Positron Emission Tomography Imaging of Mu- and Delta-Opioid Receptor Binding in Alcohol-Dependent and Healthy Control Subjects

Elise M. Weerts, Gary S. Wand, Hiroto Kuwabara, Cynthia A. Munro, Robert F. Dannals, John Hilton, J. James Frost, and Mary E. McCaul

Background: The endogenous opioid system plays a significant role in alcohol dependence. The goal of the current study was to investigate regional brain mu-opioid receptor (MOR) and delta-opioid receptor (DOR) availability in recently abstinent alcohol-dependent and age-matched healthy control men and women with positron emission tomography (PET) imaging.

Methods: Alcohol-dependent subjects completed an inpatient protocol, which included medically supervised withdrawal and PET imaging on day 5 of abstinence. Control subjects completed PET imaging following an overnight stay. PET scans with the MOR-selective ligand [^{11}C]carfentanil (CFN) were completed in 25 alcohol-dependent and 30 control subjects. Most of these same subjects (20 alcohol-dependent subjects and 18 controls) also completed PET scans with the DOR-selective ligand [^{11}C]methylnaltrindole (MeNTL).

Results: Volumes of interest and statistical parametric mapping analyses indicated that alcohol-dependent subjects had significantly higher [^{11}C]CFN binding potential (BP_{ND}) than healthy controls in multiple brain regions including the ventral striatum when adjusting for age, gender, and smoking status. There was an inverse relationship between [^{11}C]CFN BP_{ND} and craving in several brain regions in alcohol-dependent subjects. Groups did not differ in [^{11}C]MeNTL BP_{ND}; however, [^{11}C]MeNTL BP_{ND} in caudate was positively correlated with recent alcohol drinking in alcohol-dependent subjects.

Conclusions: Our observation of higher [^{11}C]CFN BP_{ND} in alcohol-dependent subjects can result from up-regulation of MOR and/or reduction in endogenous opioid peptides following long-term alcohol consumption, dependence, and/or withdrawal. Alternatively, the higher [^{11}C]CFN BP_{ND} in alcohol-dependent subjects may be an etiological difference that predisposed these individuals to alcohol dependence or may have developed as a result of increased exposure to childhood adversity, stress, and other environmental factors known to increase MOR. Although the direction of group differences in [^{11}C]MeNTL BP_{ND} was similar in many brain regions, differences did not achieve statistical significance, perhaps as a result of our limited sample size. Additional research is needed to further clarify these relationships. The finding that alcohol-dependent subjects had higher [^{11}C]CFN BP_{ND} is consistent with a prominent role of the MOR in alcohol dependence.

Key Words: Alcoholism, Abstinence, Brain Imaging, Carfentanil, Naltrindole.

IT IS GENERALLY accepted that the mesocorticolimbic system mediates the rewarding effects of most drugs of abuse including alcohol (Herz, 1998). Within this key region of the brain, the reinforcing effects of alcohol are modulated in part by an increase in the neurotransmission of opioid peptides and dopamine (for review, see Oswald and Wand, 2004). The endogenous opioid peptides (β-endorphin, enkephalins, and dynorphins) bind to different subtypes of the opioid receptor (OR). Specifically, β-endorphin binds with equal affinity to mu-opioid receptor (MOR) and delta-opioid receptor (DOR) subtypes. Enkephalins also bind to MOR and DOR subtypes but show a 20-fold greater affinity for DOR subtypes. β-endorphin and enkephalin opioid peptides increase dopamine neurotransmission within the nucleus accumbens via interactions with the MOR and DOR (Koob et al., 1998).

There is strong evidence supporting an association between the endogenous opioid system and alcohol drinking and reward in humans and laboratory animals (Gianoulakis,

From the Department of Psychiatry and Behavioral Sciences (EMW, GSW, CAM, MEM), The Johns Hopkins University School of Medicine, Baltimore, Maryland; Department of Medicine (GSW, MEM), The Johns Hopkins University School of Medicine, Baltimore, Maryland; Department of Neuroscience (JJF), The Johns Hopkins University School of Medicine, Baltimore, Maryland; and Department of Radiology (HK, RFD, JH, JJF), The Johns Hopkins University School of Medicine, Baltimore, Maryland; and Department of Neuroscience (JJF), The Johns Hopkins University School of Medicine, Baltimore, Maryland.

Received for publication December 7, 2010; accepted March 23, 2011.

Reprint requests: Elise M. Weerts, PhD, Johns Hopkins Bayview, BBRC, 5510 Nathan Shock Dr., Suite 3000, Baltimore, MD 21224; Tel.: 410-550-2781; Fax: 410-550-2780; E-mail: eweerts@jhmi.edu

DOI: 10.1111/j.1530-0277.2011.01565.x

2004). In laboratory animals, OR antagonists decrease alcohol consumption (Franck et al., 1998; Froehlich, 1995; Froehlich et al., 1991; June et al., 1999; Krishnan-Sarin et al., 1995a,b, 1998) and block alcohol-induced activation of the dopamine system (Benjamin et al., 1993; Job et al., 2007). MOR knockout mice self-administer alcohol at lower levels when compared to wild-type controls (Becker et al., 2002; Hall et al., 2001; Roberts et al., 2000). In 2 related human laboratory studies (McCaul et al., 2000, 2001), naltrexone significantly attenuated alcohol-induced increases in liking and best effects, heart rate and diastolic blood pressure, and neuroendocrine responses. These findings have been replicated (Peterson et al., 2006). Taken together, the above studies highlight the importance of the opioid system in the reward pathway for alcohol and provided support for the use of OR antagonists in the treatment for alcohol dependence. Indeed, meta-analyses of randomized clinical trials have demonstrated that the OR antagonist naltrexone has an overall small to moderate effect size in reducing drinking and relapse in alcohol-dependent subjects (Anton and Swift, 2003; Srisurapanont and Jarusuraisin, 2005).

Given the evidence for a functional involvement of the endorphin and enkephalin systems in alcohol drinking and dependence, it is highly likely that the opioid system is altered in human alcoholics. Positron emission tomography (PET) is the only technique available for examining brain receptor characteristics in living human subjects. Three PET imaging studies available to date showed conflicting results on OR in alcohol-dependent men. In the first study (Bencherif et al., 2004), MOR were lower in the right dorsal lateral prefrontal cortex, the right anterior frontal cortex, and right parietal cortex in 8 recently detoxified alcohol-dependent men when compared with 8 normal healthy men. The second study (Heinz et al., 2005) found an increase in MOR in the ventral striatum in 25 recently abstinent (1 to 3 weeks) alcohol-dependent men when compared with 10 healthy control men. A third PET study (Williams et al., 2009) examined OR in 11 alcohol-dependent and 13 healthy control men using the nonselective OR ligand [^{11}C]diprenorphine, which binds to all 3 OR subtypes. Subjects were scanned while undergoing an outpatient detoxification with chlordiazepoxide after 2 to 4 weeks of self-reported alcohol abstinence. In this study, global and regional [^{11}C]diprenorphine volumes of distribution were higher in alcohol-dependent patients when compared with controls, although this effect was not statistically significant.

The current study therefore assessed binding characteristics of MOR and DOR with PET using [^{11}C]-carfentanil (CFN), a MOR ligand, and [^{11}C]-methylnaltrindole (MeNTL), a DOR ligand, in recently abstinent alcohol-dependent and age-matched healthy control subjects. Alcohol-dependent subjects were admitted to the clinical research unit (CRU), completed medically supervised withdrawal, and completed PET scans on day 5, after withdrawal symptoms had subsided. Control subjects completed PET scans after an overnight stay on the CRU. Two types of analyses were utilized, volumes of interest (VOI) and statistical parametric mapping (SPM) analysis.

MATERIALS AND METHODS

Subjects

Current, heavy alcohol-dependent drinkers and healthy control subjects between 21 and 60 years of age were recruited via advertisement and provided informed consent, in the sober state, using an Institutional Review Board-approved informed consent document. Subjects were interviewed by a masters-level research assistant who utilized a battery of standardized diagnostic and psychological instruments. For inclusion in the study, alcohol-dependent subjects met DSM-IV criteria for alcohol dependence based on the Semi-Structured Assessment of the Genetics of Alcoholism (Bucholz et al., 1994) and were actively drinking at NIAAA-defined hazardous levels as determined by completion of a 90-day Time Line Follow Back (TLFB) (Sobell and Sobell, 1992). Healthy control subjects did not drink above the NIAAA-recommended guidelines (<8 drinks/week for women and <15 drinks/week for men) and had never meet DSM-IV criteria for either alcohol abuse or alcohol dependence. Healthy control subjects were age-matched with alcohol-dependent participants. Alcohol-dependent and healthy control subjects were excluded from study participation based any on the following criteria: (i) if they met current or lifetime DSM-IV diagnostic criteria for any other Axis I disorder, including other drug abuse/dependence (except nicotine), (ii) if urine drug toxicology was positive at screening or hospital admission, (iii) if they had other ongoing health problems, or (iv) if their mother drank during pregnancy, subject was excluded from further study participation. Alcohol-dependent subjects were excluded if they reported alcohol-related seizures or the need for medication during previous detoxifications. Using these inclusion and exclusion criteria, a total of 25 alcohol-dependent subjects and 30 healthy control subjects completed the protocol. Basic demographic characteristics for alcohol-dependent and healthy control subjects are shown in Table 1.

The Alcohol Dependence Scale (ADS) (Skinner and Allen, 1982) was administered to characterize alcohol use and associated problems. The Fagerstrom Nicotine Dependence Test (FNDT) was administered to determine nicotine dependence status in individuals who smoked cigarettes. Scores for each of these assessments are shown in Table 1. The Family History Assessment Module (Rice et al., 1995) was completed to determine the number of first- and second-degree relatives with symptoms of alcohol and drug abuse or dependence. Subjects were classified as family history positive (FHP) if at least 3 diagnostic criteria for alcohol dependence were met by either parent (father or mother) or 3 or more other first- or second-degree relatives. If mother drank during pregnancy, subject was excluded from further study participation. Subjects were classified as family history negative (FHN) if they reported (i) no first-degree relative who met alcohol dependence criteria and (ii) no or 1 second-degree relative who met alcohol dependence criteria. Subjects were designated as family history undetermined (FHU) who did not meet criteria for FHP or FHN, had multiple relatives with drug problems but no alcohol problems, or could not provide sufficient information on alcoholism status of relatives.

