase dopaminergic input to the OFC, resulting in the heightened OFC activity during decision-making that we observed. However, we do not currently know how endogenous opioids that affect the release of DA alter the BOLD signal in the human OFC. Rat studies indicate that whereas μ-opioids in the VTA increase cortical and striatal DA, κ-opioids inhibit striatal dopamine, since NTX blocks both receptors, its effect on cortical DA levels is uncertain (Herz, 1995; Margolis et al., 2006; Spanagel et al., 1992) and could depend on the relative level of μ- versus κ-mediated actions. Relative μ- versus κ-effects would be expected to differ in subjects with low circulating levels of endogenous μ-receptor ligands, such as alcoholics and their off-spring (Dai et al., 2005; del Arbol et al., 1995; Govoni et al., 1983; Vescovi et al., 1992), or in those with low levels of μ-receptor expression, as is found in subjects with low frontal DA levels due to their COMT genotype (Berthele et al., 2005), and in subjects with the A118G polymorphism of the μ-opioid receptor (Zhang et al., 2005). Combining PET imaging of NTX binding with behavioral studies of immediate reward bias shifts may shed light on this issue.

Clinical and pre-clinical data support the hypothesis that opioids regulate immediate reward bias. In rats, NTX reverses morphine induced immediate reward bias (Kieres et al., 2004). In humans, NTX shifts preference from immediate alcohol reward to delayed monetary reward in a lab bar setting (O'Malley et al., 2002). Moreover, NTX attenuates a variety of impulsive behaviors, including pathological gambling (Kim et al., 2001), binge eating in bulimics (Marrazzi et al., 1995), compulsive sexual behavior (Raymond et al., 2002), self-injurious behavior (Symons et al., 2004), and kleptomania (Grant, 2005). Together these results suggest that opioids promote impulsivity, and that opioid receptor blockade via NTX may improve self-control. The fact that immediate reward bias is reduced by acute elevation of DA levels (de Wit et al., 2002; Wade et al., 2000), further suggests that NTX may alter immediate reward bias via indirect effects on DA release, an idea consistent with studies demonstrating opioid regulation of dopamine neurons (Berthele et al., 2005; Margolis et al., 2006; Ostrowski et al., 1982; Sesack and Pickel, 1992).

4.3. BDNF and the endogenous opioid system

The Met allele of the BDNF Val66Met polymorphism is associated with reduced activity-dependent release of BDNF, impaired episodic memory, and reduced Hippocampal function (Egan et al., 2003). It has also been associated with increased anxiety (Chen et al., 2006), increased risk of eating disorders and schizophrenia, and reduced risk of substance abuse disorders (Gratacos et al., 2008). Although endogenous opioids are known to regulate BDNF expression (Zhang et al., 2007; Zhang et al., 2006), it remains unclear how variation in the BDNF system could alter response to endogenous opioid blockade. One recent study however indicates that genetic variability in the BDNF gene alters response to methadone treatment in opioid addicts (de Cid et al., 2008), providing some precedence for variability in opioid system responses based on BNDF genotype.

4.4. Limitations

4.4.1. Sample size

One limitation of the results reported here is the small sample size. This limitation is particularly critical for interpreting the genetic association data. Thus, the finding that BDNF genotype predicts NTX effects on impulsive decision-making should be considered as pilot data, which await confirmation in a larger scale study. It is also critical to acknowledge that our small sample size may have resulted in false negatives with regard to the moderating effects of the other SNPs tested.

4.4.2. Acute dose of NTX

A second limitation of the present study is the use of a single acute dose of NTX. Typical therapeutic use of NTX entails daily dosing, which may result in rather different responses to the drug. However, clinical data demonstrate equivalent or greater efficacy of acute NTX dosing, relative to daily maintenance, in reducing excessive alcohol intake (Hernandez-Avila et al., 2006).

4.4.3. No clinical outcome measures

A final limitation of the present study is the lack of any clinical outcome measures. Thus, we are unable to say whether the NTX effects on decision-making behavior or on brain activity during decision-making are directly correlated with NTX's therapeutic effects. Future studies that incorporate this decision-making task into a clinical trial would shed light on this issue.

4.5. Summary

The present findings identify actions of NTX in the human brain that may contribute to its therapeutic effect in the treatment of alcoholism. Moreover, OFC activity during subjective decisions may be a key therapeutic target for alcoholism medication development. Individual differences in NTX effects on the OFC may contribute to the variable clinical efficacy of NTX. Individual differences in BDNF genotype may also contribute to the variability in NTX's effects on behavior.

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