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Positron Emission Tomography Shows Elevated Cannabinoid CB₁ Receptor Binding in Men with Alcohol Dependence

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Abstract

Background—Several lines of evidence link cannabinoid (CB) type 1 (CB₁) receptor-mediated endogenous CB (eCB) signaling to the etiology of alcohol dependence (AD). However, to date, only peripheral measures of eCB function have been collected in living humans with AD and no human *in vivo* data on the potentially critical role of the brain CB₁ receptor in AD have been published. This is an important gap in the literature, because recent therapeutic developments suggest that these receptors could be targeted for the treatment of AD.

Methods—Medication-free participants were scanned during early abstinence 4 weeks after their last drink. Using positron emission tomography (PET) with a high resolution research tomograph and the CB₁ receptor selective radiotracer [¹¹C]OMAR, we determined [¹¹C]OMAR volume of distribution (V_T) values, a measure of CB₁ receptor density, in a-priori selected brain regions in men with AD (N=8, age 37.4± 7.9 years; 5 smokers) and healthy control (HC) men (N=8, age 32.5± 6.9 years; all non-smokers). PET images reconstructed using the MOLAR algorithm with hardware motion correction were rigidly aligned to the subject-specific magnetic resonance (MR) image, which in turn was warped to an MR template. Time-activity curves (TACs) were extracted from the dynamic PET data using a priori selected regions of interest delineated in the MR template space.

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Conflict of Interest/Financial Disclosures

The authors report no potential conflict of interest relevant to this article.

Results—In AD relative to HC, [^{11}C]OMAR V_T values were elevated by ~20% ($p=.023$) in a circuit, including the amygdala, hippocampus, putamen, insula, anterior and posterior cingulate cortices and orbitofrontal cortex. Age, body mass index or smoking status did not influence the outcome.

Conclusions—These findings agree with preclinical evidence and provide the first, albeit still preliminary *in vivo* evidence suggesting a role for brain CB $_1$ receptors in AD. The current study design does not answer the important question of whether elevated CB $_1$ receptors are a pre-existing vulnerability factor for AD or whether elevations develop as a consequence of AD.

Keywords

alcohol dependence; brain imaging; positron emission tomography; endogenous cannabinoids; CB $_1$ receptor

INTRODUCTION

Almost 2 in 3 alcoholics resume drinking after engaging in alcohol treatment (Brown et al., 1995; Hunt et al., 1971), highlighting the substantial need to improve treatments for this patient population. At a neurobiological level, studies show impairments in decision-making in alcohol-dependent (AD) patients (Dom et al., 2006), which is associated with altered functions in cortico-limbic-striatal circuitry including the amygdala, hippocampus, anterior and posterior cingulate cortices, insula, and subregions of the striatum, i.e. putamen (Goldstein et al., 2004; Makris et al., 2008). At least three sets of factors may contribute to high alcohol relapse rates. First, individual differences in the positive, reinforcing properties of alcohol may increase risk of alcoholism and possibly alcohol relapse (Schuckit and Smith, 1996). Second, stimuli previously associated with alcohol use and its physiological and subjective effects may become paired with alcohol and serve as “conditioned cues” that can increase alcohol craving and subsequent alcohol use (O'Brien et al., 1998). Finally, differences in stress responsiveness may contribute to the development and maintenance of alcohol addiction (Brown et al., 1990; Miller et al., 1996; Sinha, 2001). All three factors can be linked to the endogenous cannabinoid (eCB) system and its attending CB $_1$ receptor (Serrano and Parsons, 2011), and a growing body of evidence points to an involvement of CB $_1$ receptors in the acquisition and maintenance of AD (Vinod and Hungund, 2006).

The behavioral effects of CB $_1$ receptor knock-out (Hungund et al., 2003; Naassila et al., 2004; Poncelet et al., 2003; Thanos et al., 2005) and pharmacological manipulation of CB $_1$ receptor function (Femenia et al., 2010; Maccioni et al., 2010; Maccioni et al., 2008; Malinen and Hyytia, 2008) result in reduced voluntary alcohol intake. In contrast, administration of a CB $_1$ receptor agonist (Colombo et al., 2002; Gallate et al., 1999; Vinod et al., 2008) enhances alcohol consumption. These findings suggest that upregulation of CB $_1$ receptor-mediated G- protein signaling in a circuit involving the amygdala, hippocampus, ventromedial prefrontal and orbitofrontal cortex, insula and striatum (Sullivan and Pfefferbaum, 2005) might contribute to the increased alcohol consummatory behavior in patients with AD.