Inpatient Procedures Following Admission to CRU

Healthy control subjects completed PET imaging following an overnight stay in the hospital or under an inpatient protocol. Alcohol-dependent subjects completed the study under an inpatient protocol that included hospital admission and medically supervised alcohol withdrawal prior to PET imaging on day 5 of supervised abstinence. Alcohol-dependent subjects remained on the CRU for subsequent naltrexone treatment (50 mg per day) and PET imaging to determine naltrexone blockade of mu and delta receptors. The methodology and results for [^{11}C]CFN binding potential (BP_{ND}) in the context of naltrexone treatment in 21 of 25 alcohol-dependent subjects were reported in a separate paper (Weerts et al., 2008). The

Table 1. Demographics for AD and HC Subjects

	AD (n = 25)	HC (n = 30)
Mean years of age (SD)	43.8 (7.4)	43.5 (9.4)
Gender (n)		
Male	18	20
Female	7	10
Race (n)		
Caucasian	15	16
Black	10	14
Family history of alcoholism (n)		
FHP	14	9
FHN	10	16
FHU	1	5
Smoking status (n)		
Nonsmokers	5	21
Smokers	20	9
Nicotine dependent (DSM-IV)	13	5
No nicotine dependence (DSM-IV)	7	4
Smoking measures in smokers only: mean (SD)		
Peak number of cigarettes/d	17.7 (10.3)*	15.1 (5.5)
Years of cigarette use	18.3 (11.3)	19.3 (9.0)
Fagerstrom score	4.4 (2.5)	4.4 (2.3)
Age of 1st cigarette use	15.4 (5.6)	16.6 (9.2)
Alcohol-related measures: mean (SD)		
Age met criteria for alcohol dependence	28.7 (7.1)	N/A
Years of dependent alcohol drinking	15.3 (9.2)	N/A
ADS score	19.6 (6.7)*	0.3 (0.7)
Number of drinks per drinking day	12.4 (6.5)*	1.6 (1.6)
Number of drinking d/wk	5.5 (1.4)*	0.7 (1.3)
Peak Penn Alcohol Craving VAS days 1–5	20.1 (6.9)	N/A
Peak Alcohol Craving	23.4 (7.9)	N/A
Peak CIWA days 1–5	5.0 (2.6)	N/A
Peak OCDS days 1–5	28.1 (10.1)	N/A
Pre-PET Penn Alcohol Craving	7.8 (7.3)*	0.2 (0.6)
Pre-PET Alcohol Craving VAS	9.2 (10.6)*	0.1 (0.4)
Pre-PET CIWA	0.6 (1.5)	0.2 (0.7)
Pre-PET OCDS	15.6 (13.1)*	1.1 (2.0)
Psychological assessments on admission: mean (SD)		
BDI-II	12.8 (9.6)*	1.0 (1.4)
BAI	9.6 (8.1)*	1.1 (2.1)
BSI	0.5 (0.6)*	0.1 (0.1)

Data shown are group means (SD) or number (n) of subjects as indicated.

*Significant t-test comparison between AD and HC groups ($p \leq 0.0001$).

AD, alcohol-dependent subjects; HC, healthy control subjects; FHP, family history positive; FHN, family history negative; FHU, family history undetermined; DSM, Diagnostic and Statistical Manual of Mental Disorders; ADS, Alcohol Dependence Scale; VAS, Visual Analog Scale; CIWA, Clinical Institute Withdrawal Assessment; OCDS, Obsessive Compulsive Drinking Scale; BDI-II, Beck Depression Inventory; BAI, Beck Anxiety Inventory; BSI, Brief Symptom Inventory.

analysis of the basal scan data in the control subjects and comparison with basal scan data in the alcohol-dependent subjects are unique to the current article.

Following admission to the CRU and regularly throughout their hospital stay, subjects completed a second battery of psychological assessments, which included a Visual Analog Scale (VAS) of alcohol craving, Penn Craving Scale (Flannery et al., 1999), the Obsessive Compulsive Drinking Scale (OCDS) (Anton et al., 1996), Beck Depression Inventory (BDI-II) (Beck et al., 1996), the Beck Anxiety Inventory (BAI) (Beck et al., 1988), and the Brief Symptom Inventory (BSI) (Derogatis and Melisaratos, 1983).

To monitor the severity of withdrawal symptoms, CRU nursing staff completed the Clinical Institute Withdrawal Assessment—Alcohol Revised (CIWA-Ar) (Sullivan et al., 1989) with alcohol-dependent participants 3 times each day for the first 5 days. CIWA-Ar items were scored to reflect the time period since the last measurement. No subject required withdrawal medication based on CIWA scores, vital signs, and physician assessment.

During hospitalization and all study procedures, cigarette smoking was prohibited. Smokers who were nicotine dependent as determined by an FNDT score of 3 or higher received a new transdermal nicotine patch (21 mg nicotine) at the time of hospital admission, in the morning of each day while on the CRU and 3 hours prior to the PET scan. This standardized approach was used to limit the possible impact of nicotine withdrawal on the day of the scan. All subjects received a calorie-controlled breakfast 3 hours before the first scan. Before PET procedures and randomly during the hospital stay, urine toxicology screens and breath alcohol tests were conducted in all subjects to verify abstinence from alcohol and drugs.

PET Procedures

Subjects underwent 2 PET scans in a fixed order on the same day; the [^{11}C]MeNTL, a specific DOR antagonist (Lever et al., 1992; Madar et al., 1996), and [^{11}C]CFN, a specific MOR agonist (Frost et al., 1985; Titeler et al., 1989), scans were conducted at 8:30 and 10:45 AM, respectively. A total of 25 alcohol-dependent subjects and 24 healthy control subjects completed [^{11}C]CFN scans concurrently between July 2001 and July 2008. Among these subjects who completed the [^{11}C]CFN scan, 20 alcohol-dependent and 18 healthy controls completed the [^{11}C]MeNTL scan. Specifically, [^{11}C]MeNTL scans were lost owing to problems with the arterial line and blood sampling ($N = 4$ healthy control and $N = 3$ alcohol-dependent subjects) and failure of radioligand synthesis ($N = 2$ alcohol-dependent and $N = 2$ healthy control subjects). Six additional healthy controls who were smokers without alcohol problems were recruited specifically to control for possible effects of smoking on [^{11}C]CFN BP_{ND} and completed [^{11}C]CFN scans between April 2009 and February 2010; these subjects also did not complete the [^{11}C]MeNTL scans. The decrease in subjects that completed the [^{11}C]MeNTL did not alter the demographic distributions shown in Table 1. Alcohol-dependent subjects were about 44 years old, mostly men ($n = 15$) and Caucasian ($n = 12$), with 55% ($n = 11$) reporting a positive family history of alcoholism. Healthy controls were also about 44 years old, mostly men ($n = 11$) and Caucasian ($n = 10$), with 28% ($n = 5$) reporting a positive family history of alcoholism.

PET scans were acquired in 3D mode on a GE Advance PET scanner (GE Medical Systems, Milwaukee, WI). A thermoplastic mask was individually fitted to each subject's face for immobilization and positioning during imaging. A transmission scan of 10-minutes duration was obtained using rotating germanium-68 rods before the injection of the radiotracer. After intravenous bolus administration of the radiotracer, a set of 25 images with variable time period (6×30, 5×60, 5×120, 9×480 seconds) was acquired during a 90-minute period for each study. Table 2 shows the injected dose, specific activity, and mass for alcohol-dependent and control subjects. During [^{11}C]MeNTL scans, small amounts of arterial blood samples were collected every 5 seconds initially, and then, increasing intervals throughout the scan (e.g., 1, 2, 5, 10, and 15 minutes), counted for the radioactivity. Selected samples were analyzed with high performance liquid chromatography for metabolites (Hilton et al., 2000). PET images were reconstructed using the back-projection algorithm with a ramp filter using the software provided by the manufacturer correcting for attenuation, scatter, and dead time (Kinahan and Rogers, 1989). The radioactivity was corrected for physical decay to the injection time. Each PET frame consisted of a $128 \times 128 \times 35$ matrix with voxel size of $2 \times 2 \times 4.25$ mm in a spatial resolution of 5.5 and 6.1 mm full width at half maximum (FWHM) in the radical and tangential directions, respectively, at 10 cm radius from the center of the field-of-view (Lewellen et al., 1996).

Table 2. Mean and Standard Deviation (SD) of Drug, Injected Specific Activity, Body Weights (BW), and Mass of (a) [^{11}C]CFN and (b) [^{11}C]MeNTL in Alcohol-Dependent (AD) and Healthy Control (HC) Groups

(a) [^{11}C]CFN	AD (N = 25)		HC (N = 30)	
	Mean	SD	Mean	SD
μg CFN	0.82	0.70	0.63	0.47
Inj. Activity (mCi)	19.30	2.23	19.99	3.09
mCi/μmole	18,444.1	14,248.0	24,955.5	24,467.2
BW (kg)	74.7	13.4	79.3	13.8
Dose (μg/kg)	0.011	0.009	0.008	0.006
(b) [^{11}C]MeNTL	AD (N = 20)		HC (N = 18)	
	Mean	SD	Mean	SD
μg MeNTL	2.48	1.29	2.40	1.76
Inj. Activity (mCi)	18.87	3.07	17.52	2.19
mCi/μmole	4,719.6	3,054.8	5,050.4	3,981.3
BW (kg)	73.9	13.4	76.3	13.1
Dose (μg/kg)	0.033	0.030	0.035	0.019

t-Test comparison between AD and HC groups indicated no significant differences (all $p > 0.15$).

About 1 week before CRU admission, subjects underwent magnetic resonance imaging (MRI) to allow anatomical localization and alignment of PET imaging planes within subjects (Meltzer et al., 1990).

VOI Analyses

VOI were limited in this study to the ventral striatum, cingulate cortex, caudate nucleus, putamen, insula, globus pallidus, thalamus, and amygdala. The VOI were manually defined on spoiled gradient-recalled echo MRI for putamen, caudate nucleus, and thalamus using a locally developed VOI-defining tool. The ventral striatum was defined as described previously (Baumann et al., 1999; Martinez et al., 2003; Oswald et al., 2005). For the remaining VOI, a standard VOI template (Hammers et al., 2003; Mazziotta et al., 1995; available at http://www.loni.ucla.edu) was spatially transferred to individual subjects' MRI, using the MRI-to-MRI spatial normalization parameters obtained with the SPM spatial normalization module Ashburner and Friston, 2004; available at http://www.fil.ion.ucl.ac.uk/spm), and edited to suit outlines of the structures given by the MRI. Those VOI were transferred to PET space according to the MRI-to-PET coregistration parameters obtained with the SPM2 coregistration module and applied to individual PET frames to obtain time–radioactivity curves of VOI.

Pharmacokinetic Modeling

The primary outcome variable of interest for MOR and DOR was BP_{ND} (Innis et al., 2007) of [^{11}C]CFN and [^{11}C]MeNTL, respectively. For [^{11}C]CFN, the reference tissue graphical analysis (RTGA) (Logan et al., 1996) was used, with occipital lobe as the reference region and setting the brain-to-blood clearance rate constant of the reference region (k_2^R) at 0.104 per minute (Endres et al., 2003; Frost et al., 1990). Estimates of BP_{ND} using RTGA have been shown to be highly correlated with those obtained from the arterial input-based kinetic model (Endres et al., 2003). The analyses for [^{11}C]MeNTL differed from that used for [^{11}C]CFN. First, we analyzed data using a 2-tissue compartmental model (TTCM). It was determined that, although TTCM fit the data well, there were occasional outliers (< 10% of regions). We then examined data using the plasma reference graphical analysis (PRGA) (Logan et al., 1990) to obtain distribution volume (V_T); [^{11}C]MeNTL BP_{ND} was obtained as target reference V_T ratio less 1. Regional [^{11}C]MeNTL BP_{ND} values of PRGA correlated with those of TTCM (TTCM = 0.94 PRGA + 0.15; $R_2 = 0.601$) without yielding apparent outliers. For this reason, PRGA was selected for [^{11}C]MeNTL. Reference Logan plots for [^{11}C]CFN approached linear starting 10 minutes after the injection, as described previously (Zubieta et al., 2001). Plasma Logan plots for [^{11}C]MeNTL approached linear in all regions examined by 20 minutes in all subjects. t^* was set at 20 minutes for both ligands.

VOI Statistical Analyses

All statistical analyses were carried out using SAS version 9.2 (SAS Institute Inc., Cary, NC). Possible differences in regional binding of [^{11}C]CFN and [^{11}C]MeNTL between groups (alcohol-dependent and healthy control subjects) were determined using independent analyses of covariance (ANCOVAs) for each of the 8 brain volumes. We included both age and gender as covariates in the model because both have been shown to influence [^{11}C]CFN BP_{ND} (Zubieta et al., 1999). Smoking status was added as covariate in a secondary analysis, based on findings as indicated in the results. Independent ANCOVAs for each brain volume were performed over a model including the VOI as an independent variable because of nonhomogenous variance between VOI. The adaptive step-down Bonferroni adjustment (Hochberg and Benjamini, 1990), which is based on the Bonferroni-Holm (Holm, 1979) approach, was applied to correct for multiple comparisons. The unadjusted p-values and the adjusted p-values (shown as Q) are reported. The association of regional binding of [^{11}C]CFN and [^{11}C]MeNTL with family history of alcoholism, psychological problems (BDI, BAI, BSI scores), craving (OCDS, VAS, and Penn Craving Scale scores), recent drinking from the TLFB (drinks per episode, episodes per week, total drinks), and alcohol withdrawal severity (CIWA scores) were each analyzed as independent ANCOVAs. The adaptive step-down Bonferroni adjustment (Hochberg and Benjamini, 1990) was applied to correct for multiple comparisons (i.e., all 8 VOI), and the adjusted p-values (shown as Q) are reported.

SPM Analyses

SPM analyses were conducted to corroborate VOI findings and determine whether the regional increases in [^{11}C]CFN BP_{ND} in alcohol-dependent subjects were more generalized and extended to other regions not examined in our VOI analysis. Functional volumes (voxel-by-voxel maps) of BP_{ND} were spatially normalized to a standard brain using MRI-to-MRI spatial normalization and PET-to-MRI coregistration parameters using SPM5 modules and smoothed with a Gaussian filter of 12 mm FWHM. Parametric statistical models are assumed at each voxel, using the general linear model. Additional analysis was conducted controlling for current smoking status as a nuisance variable. To reduce chances of false positives, the search volume was limited to gray-matter voxels to eliminate white-matter clusters. Voxel-wise statistical tests were performed to examine the differences in BP_{ND} between alcohol-dependent and healthy control subjects. A significance level of $p < 0.05$, family-wise error corrected, was employed for the group difference ($t > 4.6$).

RESULTS

VOI Analysis of Covariance of Alcohol-Dependent Versus Healthy Control Subjects

When controlling for age and gender, alcohol-dependent subjects had higher [^{11}C]CFN BP_{ND} than healthy control subjects across all VOI. This effect was highly significant across regions [amygdala (p and $Q = 0.004$), cingulate (p and $Q = 0.002$), insula, ventral striatum, caudate, globus

pallidus, putamen, and thalamus (all p and $Q < 0.001$)]. In contrast, [^{11}C]MeNTL BP_{ND} did not differ between alcohol-dependent and healthy controls subjects in any region. The mean ($\pm$SD) V_T of cerebellum was not different between alcohol-dependent subjects (7.75 $\pm$ 1.57 ml/ml) and healthy control subjects (8.0 $\pm$ 1.53 ml/ml; $t = 0.47$; df = 36; $p = 0.638$).

ANCOVA also confirmed an overall effect for gender on [^{11}C]CFN BP_{ND}. When compared with men, women had lower mean [^{11}C]CFN BP_{ND} in cingulate (0.74 $\pm$ 0.02 vs. 0.65 $\pm$ 0.3, p and $Q = 0.01$) and ventral striatum (1.76 $\pm$ 0.06 vs. 1.47 + 0.08, p and $Q = 0.005$). Women also had a trend toward higher in [^{11}C]MeNTL BP_{ND} in amygdala than men (0.91 $\pm$ 0.08 vs. 0.64 $\pm$ 0.06, $p = 0.009$, $Q = 0.07$).

Adding smoking as a covariate did not change the increases in [^{11}C]CFN BP_{ND} in alcohol-dependent when compared with healthy control subjects. Alcohol-dependent subjects had significantly higher [^{11}C]CFN BP_{ND} when compared with healthy control subjects in amygdala (p and $Q = 0.002$), cingulate, insula, ventral striatum, caudate, globus pallidus, putamen, and thalamus (all p and $Q < 0.001$). Figure 1 shows mean [^{11}C]CFN BP_{ND} adjusted for age, gender, and smoking status. As shown in Fig. 1, the mean difference in [^{11}C]CFN BP_{ND} between groups was greatest in the globus pallidus and ventral striatum. The greater [^{11}C]CFN BP_{ND} in alcohol-dependent compared with healthy control subjects can be seen clearly in the averaged [^{11}C]CFN BP_{ND} images shown at the level of ventral striatum in Fig. 2. As shown in Fig. 3, although the direction of effects in several brain regions was similar to that observed for [^{11}C]CFN BP_{ND}, mean [^{11}C]MeNTL BP_{ND} was not significantly different in alcohol-dependent and healthy control subjects when adjusted for age, gender, and smoking status. When adjusting for age, gender, and group (alcohol-dependent vs. control), [^{11}C]CFN BP_{ND} did not differ between smokers ($n = 29$) and nonsmokers ($n = 26$) except that smokers had lower [^{11}C]CFN BP_{ND} in the globus pallidus (Table 3). When adjusting for age and gender, [^{11}C]MeNTL BP_{ND} did not differ between smokers ($n = 18$) and nonsmokers ($n = 20$) in any of the VOI (data not shown). Table 4*a,b*, respectively, show mean [^{11}C]CFN BP_{ND} and [^{11}C]MeNTL BP_{ND} in the 8 VOI in alcohol-dependent and healthy control subjects when adjusted for age, gender, and smoking.

SPM Analyses of Alcohol-Dependent Versus Healthy Control Subjects

SPM analysis of [^{11}C]CFN BP_{ND} in alcohol-dependent and healthy control subjects confirmed that alcohol-dependent subjects had higher [^{11}C]CNF BP_{ND}. The addition of smoking status as a nuisance variable in the contrast analysis of alcohol-dependent and healthy control subjects did not change this result. Specifically, group differences were identified as 2 large, symmetrical volumes of 218 ml (left) and 222 ml (right), which peaked at thalamus (Fig. 4). The x, y, z coordinates were 20, −12, 4 (Peak $T = 7.5$) for left and −22, 12, 6 (Peak $T = 7.49$) for right volumes, respectively (Fig. 4). There were no differences between alcohol-dependent and healthy control subjects in [^{11}C]CNF BP_{ND} in para-sagittal areas.