Positron emission tomography (PET) imaging is a direct, sensitive and straightforward means of probing the functional neurochemistry of humans and assessing molecular targets in the brain *in vivo*. The recent development of a CB $_1$ receptor selective radiotracer, designated [^{11}C]OMAR (Horti et al., 2006; Wong et al., 2010), facilitates *in vivo* assessment of [^{11}C]OMAR volumes of distribution (V_T), a measure of total binding in the brain, and proportional to CB $_1$ receptor density. Given the higher rates of AD among men compared with women (Nolen- Hoeksema and Hilt, 2006) and important sex differences in the biological underpinnings of AD (Fox et al., 2009), we measured CB $_1$ receptors using PET

and [^{11}C]OMAR in men with AD during early abstinence defined as 4 weeks after their last drink and in healthy control (HC) subjects. Based on preclinical data, we hypothesized that CB₁ receptor binding will be increased in men with AD compared to matched HC participants.

METHODS

Subjects

Eight men with AD, all medication-free in early abstinence 4 weeks after their last drink and eight male age- and body mass index (BMI)-matched HC participants without a history of psychiatric or substance use disorder were recruited through public advertisement. After giving informed consent, participants were evaluated and diagnosed using Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria and the Structured Clinical Interview for DSM-IV (SCID) (First et al., 1996). AD patients did not have any additional axis-I diagnosis and were treatment engaged. They were participating in standard 3 times weekly substance abuse counseling and had been abstinent from alcohol for four weeks at the time of the PET scan. Abstinence was confirmed with regular breathalyzer and urine toxicology tests at each clinic visit to ensure abstinence before the PET scan. Signs and symptoms of alcohol withdrawal were quantified with the Clinical Institute Withdrawal Assessment of Alcohol Scale, Revised (CIWA-Ar). AD patients were past the acute alcohol-withdrawal period at the time of the PET scan. All participants were evaluated by physical examination, electrocardiogram, standard laboratory tests, urine analysis and toxicology and were free of significant medical or neurological conditions. Cannabis users were excluded given recent evidence showing reversible and regionally selective downregulation of brain CB₁ receptors in human subjects who chronically smoke cannabis (Hirvonen et al., 2011). No participants received medication for at least 4 weeks prior to PET scanning. The protocol was approved by the Yale University School of Medicine Human Investigation Committee, the Human Subjects Subcommittee of the Veterans Affairs Connecticut Healthcare System, the Magnetic Resonance Research Center and the Yale New Haven Hospital Radiation Safety Committee.

Scanning and Imaging Procedures

[^{11}C]OMAR was prepared at high specific activity (212 ± 95 MBq/nmol at end of synthesis) by methods previously described but adopted for synthesis using the TRACERlab FXC Pro automated synthesis module (GE Healthcare, Milwaukee, WI) (Horti et al., 2006). The radiotracer (651 ± 69 MBq, 45 ± 27 ng/kg) was infused as a slow bolus over 1 minute through the antecubital vein. The radioactivity concentration of blood from the radial artery was measured continuously using an integrated peristaltic pump and calibrated radioactivity detector (PBS101, Veenstra Instruments, Joure, The Netherlands) for the first 7 min after radiotracer administration with discrete sample manually obtained at 3, 5, 15, 30, 60, and 90 minutes post-injection. Manual samples were measured on a gamma counter (Wizard 1480, Perkin-Elmer, Waltham, Massachusetts, United States) to determine radioactivity concentration in whole blood and in plasma. The whole blood-plasma ratio was calculated and used to scale the continuous whole blood concentration data measured by the continuous blood counter used at the beginning of the scan. Selected discrete blood samples were analyzed for the fraction of unchanged [^{11}C]OMAR and its radiometabolites using a column-switching high performance liquid chromatography assay (Hilton et al., 2000).

Listmode emission data were collected for 120 minutes using the HRRT PET scanner (Siemens Medical Systems, Knoxville, Tennessee, United States). Subject motion was measured using the Polaris Vicra optical tracking system (Northern Digital Inc., Waterloo, Ontario, Canada). Vicra recordings were used to correct listmode data in the Motion-

compensation Ordered subsets expectation maximization Algorithm for resolution-recovery Reconstruction (Carson et al., 2003) which produced dynamic images corrected for motion, photon scatter and attenuation, random coincidences, scanner dead-time, and detector inhomogeneity. The PET image sequences were registered to subject-specific T1-weighted magnetic resonance images acquired on a 3 Tesla Trio imaging system (Siemens Medical Systems, Erlangen, Germany). Anatomical MR images were in turn non-linearly warped to an MR template where regions of interest (ROIs) were defined (Tzourio-Mazoyer et al., 2002). These regions (Figure 1., Table 2.) constitute a neural circuit consistently implicated in the pathophysiology of AD, and are known to contain high levels of CB₁ receptors. Regional time activity curves (TACs) were extracted from the dynamic PET data and analyzed using multilinear analysis (Ichise et al., 2002) with metabolite-corrected arterial input functions and cutoff time of $t^*=30$ minutes. This kinetic analysis yields regional estimates of total V_T , the equilibrium ratio of radioligand in tissue relative to arterial plasma (Innis et al., 2007), which reflects binding of [¹¹C]OMAR to CB₁ receptors plus nondisplaceable binding. The multilinear analysis method used here was found to yield V_T values in excellent agreement with full compartmental modeling (Normandin et al., 2010a). The cutoff time of $t^*=30$ min was the shortest that adequately fit data from all regions, and exhibited the best test-retest reproducibility in an initial cohort of healthy subjects (Normandin et al., 2010b). Since no region without specific binding has been found for [¹¹C]OMAR, binding potential cannot be determined.