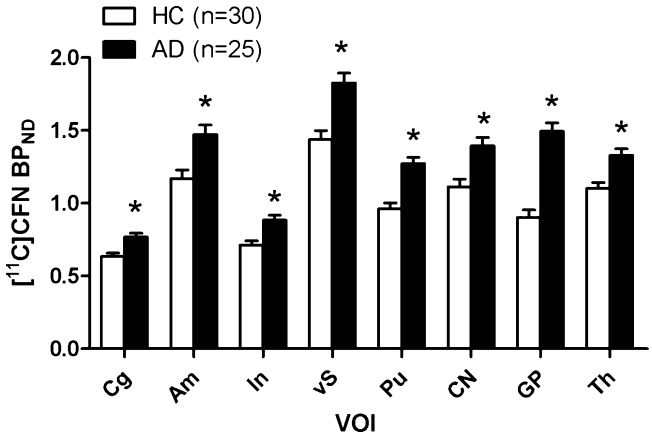

Fig. 1. [^{11}C]CFN BP_{ND} in alcohol-dependent (AD) versus healthy control (HC) subjects. Bars are the mean ± SEM [^{11}C]CFN BP_{ND} adjusted for age, gender, and smoking for cingulate (Cg), amygdala (Am), insula (In), ventral striatum (vS), putamen (Pu), caudate nucleus (CN), globus pallidus (GP), and thalamus (Th). The asterisks above a volumes of interest (VOI) indicate the difference in [^{11}C]CFN BP_{ND} between AD and HC subjects, which was significant as determined by Bonferroni-Holm post hoc tests (all VOI shown, $p \leq 0.004$).

In contrast, consistent with the VOI analysis, SPM analysis did not reveal any significant differences in [^{11}C]MeNTL BP_{ND} between alcohol-dependent and healthy control subjects.

Secondary Analyses of Impact of Severity of Alcohol Dependence and Drinking History

As shown in Table 1, alcohol-dependent subjects scored significantly higher than healthy controls on measures of alcohol dependence severity (ADS score) and recent alcohol drinking recorded on the TLFB (drinks per drinking day, drinks per week, total drinks last 90 days; all $p < 0.0001$, t-test). There was a significant positive association of recent drinking and [^{11}C]MeNTL BP_{ND} in the caudate [mean drinks per drinking day (coefficient 20.4, $p = 0.001$, $Q = 0.003$), mean drinks per week (coefficient 15.5, $p = 0.002$, $Q = 0.01$), and total number of drinks (coefficient 15.8, $p = 0.001$, $Q = 0.01$)]. There was, however, no association of drinking reported in the 90-day TLFB and [^{11}C]CFN BP_{ND} in any of the VOI. [^{11}C]CFN BP_{ND} and [^{11}C]MeNTL BP_{ND} were also not significantly associated with ADS score, age first met criteria for alcohol dependence, or years of heavy drinking in alcohol-dependent subjects.

During the inpatient protocol, moderate alcohol withdrawal symptoms, as determined by peak scores on the CIWA-AR across days 1 to 5 postadmission, were observed and ranged from 1 to 12 (mean peak score = 5 $\pm$ 2.6 SD) for alcohol-dependent subjects. Alcohol withdrawal symptoms had

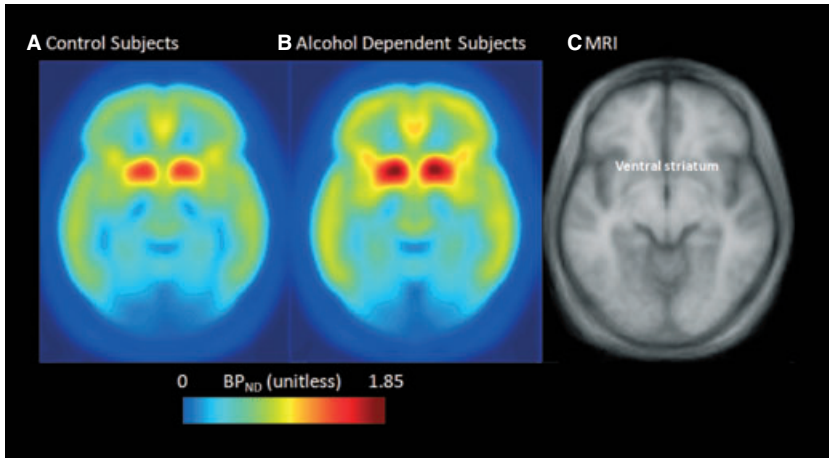

Fig. 2. Mean [^{11}C]CFN BP_{ND} images in control (a) and alcohol-dependent subjects (b). Colored legend depicts [^{11}C]CFN BP_{ND} from 0 (light blue) to 1.85 (red). Mean magnetic resonance imaging image of all subjects is shown (c). Images are taken at the level of ventral striatum.

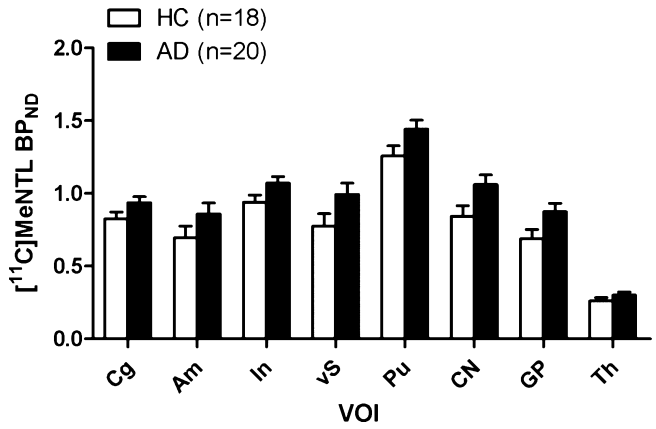

Fig. 3. [^{11}C]MeNTL BP_{ND} in alcohol-dependent (AD) versus healthy control (HC) subjects. Bars are the mean ± SEM [^{11}C]MeNTL BP_{ND} adjusted for age, gender, and smoking for cingulate (Cg), amygdala (Am), insula (In), ventral striatum (vS), putamen (Pu), caudate nucleus (CN), globus pallidus (GP), and thalamus (Th). There were no significant differences in [^{11}C]MeNTL BP_{ND} between AD and HC subjects in any of the volumes of interest (VOI) shown.

Table 3. Effects of Smoking Status on Mean [^{11}C]CFN BP_{ND}

	Smoker (N = 29)		Nonsmoker (N = 26)			
VOI	Mean	SEM	Mean	SEM	p	Q(Holm)
Cingulate	0.670	0.025	0.732	0.025	0.099	0.099
Amygdala	1.261	0.063	1.382	0.064	0.204	0.204
Insula	0.781	0.030	0.817	0.031	0.432	0.432
Ventral striatum	1.590	0.065	1.673	0.066	0.396	0.396
Putamen	1.066	0.042	1.169	0.043	0.107	0.107
Caudate	1.206	0.055	1.302	0.056	0.243	0.243
Globus pallidus	**1.016**	**0.056**	**1.380**	**0.058**	**<0.001**	**<0.001**
Thalamus	1.189	0.043	1.243	0.044	0.400	0.400

Data shown are group means and SEM with p-values for smokers and nonsmokers adjusted for age, gender, and group (alcohol-dependent vs. control) for each volumes of interest (VOI). Q-values show adjusted p-values using the step-down Bonferroni-Holm adjustment (Hochberg and Benjamini, 1990) to correct for multiple comparisons. Significant VOI with adjusted $p < 0.05$ are highlighted in bold type.

subsided by day 5 (mean CIWA score on day 5 = 0.44 + 1.1 SD) when PET scans were conducted. CIWA scores on the day of the PET scans did not differ between alcohol-dependent and healthy control groups (Table 1). When controlling for smoking status, age, and gender, neither peak CIWA across days 1 to 5 nor prescan CIWA scores predicted [^{11}C]CFN BP_{ND} or [^{11}C]MeNTL BP_{ND}.

Secondary Analyses of Family History of Alcoholism

Because family history is a known risk factor for alcoholism, we examined whether family history predicted [^{11}C]CFN BP_{ND} or [^{11}C]MeNTL BP_{ND}. We adjusted for age, gender, smoking status, and group in the analyses comparing FHP and FHN subjects. FHP subjects (n = 23) did not differ from FHN subjects (n = 26) in [^{11}C]CFN BP_{ND} in any of the VOI ($p > 0.09$, $Q > 0.8$). When stratified by group, alcohol-dependent FHP subjects (n = 14) did not differ from FHN subjects (n = 10) in [^{11}C]CFN BP_{ND} in any of the VOI (all $p > 0.1$, all $Q > 0.2$). Likewise, healthy control FHP subjects (n = 9) did not differ from FHN subjects (n = 16) in [^{11}C]CFN BP_{ND} in any of the VOI ($p > 0. .2$, Q = 1.0). When compared with FHN subjects (n = 19), FHP subjects (n = 16) had a trend toward lower mean [^{11}C]MeNTL BP_{ND} in the insula (FHN: 0.95 ± 0.04 vs. FHP:1.07 ± 0.04, p = 0.04, Q = 0.073). Using the observed group means and standard deviations, we then completed a sample size analysis to detect an effect with 0.90% power. A sample size of 70 (or 35 in each group) was estimated for p = 0.05 in insula. When stratified by group, alcohol-dependent FHP subjects (n = 11) did not differ from FHN subjects (n = 8) in any of the VOI ($p > 0.1$, Q = 1.0), and healthy control FHP subjects

Table 4. (a) Mean [^{11}C]CFN BP_{ND} and (b) Mean [^{11}C]MeNTL BP_{ND} in Alcohol-Dependent (AD) and Healthy Control (HC) Subjects

(a) [^{11}C]CFN BP_{ND}	AD (N = 25)		HC (N = 30)				
VOI	Mean	SEM	Mean	SEM	F	p	Q(Holm)
Cingulate	**0.768**	**0.026**	**0.634**	**0.023**	**13.787**	**0.001**	**0.001**
Amygdala	**1.472**	**0.066**	**1.170**	**0.060**	**10.897**	**0.002**	**0.002**
Insula	**0.885**	**0.032**	**0.713**	**0.029**	**15.379**	**<0.001**	**<0.001**
Ventral striatum	**1.826**	**0.068**	**1.438**	**0.061**	**17.106**	**<0.001**	**<0.001**
Putamen	**1.272**	**0.044**	**0.962**	**0.040**	**25.716**	**<0.001**	**<0.001**
Caudate	**1.395**	**0.057**	**1.113**	**0.052**	**12.677**	**0.001**	**0.001**
Globus pallidus	**1.494**	**0.059**	**0.902**	**0.053**	**52.052**	**<0.001**	**<0.001**
Thalamus	**1.329**	**0.045**	**1.102**	**0.041**	**13.201**	**0.001**	**0.001**
(b) [^{11}C]MeNTL BP_{ND}	AD (N = 20)		HC (N = 18)				
VOI	Mean	SEM	Mean	SEM	F	p	Q(Holm)
Cingulate	0.935	0.042	0.826	0.046	2.389	0.132	0.132
Amygdala	0.859	0.075	0.694	0.082	1.738	0.196	0.196
Insula	1.070	0.045	0.939	0.050	3.038	0.091	0.091
Ventral striatum	0.993	0.078	0.776	0.085	2.835	0.102	0.102
Putamen	1.442	0.063	1.258	0.069	3.103	0.087	0.087
Caudate	1.061	0.067	0.843	0.073	3.846	0.058	0.058
Globus pallidus	0.874	0.059	0.687	0.065	3.677	0.064	0.064
Thalamus	0.300	0.022	0.261	0.024	1.108	0.300	0.300

Data shown are group means and SEM, F and p values adjusted for age, gender, and smoking for each volumes of interest (VOI). Q(Holm) values show adjusted p values using the step-down Bonferroni-Holm adjustment (Hochberg and Benjamini, 1990) to correct for multiple comparisons. Significant VOI with adjusted $p < 0.05$ are highlighted in bold type.

($n = 5$) did not differ from FHN subjects ($n = 11$) in any of the VOI ($p > 0.08$, $Q > 0.3$).

Secondary Analyses on Impact of Psychological Problems and Alcohol Craving

As shown in Table 1, alcohol-dependent subjects reported significantly more symptoms of anxiety (BAI), depression (BDI), and psychological problems (BSI) than healthy control subjects (all $p < 0.0001$, t-test). ANCOVA of these data indicated there was not a direct relationship between anxiety, depression, and global measures of psychological problems as measured by the BAI, BDI-II and BSI, and [^{11}C]CFN BP_{ND} or [^{11}C]MeNTL BP_{ND}.

Alcohol-dependent subjects reported higher alcohol craving in the Penn Craving Scale, VAS, and OCDS than healthy control subjects (Table 1, all $p < 0.0001$, t-test). There was a negative correlation with peak VAS alcohol craving scores across days 1 to 5 postadmission and [^{11}C]CFN BP_{ND} in the amygdala ($F = 5.0$, p and $Q = 0.04$), ventral striatum ($F = 10.6$, p and $Q = 0.004$), and thalamus ($F = 4.5$, p and $Q = 0.05$); there was also a trend for the cingulate (p and $Q = 0.055$). There was no relationship between scores on other craving instruments (the OCDS or the Penn Craving Scale) for either [^{11}C]CFN BP_{ND} or [^{11}C]MeNTL BP_{ND} in alcohol-dependent subjects.

DISCUSSION

There were 5 primary findings of the current study. First, 5-day abstinent alcohol-dependent men and women had higher [^{11}C]CFN BP_{ND} when compared with age-matched healthy control men and women in brain regions, which included the ventral striatum, amygdala, caudate, globus pallidus, insula, putamen, and thalamus. This observation remained after adjusting for age, gender, and smoking status. The SPM analysis corroborated this finding and indicated that the alcohol effect is even more global than the VOI analyses suggest. Second, although the direction of effects in several brain regions was similar to that observed for [^{11}C]CFN BP_{ND}, VOI and SPM analyses did not reveal significant differences in [^{11}C]MeNTL BP_{ND} between alcohol-dependent and healthy control subjects. Third, [^{11}C]MeNTL BP_{ND} in the caudate was positively correlated with recent alcohol drinking in alcohol-dependent subjects. Fourth, there was a significant negative correlation between [^{11}C]CFN BP_{ND} and peak VAS alcohol craving in several VOI. Fifth, other measures of alcohol dependence and withdrawal severity, mood, and other psychological symptoms, were not associated with [^{11}C]CFN BP_{ND} or [^{11}C]MeNTL BP_{ND}. Each of these findings is discussed below.

The findings of our current study, which compared [^{11}C]CFN BP_{ND} in 25 alcohol-dependent and 30 age-matched healthy control men and women, are consistent with the higher [^{11}C]CFN BP_{ND} in ventral striatum in 25 alcohol-dependent men compared with 10 healthy controls reported by Heinz and colleagues (2005). In addition, alcohol-dependent subjects had significantly higher [^{11}C]CFN BP_{ND} in amygdala, caudate, globus pallidus, insula, putamen, and thalamus. Our finding that the increase in [^{11}C]CFN BP_{ND} may be more extensive is consistent with the reported trend toward an increase in regional and global [^{11}C]diprenorphine volumes of distribution in alcohol-dependent patients ($n = 11$) when compared with controls ($n = 13$), although this effect was not statistically significant (Williams et al., 2009). The authors note the study may have been under-powered and that there was substantial variability possibly related to [^{11}C]diprenorphine binding to all 3 subunits (μ, δ, and κ) of the OR.

The methodology for the current study differs from these previous studies in the following important ways. First, we enrolled both male and female alcohol-dependent and age-matched healthy subjects and used stringent psychological assessment procedures to ensure no current drug use, no other drug use disorders, and no other current or lifetime Axis I psychiatric disorders. Age, gender, other drug use disorders, and psychiatric problems are important confounders that are known to influence the endogenous opioid system. Second, all subjects were housed in the same inpatient research unit where diet was controlled, smoking was prohibited, and urine toxicology screens verified no other drug use prior to imaging. For alcohol-dependent subjects, all scans were conducted under an inpatient detoxification protocol where alcohol abstinence was supervised, and the timing of onset of alcohol abstinence and imaging was fixed. Alcohol-dependent subjects were enrolled in the study while actively drinking, and abstinence was initiated at the time of inpatient admission. Third, both MOR and DOR were examined via PET scans

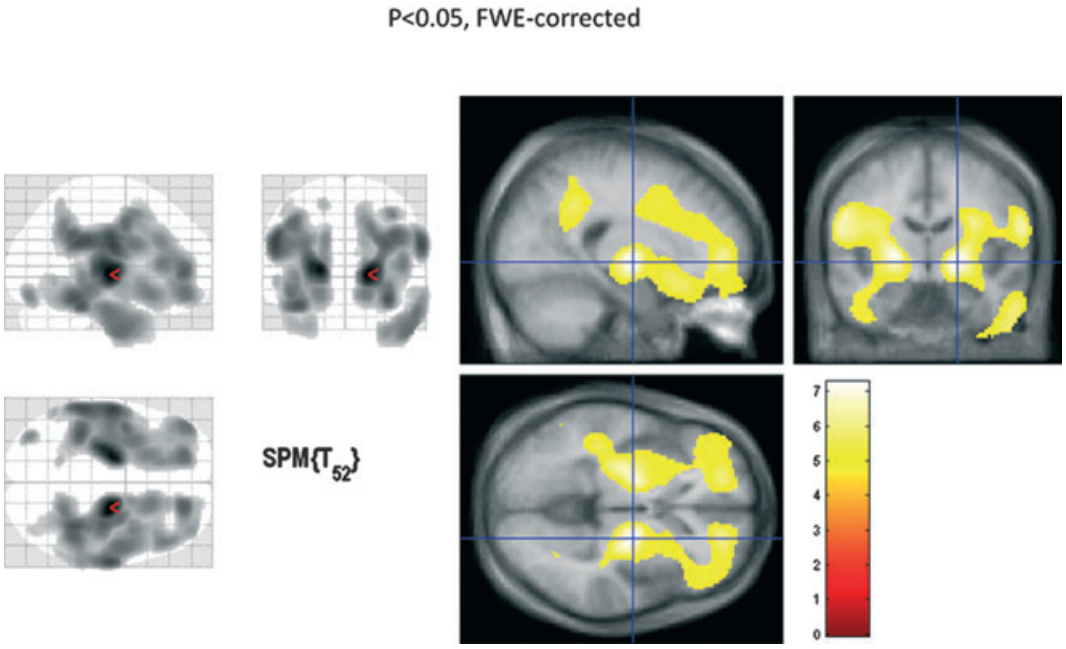

Fig. 4. [^{11}C]CFN BP_{ND} brain images shown in lateral, anterior, and superior views of bihemispheric clusters of statistical parametric mapping (SPM). The intersection of the blue lines targets the peak in the thalamus. Smoking status was added a nuisance variable in the contrast analysis of alcohol-dependent and healthy control subjects. Colored legend depicts peak *t*-values ranging from 0 (red) to 7.5 (yellow/white). Significant differences between alcohol-dependent and healthy control subjects are shown in yellow (*t*-values >4.6, $p < 0.05$ family-wise error [FWE]).

with carbon 11-labeled [^{11}C]CFN, a MOR-selective radioligand, and [^{11}C]MeNTL, a DOR-selective radioligand, in the same subjects under conditions of validated alcohol abstinence and on the same day (day 5) of abstinence. Change in the endogenous opioid system following alcohol abstinence is a dynamic process, and these changes are likely greatest during early abstinence. Thus, fixing the time of scanning to a specific day during early abstinence minimizes the variance in the data introduced when the scanning time is allowed to vary by days or weeks. Fourth, withdrawal medications (e.g., benzodiazepines), which can alter OR function (Cox and Collins, 2001), were not used. Fifth, we examined 8 brain VOI in mesolimbic opioid-rich regions, including the ventral striatum and the amygdala, which have been associated with alcohol reinforcement, dependence, and craving. Last, as many alcohol-dependent subjects were also smokers, we specifically recruited healthy smokers without heavy drinking or alcohol problems to balance and control for smoking. This point is particularly relevant as approximately 80% of alcohol-dependent subjects report regular tobacco use (Batel et al., 1995; DiFranza and Guerrera, 1990) and smoke at high rates (Dawson, 2000), when compared with social drinkers. Comparison subjects used in previous PET studies did not include smokers without alcohol problems. Thus, the current study used a rigorous level of control over other drug use and psychiatric disorders, the duration of alcohol abstinence and cigarette smoking. It is likely that significance was achieved in the current study because of the larger sample size and control over these potential confounding effects.

Previous studies in healthy human volunteers using [C^{11}]-MeNTL PET imaging have shown that DOR rich areas include neocortical regions (insular, parietal, frontal, cingulate, and occipital), caudate nucleus, putamen, and amygdala (Madar et al., 1996). In addition, [^{11}C]MeNTL PET imaging has been utilized successfully to examine group differences in other disease states such as epilepsy (Madar et al., 1997) and carcinoma (Madar et al., 2007). This is the first study to compare DOR availability in recently abstinent alcohol-dependent and healthy control human subjects. Interestingly, we found a positive association of recent drinking (average drinks per drinking day) with [^{11}C]MeNTL BP_{ND} in the caudate for alcohol-dependent subjects. These data suggest that the delta receptor may be sensitive to recent alcohol drinking history. These data provide evidence of some role of the DOR in alcoholism, particularly when taken together with our previous report showing that the clinical dose of naltrexone (50 mg) produced only partial inhibition (21%) and substantial intersubject variability of [^{11}C]MeNTL binding in alcohol-dependent subjects (Weerts et al., 2008). Because this same dose of naltrexone produces near-complete inhibition (95%) of [^{11}C]CFN binding, it seems likely that the magnitude of DOR blockade by naltrexone may contribute to the variability of naltrexone treatment outcomes and may be influenced by baseline differences in DOR availability prior to the treatment. The current study did not reveal significant [^{11}C]MeNTL BP_{ND} differences between groups. These data are in contrast to preclinical studies in alcohol-preferring and alcohol-nonpreferring rodent strains, which have shown increases and decreases in DOR density in mesolimbic regions (de Waele et al., 1995; Marinelli et al., 2000; McBride et al., 1998; Soini et al., 1998). Because intersubject variations (measured as coefficient of variation) were similar between

[^{11}C]CFN and [^{11}C]MeNTL for examined regions, the lack of group differences for [^{11}C]MeNTL cannot be attributed to greater variability in binding. A possible caveat is that the regional estimates of [^{11}C]MeNTL BP_{ND} were lower than estimates of [^{11}C]CFN BP_{ND}. Thus, it may be argued that the observed lower signal-to-noise (i.e., specific-to-nonspecific binding) ratio of [^{11}C]MeNTL may mask potential group differences. Alternatively, the lack of differences between groups may be related to the smaller sample size for the [^{11}C]MeNTL scans. It is possible that a larger sample size might reveal a significant increase in [^{11}C]MeNTL BP_{ND}.

The higher [^{11}C]CFN BP_{ND} in alcohol-dependent subjects can be interpreted in several ways. It may reflect greater MOR availability owing to decreased mu receptor occupancy by endogenous opioids. Alternatively, the increase in [^{11}C]CFN BP_{ND} in alcohol-dependent subjects also may reflect an increase in MOR density (e.g., an up-regulation of MOR) compared with controls. This elevation in [^{11}C]CFN BP_{ND} in alcohol-dependent subjects compared with healthy controls could be a consequence of (i) alcohol withdrawal, (ii) long-term hazardous alcohol drinking/dependence, (iii) inherited differences in the opioid system, and/or (iv) acquired differences owing to environmental factors (e.g., childhood adversity, chronic stress) that might alter [^{11}C]CFN BP_{ND}. There is support for some of these possibilities. Studies in rodents have reported increased MOR binding in limbic areas, including the nucleus accumbens, after extended alcohol consumption (5 weeks) (Cowen et al., 1998, 1999; Djouma and Lawrence, 2002) and during alcohol withdrawal (1 to 10 days) (Djouma and Lawrence, 2002). Studies examining genetic variations in opioid activity in rodent lines have also demonstrated greater MOR density in limbic structures, such as the nucleus accumbens and amygdala in the alcohol-preferring lines, when compared with the nonpreferring lines (de Waele et al., 1995; Marinelli et al., 2000; McBride et al., 1998), although not in all studies (Fadda et al., 1999). Although we did not find a direct relationship between [^{11}C]CFN BP_{ND} and measures of anxiety, depression, and psychological problems, alcohol-dependent subjects reported significantly greater symptoms for all of these measures than healthy control subjects, even after exclusion of people with a history of other Axis I disorders.

In the current study, detailed family histories were obtained from the participants, and subjects were classified according to family histories of alcoholism. The increase in [^{11}C]CFN BP_{ND} does not appear to be directly related to family history of alcoholism. Subjects with positive family histories of alcoholism did not differ in [^{11}C]CFN BP_{ND} from subjects with negative family histories of alcoholism in any of the VOI. Heinz and colleagues (2005) also did not observe an effect of family history of alcoholism on [^{11}C]CFN BP_{ND}. Likewise, previously we reported there were no significant differences in amphetamine-induced mesolimbic dopamine release, subjective responses, or stress hormone measures as a function of family history of alcoholism (Munro et al., 2006). It seems unlikely that the observed elevation in [^{11}C]CFN BP_{ND} can be attributed to acute abstinence alone. We did not observe a relationship between BP_{ND} and alcohol withdrawal severity as measured by the CIWA-Ar in the current study. Our selection of alcohol-dependent subjects with relatively mild alcohol withdrawal symptoms may have diminished our ability to observe such an effect. In the study by Heinz and colleagues (2005), increased MOR availability in the ventral striatum was observed after 1 to 3 weeks of alcohol abstinence and remained elevated and stable 5 weeks later when [^{11}C]CFN PET scans were repeated in a subset of alcohol-dependent subjects. Similarly, [^{11}C]diprenorphine volumes of distribution were stable when examined at 2 weeks and again 2 months after alcohol abstinence (Williams et al., 2009).

Neither our study nor the study by Heinz and colleagues (2005) observed any relationship between MOR availability and alcohol drinking history or severity of alcohol dependence. Likewise, Williams et al. (2009) did not see a correlation between alcohol drinking history or severity of alcohol dependence and the volumes of distribution of the nonselective tracer [^{11}C]diprenorphine. We did, however, observe a positive correlation between recent alcohol drinking and [^{11}C]MeNTL BP_{ND} in caudate. It should be acknowledged, however, that the ability to observe a relationships between drinking measures and [^{11}C]CFN BP_{ND} within the alcohol-dependent subjects may be compromised by the homogeneity and chronicity of the sample (i.e., all were long-term heavy drinkers). Increases in MOR binding were observed in rodents after only 5 weeks of alcohol consumption. If chronic alcohol drinking produced an up-regulation of MOR, it likely occurred earlier in the progression from regular drinking to dependence and could not be detected in our relatively homogonous sample of long-term alcohol-dependent drinkers.

We found an inverse relationship between craving scores on the VAS and [^{11}C]CFN BP_{ND} in several brain regions including the ventral striatum, thalamus, and cingulate. Interestingly, alcohol-dependent subjects show greater activation of these same brain regions in response to alcohol cues (a sip of alcohol and pictures of alcoholic beverages) when compared with control cues in functional MRI studies (George et al., 2001; Myrick et al., 2004); activation in cingulate and nucleus accumbens was correlated with higher craving in alcoholic and not social drinkers (Myrick et al., 2004). The inverse correlation with craving in the current study was unexpected, as alcohol-dependent subjects reported higher craving and had higher [^{11}C]CFN BP_{ND} when compared with controls. In addition, our data contrast with positive correlations of self-reported craving with [^{11}C]CFN and [^{11}C]diprenorphine receptor availability reported previously (Heinz et al., 2005; Williams et al., 2009). The OCDS scores obtained on the day of the PET scans in the current study were comparable to those in the Heinz and colleagues (2005) study. An inverse relationship between craving and dopamine D2 receptor ligand [^{18}F]desmethoxyfallypride BP_{ND} in the ventral striatum in alcohol-dependent subjects has been reported (Heinz et al., 2004). Although it appears that D2 and MOR receptors behave oppositely in alcohol dependence, a similar inverse

relationship with craving may occur. For example, if opioid peptides are reduced by chronic alcohol drinking leading to the up-regulation of MOR, then greater up-regulation of MOR may result in greater opioid transmission and less craving compared with individuals with less up-regulation. In the case of alcohol-related reductions in endogenous opioid release, the up-regulation of receptors would have to be proportionally more than the reduction in opioids for there to be net increase in opioid neurotransmission. This would bring craving closer to that in normal individuals, but not normalize it completely. This mechanism is speculative and further studies are needed to determine whether this hypothesis is supported.

There were some study limitations that may limit generalization of these findings. We selected alcohol-dependent subjects without prior histories of serious withdrawal symptoms and excluded subjects who had previously required benzodiazepine treatment for withdrawal symptoms. Thus, subjects who experienced more severe forms of alcohol withdrawal were excluded from participation in the study. Yet, despite this conservative selection of subjects with modest withdrawal symptoms, differences in [^{11}C]CFN BP_{ND} between alcohol-dependent subjects and controls were highly significant across multiple brain volumes. An additional consideration is subjects were long-term alcohol-dependent drinkers (i.e., an average of 15 years of alcohol-dependent drinking). Thus, similar long-term exposure to alcohol for this homogenous sample may have reduced the ability to show relationships between [^{11}C]CFN BP_{ND} and behavior/clinical measures. Additional studies in subjects with a wider range of drinking levels and with and without diagnosis of alcohol use disorders are needed to better understand the transitions in the opioid system as alcohol consumption progresses from social drinking to heavy drinking and then to alcohol dependence.

In summary, our observations that [^{11}C]MeNTL BP_{ND} was associated with recent drinking and that [^{11}C]MeNTL BP_{ND} showed the same direction of group differences as [^{11}C]CFN BP_{ND} suggest a potential role of DOR in alcohol dependence and clearly warrant further investigation. When taken together with our previous report showing the clinical dose of naltrexone (50 mg) produced only partial blockade of [^{11}C]MeNTL binding, these data suggest the DOR likely contributes to the variability of naltrexone treatment outcomes as well alcohol dependence. The higher [^{11}C]CFN BP_{ND} in alcohol-dependent subjects may represent a predisposing risk factor for alcohol dependence or could be a result of long-term drinking, alcohol dependence, or withdrawal. The finding that [^{11}C]CFN BP_{ND} was increased in alcoholics provides evidence of a prominent role of the MOR in alcohol dependence.

ACKNOWLEDGMENTS

The authors acknowledge the technical support of Dr. Hayden T. Ravert, Mr. Robert Smoot, and Mr. Daniel Holt for their radiochemistry expertise and Ms. Karen Edmonds, Mr. David Clough, Mr. Michael Hans, and Mr. Bineyam Gebrewold for PET acquisition. The authors also acknowledge the excellent technical assistance of Courtney Cook for data management and Xiaoqiang Xu for statistical analyses. The National Institute of Alcohol Abuse and Alcoholism (NIAAA) provided financial support for research related to the subject matter of this manuscript from the grants R01AA11872 (PI: J.J. Frost), R01AA11855 (PI: M.E. McCaul), and R37AA12303 (PI: G.S. Wand). Dr. Wand is the recipient of a gift fund from the Kenneth Lattman Foundation. Dr. McCaul was principal investigator on a contract (A Phase 2 Study of LY2196044 Compared with Naltrexone and Placebo in the Treatment of Alcohol Dependence) funded by Lilly Research Laboratories; Drs. Weerts and Wand were coinvestigators on this project.

REFERENCES

Anton RF, Moak DH, Latham PK (1996) The obsessive compulsive drinking scale: a new method of assessing outcome in alcoholism treatment studies [published erratum appears in Arch Gen Psychiatry 1996 Jul;53(7):576]. Arch Gen Psychiatry 53:225–231.

Anton RF, Swift RM (2003) Current pharmacotherapies of alcoholism: a U.S. perspective. Am J Addict 12(Suppl 1):S53–S68.

Ashburner JT, Friston KJ (2004) High-dimensional image warping, in *Human Brain Function*, 2nd ed (Frackowiak RSJ, Friston KJ, Frith CD, Dolan RJ, Price CJ, Zeki S, Ashburner JT, Penny WD eds), pp 673–694. Academic Press, San Diego, CA.

Batel P, Pessione F, Maitre C, Rueff B (1995) Relationship between alcohol and tobacco dependencies among alcoholics who smoke. Addiction 90:977–980.

Baumann B, Danos P, Krell D, Diekmann S, Leschinger A, Stauch R, Wurthmann C, Bernstein HG, Bogerts B (1999) Reduced volume of limbic system-affiliated basal ganglia in mood disorders: preliminary data from a postmortem study. J Neuropsychiatry Clin Neurosci 11:71–78.

Beck AT, Epstein N, Brown G, Steer RA (1988) An inventory for measuring clinical anxiety: psychometric properties. J Consult Clin Psychol 56:893–897.

Beck AT, Steer RA, Brown GK (1996) Manual for the Beck Depression Inventory-II. Psychological Corporation, San Antonio, TX.

Becker A, Grecksch G, Kraus J, Loh HH, Schroeder H, Hollt V (2002) Rewarding effects of ethanol and cocaine in mu opioid receptor-deficient mice. Naunyn Schmiedebergs Arch Pharmacol 365:296–302.

Bencherif B, Wand GS, McCaul ME, Kim YK, Ilgin N, Dannals RF, Frost JJ (2004) Mu-opioid receptor binding measured by [11C]carfentanil positron emission tomography is related to craving and mood in alcohol dependence. Biol Psychiatry 55:255–262.

Benjamin D, Grant ER, Pohorecky LA (1993) Naltrexone reverses ethanol-induced dopamine release in the nucleus accumbens in awake, freely moving rats. Brain Res 621:137–140.

Bucholz KK, Cadoret R, Cloninger CR, Dinwiddie SH, Hesselbrock VM, Nurnberger JI Jr, Reich T, Schmidt I, Schuckit MA (1994) A new, semi-structured psychiatric interview for use in genetic linkage studies: a report on the reliability of the SSAGA. J Stud Alcohol 55:149–158.

Cowen MS, Rezvani A, Jarrott B, Lawrence AJ (1998) Distribution of opioid peptide gene expression in the limbic system of Fawn-Hooded (alcohol-preferring) and Wistar-Kyoto (alcohol-non-preferring) rats. Brain Res 796:323–326.

Cowen MS, Rezvani AH, Jarrott B, Lawrence AJ (1999) Ethanol consumption by Fawn-Hooded rats following abstinence: effect of naltrexone and changes in mu-opioid receptor density. Alcohol Clin Exp Res 23:1008–1014.

Cox RF, Collins MA (2001) The effects of benzodiazepines on human opioid receptor binding and function. Anesth Analg 93:354–358.

Dawson DA (2000) Drinking as a risk factor for sustained smoking. Drug Alcohol Depend 59:235–249.

Derogatis LR, Melisaratos N (1983) The Brief Symptom Inventory: an introductory report. Psychol Med 13:595–605.

de Waele JP, Kiianmaa K, Gianoulakis C (1995) Distribution of the mu and delta opioid binding sites in the brain of the alcohol-preferring AA and alcohol-avoiding ANA lines of rats. J Pharmacol Exp Ther 275:518–527.

DiFranza JR, Guerrera MP (1990) Alcoholism and smoking. J Stud Alcohol 51:130–135.

Djouma E, Lawrence AJ (2002) The effect of chronic ethanol consumption and withdrawal on mu-opioid and dopamine D(1) and D(2) receptor density in Fawn-Hooded rat brain. J Pharmacol Exp Ther 302:551–559.

Endres CJ, Bencherif B, Hilton J, Madar I, Frost JJ (2003) Quantification of brain mu-opioid receptors with [11C]carfentanil: reference-tissue methods. Nucl Med Biol 30:177–186.

Fadda P, Tronci S, Colombo G, Fratta W (1999) Differences in the opioid system in selected brain regions of alcohol-preferring and alcohol-nonpreferring rats. Alcohol Clin Exp Res 23:1296–1305.

Flannery BA, Volpicelli JR, Pettinati HM (1999) Psychometric properties of the Penn Alcohol Craving Scale [In Process Citation]. Alcohol Clin Exp Res 23:1289–1295.

Franck J, Lindholm S, Raaschou P (1998) Modulation of volitional ethanol intake in the rat by central delta-opioid receptors. Alcohol Clin Exp Res 22:1185–1189.

Froehlich JC (1995) Genetic factors in alcohol self-administration. J Clin Psychiatry 56(Suppl 7):15–23.

Froehlich JC, Zweifel M, Harts J, Lumeng L, Li TK (1991) Importance of delta opioid receptors in maintaining high alcohol drinking. Psychopharmacology 103:467–472.

Frost JJ, Mayberg HS, Sadzot B, Dannals RF, Lever JR, Ravert HT, Wilson AA, Wagner HN Jr, Links JM (1990) Comparison of [11C]diprenorphine and [11C]carfentanil binding to opiate receptors in humans by positron emission tomography. J Cereb Blood Flow Metab 10:484–492.

Frost JJ, Wagner HN Jr, Dannals RF, Ravert HT, Links JM, Wilson AA, Burns HD, Wong DF, McPherson RW, Rosenbaum AE, Kuhar MJ, Snyder SH (1985) Imaging opiate receptors in the human brain by positron tomography. J Comput Assist Tomogr 9:231–236.

George MS, Anton RF, Bloomer C, Teneback C, Drobes DJ, Lorberbaum JP, Nahas Z, Vincent DJ (2001) Activation of prefrontal cortex and anterior thalamus in alcoholic subjects on exposure to alcohol-specific cues. Arch Gen Psychiatry 58:345–352.

Gianoulakis C (2004) Endogenous opioids and addiction to alcohol and other drugs of abuse. Curr Top Med Chem 4:39–50.

Hall FS, Sora I, Uhl GR (2001) Ethanol consumption and reward are decreased in mu-opiate receptor knockout mice. Psychopharmacology (Berl) 154:43–49.

Hammers A, Allom R, Koepp MJ, Free SL, Myers R, Lemieux L, Mitchell TN, Brooks DJ, Duncan JS (2003) Three-dimensional maximum probability atlas of the human brain, with particular reference to the temporal lobe. Hum Brain Mapp 19:224–247.

Heinz A, Reimold M, Wrase J, Hermann D, Croissant B, Mundle G, Dohmen BM, Braus DF, Schumann G, Machulla HJ, Bares R, Mann K (2005) Correlation of stable elevations in striatal mu-opioid receptor availability in detoxified alcoholic patients with alcohol craving: a positron emission tomography study using carbon 11-labeled carfentanil. Arch Gen Psychiatry 62:57–64.

Heinz A, Siessmeier T, Wrase J, Hermann D, Klein S, Grusser SM, Flor H, Braus DF, Buchholz HG, Grunder G, Schreckenberger M, Smolka MN, Rosch F, Mann K, Bartenstein P (2004) Correlation between dopamine D(2) receptors in the ventral striatum and central processing of alcohol cues and craving. Am J Psychiatry 161:1783–1789.

Herz A (1998) Opioid reward mechanisms: a key role in drug abuse? Can J Physiol Pharmacol 76:252–258.

Hilton J, Yokoi F, Dannals RF, Ravert HT, Szabo Z, Wong DF (2000) Column-switching HPLC for the analysis of plasma in PET imaging studies. Nucl Med Biol 27:627–630.

Hochberg Y, Benjamini Y (1990) More powerful procedures for multiple significance testing. Stat Med 9:811–818.

Holm S (1979) A simple sequentially rejective multiple test procedure. Scand J Statist 6:65–70.

Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN, Holden J, Houle S, Huang SC, Ichise M, Iida H, Ito H, Kimura Y, Koeppe RA, Knudsen GM, Knuuti J, Lammertsma AA, Laruelle M, Logan J, Maguire RP, Mintun MA, Morris ED, Parsey R, Price JC, Slifstein M, Sossi V, Suhara T, Votaw JR, Wong DF, Carson RE (2007) Consensus nomenclature for in vivo imaging of reversibly binding radioligands. J Cereb Blood Flow Metab 27:1533–1539.

Job MO, Tang A, Hall FS, Sora I, Uhl GR, Bergeson SE, Gonzales RA (2007) Mu (mu) opioid receptor regulation of ethanol-induced dopamine response in the ventral striatum: evidence of genotype specific sexual dimorphic epistasis. Biol Psychiatry 62:627–634.

June HL, McCane SR, Zink RW, Portoghese PS, Li TK, Froehlich JC (1999) The delta 2-opioid receptor antagonist naltriben reduces motivated responding for ethanol. Psychopharmacology (Berl) 147:81–89.

Kinahan PE, Rogers JG (1989) Analytic 3D image reconstruction using all detected events. IEEE Trans Nucl Sci 36:964–968.

Koob GF, Roberts AJ, Schulteis G, Parsons LH, Heyser CJ, Hyytia P, Merlo-Pich E, Weiss F (1998) Neurocircuitry targets in ethanol reward and dependence. Alcohol Clin Exp Res 22:3–9.

Krishnan-Sarin S, Jing SL, Kurtz DL, Zweifel M, Portoghese PS, Li TK, Froehlich JC (1995a) The delta opioid receptor antagonist naltrindole attenuates both alcohol and saccharin intake in rats selectively bred for alcohol preference. Psychopharmacology (Berl) 120:177–185.

Krishnan-Sarin S, Portoghese PS, Li TK, Froehlich JC (1995b) The delta 2-opioid receptor antagonist naltriben selectively attenuates alcohol intake in rats bred for alcohol preference. Pharmacol Biochem Behav 52:153–159.

Krishnan-Sarin S, Wand GS, Li XW, Portoghese PS, Froehlich JC (1998) Effect of mu opioid receptor blockade on alcohol intake in rats bred for high alcohol drinking. Pharmacol Biochem Behav 59:627–635.

Lever JR, Scheffel U, Kinter CM, Ravert HT, Dannals RF, Wagner HN Jr, Frost JJ (1992) In vivo binding of N1′-([11C]methyl)naltrindole to delta-opioid receptors in mouse brain. Eur J Pharmacol 216:459–460.

Lewellen TK, Kohlmyer SG, Miyaoka RS, Kaplan MS, Stearns CW, Schubert SF (1996) Investigation of the performance of the General Electric ADVANCE positron emission tomograph in 3D mode. IEEE Trans Nucl Sci 43:2199–2206.

Logan J, Fowler JS, Volkow ND, Wang GJ, Ding YS, Alexoff DL (1996) Distribution volume ratios without blood sampling from graphical analysis of PET data. J Cereb Blood Flow Metab 16:834–840.

Logan J, Fowler JS, Volkow ND, Wolf AP, Dewey SL, Schlyer DJ, MacGregor RR, Hitzemann R, Bendriem B, Gatley SJ, Christman DR (1990) Graphical analysis of reversible radioligand binding from time-activity measurements applied to [N-11C-methyl]-(-)-cocaine PET studies in human subjects. J Cereb Blood Flow Metab 10:740–747.

Madar I, Bencherif B, Lever J, Heitmiller RF, Yang SC, Brock M, Brahmer J, Ravert H, Dannals R, Frost JJ (2007) Imaging delta- and mu-opioid receptors by PET in lung carcinoma patients. J Nucl Med 48(2):207–213.

Madar I, Lesser RP, Krauss G, Zubieta JK, Lever JR, Kinter CM, Ravert HT, Musachio JL, Mathews WB, Dannals RF, Frost JJ (1997) Imaging of delta- and mu-opioid receptors in temporal lobe epilepsy by positron emission tomography. Ann Neurol 41(3):358–367.

Madar I, Lever JR, Kinter CM, Scheffel U, Ravert HT, Musachio JL, Mathews WB, Dannals RF, Frost JJ (1996) Imaging of delta opioid receptors in human brain by N1′-([11C]methyl)naltrindole and PET. Synapse 24:19–28.

Marinelli PW, Kiianmaa K, Gianoulakis C (2000) Opioid propeptide mRNA content and receptor density in the brains of AA and ANA rats. Life Sci 66:1915–1927.

Martinez D, Slifstein M, Broft A, Mawlawi O, Hwang DR, Huang Y, Cooper T, Kegeles L, Zarahn E, Abi-Dargham A, Haber SN, Laruelle M (2003) Imaging human mesolimbic dopamine transmission with positron emission

tomography. Part II: amphetamine-induced dopamine release in the functional subdivisions of the striatum. J Cereb Blood Flow Metab 23:285–300.

Mazziotta JC, Toga AW, Evans A, Fox P, Lancaster J (1995) A probabilistic atlas of the human brain: theory and rationale for its development. The International Consortium for Brain Mapping (ICBM). Neuroimage 2:89–101.

McBride WJ, Chernet E, McKinzie DL, Lumeng L, Li TK (1998) Quantitative autoradiography of mu-opioid receptors in the CNS of alcohol-naive alcohol-preferring P and -nonpreferring NP rats. Alcohol 16:317–323.

McCaul ME, Wand GS, Eissenberg T, Rohde CA, Cheskin LJ (2000) Naltrexone alters subjective and psychomotor responses to alcohol in heavy drinking subjects. Neuropsychopharmacology 22:480–492.

McCaul ME, Wand GS, Stauffer R, Lee SM, Rohde CA (2001) Naltrexone dampens ethanol-induced cardiovascular and hypothalamic-pituitary-adrenal axis activation. Neuropsychopharmacology 25:537–547.

Meltzer CC, Bryan RN, Holcomb HH, Kimball AW, Mayberg HS, Sadzot B, Leal JP, Wagner HN Jr, Frost JJ (1990) Anatomical localization for PET using MR imaging. J Comput Assist Tomogr 14:418–426.

Munro CA, McCaul ME, Oswald LM, Wong DF, Zhou Y, Brasic J, Kuwabara H, Kumar A, Alexander M, Ye W, Wand GS (2006) Striatal dopamine release and family history of alcoholism. Alcohol Clin Exp Res 30:1143–1151.

Myrick H, Anton RF, Li X, Henderson S, Drobes D, Voronin K, George MS (2004) Differential brain activity in alcoholics and social drinkers to alcohol cues: relationship to craving. Neuropsychopharmacology 29:393–402.

Oswald LM, Wand GS (2004) Opioids and alcoholism. Physiol Behav 81:339–358.

Oswald LM, Wong DF, McCaul M, Zhou Y, Kuwabara H, Choi L, Brasic J, Wand GS (2005) Relationships among ventral striatal dopamine release, cortisol secretion, and subjective responses to amphetamine. Neuropsychopharmacology 30:821–832.

Peterson JB, Conrod P, Vassileva J, Gianoulakis C, Pihl RO (2006) Differential effects of naltrexone on cardiac, subjective and behavioural reactions to acute ethanol intoxication. J Psychiatry Neurosci 31:386–393.

Rice JP, Reich T, Bucholz KK, Neuman RJ, Fishman R, Rochberg N, Hesselbrock VM, Nurnberger JI Jr, Schuckit MA, Begleiter H (1995) Comparison of direct interview and family history diagnoses of alcohol dependence. Alcohol Clin Exp Res 19:1018–1023.

Roberts AJ, McDonald JS, Heyser CJ, Kieffer BL, Matthes HW, Koob GF, Gold LH (2000) mu-Opioid receptor knockout mice do not self-administer alcohol. J Pharmacol Exp Ther 293:1002–1008.

Skinner HA, Allen BA (1982) Alcohol dependence syndrome: measurement and validation. J Abnorm Psychol 91:199–209.

Sobell LC, Sobell MB (1992) Timeline followback: a technique for assessing self-reported alcohol consumption, in *Measuring Alcohol Consumption: Psychosocial and Biological Methods* (Litten RZ, Allen J eds), pp 41–72. Humana Press, Totowa, NJ.

Soini SL, Ovaska T, Honkanen A, Hyytia P, Korpi ER (1998) Brain opioid receptor binding of [3H]CTOP and [3H]naltrindole in alcohol-preferring AA and alcohol-avoiding ANA rats. Alcohol 15:227–232.

Srisurapanont M, Jarusuraisin N (2005) Naltrexone for the treatment of alcoholism: a meta-analysis of randomized controlled trials. Int J Neuropsychopharmacol 8:267–280.

Sullivan JT, Sykora K, Schneiderman J, Naranjo CA, Sellers EM (1989) Assessment of alcohol withdrawal: the revised clinical institute withdrawal assessment for alcohol scale (CIWA-Ar). Br J Addict 84:1353–1357.

Titeler M, Lyon RA, Kuhar MJ, Frost JF, Dannals RF, Leonhardt S, Bullock A, Rydelek LT, Price DL, Struble RG (1989) Mu opiate receptors are selectively labelled by [3H]carfentanil in human and rat brain. Eur J Pharmacol 167:221–228.

Weerts EM, Kim YK, Wand GS, Dannals RF, Lee JS, Frost JJ, McCaul ME (2008) Differences in delta- and mu-opioid receptor blockade measured by positron emission tomography in naltrexone-treated recently abstinent alcohol-dependent subjects. Neuropsychopharmacology 33:653–665.

Williams TM, Davies SJ, Taylor LG, Daglish MR, Hammers A, Brooks DJ, Nutt DJ, Lingford-Hughes A (2009) Brain opioid receptor binding in early abstinence from alcohol dependence and relationship to craving: an [11C]diprenorphine PET study. Eur Neuropsychopharmacol 19:740–748.

Zubieta JK, Dannals RF, Frost JJ (1999) Gender and age influences on human brain mu-opioid receptor binding measured by PET. Am J Psychiatry 156:842–848.

Zubieta JK, Smith YR, Bueller JA, Xu Y, Kilbourn MR, Jewett DM, Meyer CR, Koeppe RA, Stohler CS (2001) Regional mu opioid receptor regulation of sensory and affective dimensions of pain. Science 293:311–315.