Statistical Analysis

Independent sample t-tests were used to compare continuous clinical and demographic variables between AD and HC. Data were normally distributed as determined by visual inspection and the Kolmogorov-Smirnov D test. Chi-square was used in the case of dichotomous variables. [¹¹C]OMAR V_T values were subjected to a repeated measures analysis of variance (rmANOVA) with group as a between-subject factor and brain region as a within-subject factor. Significant results for the omnibus test were followed-up with post-hoc analyses using ANOVA with regional [¹¹C]OMAR V_T as dependent variable and group as predictor variable to determine significant differences between groups in individual ROIs. Tests of association between continuous variables were performed using Pearson's product-moment correlations. All tests were performed two-tailed, with results considered significant at $p<0.05$. Means and standard deviations are reported. All statistical analyses were conducted using SPSS version 16.0 (SPSS Inc, Chicago, IL, USA).

RESULTS

Demographics and Clinical Characteristics

Participants in the AD and HC groups were matched for age and body mass index (BMI); smoking frequency was greater in the AD compared to HC group. As expected, AD patients relative to HC had a significantly earlier age at their first drink (15.8 ± 2.4 versus 21.9 ± 3.7 years) and had a mean illness duration of 16.6 ± 9.0 years. Participants in the AD group experienced low levels of alcohol withdrawal at the time of the PET scan as indicated by a mean CIWA score of 4.6 ± 4.2 (Table 1.).

Neuroreceptor Imaging

[¹¹C]OMAR V_T values within the circuit were significantly higher in the AD group compared to the HC group (main effect of group, $F=6.56$; $df=1,14$; $p=0.023$). Post-hoc testing revealed elevated [¹¹C]OMAR V_T values in the a priori selected ROIs in patients with AD compared to the HC group (Figure 1., upper panel; Table 2.) which is evident also in an example comparing amygdala [¹¹C]OMAR V_T in a patient with AD relative to a HC subject (Figure 1., lower panel). Including smoking status as a co-variate in the rmANOVA

did not influence the outcome. Exploratory analyses in other regions outside of the circuit including caudate, pallidum, parietal cortex or thalamus did not yield any significant between-group differences.

There were no associations between [^{11}C]OMAR V_T values and age or BMI in either group. There were no associations between [^{11}C]OMAR V_T values and CIWA score, age at first drink or illness duration in the AD group.

DISCUSSION

Herein we demonstrate for the first time *in vivo* elevated V_T values of [^{11}C]OMAR in chronic AD patients during early abstinence relative to matched HC subjects. Age at onset of drinking, number of drinks or illness duration did not correlate with [^{11}C]OMAR V_T . We interpret these findings as reflecting elevations in CB $_1$ receptor density in AD patients.

Regional V_T values showed good agreement with the known density and distribution of CB $_1$ receptors in human autoradiography studies (Glass et al., 1997; Herkenham et al., 1990). Relatively lower hippocampus [^{11}C]OMAR V_T values despite highest [^3H]CP55,940 binding shown in the autoradiography studies was also reported by other groups in their *in vivo* PET studies using different CB $_1$ receptor specific radioligands (Burns et al., 2007; Terry et al.; Terry et al., 2010; Terry et al., 2009; Van Laere et al., 2008; Wong et al., 2010) and could be explained by the limited availability of the hippocampus CB $_1$ receptor for *in vivo* binding as suggested by data showing that approximately 85% of these receptors are located in intracellular vesicles (Burns et al., 2007; Coutts et al., 2001).

AD is characterized by episodes of alcohol deprivation alternating with episodes of increased alcohol intake. Therefore, the timing of the PET studies in relation to alcohol consumption and deprivation is important to consider. Consistent with the alcohol deprivation phase in animals exposed to chronic alcohol intake which is associated with an upregulation of CB $_1$ receptor-mediated G-protein signaling in response to reduced eCB levels (Vinod et al., 2006), we found elevated [^{11}C]OMAR V_T values in AD subjects, indicating a possible upregulation of CB $_1$ receptors. Similarly to animal studies, CB $_1$ receptor upregulation could be associated with enhanced CB $_1$ receptor mediated G-protein signaling. We recently showed decreased serum anandamide levels in AD patients (Mangieri et al., 2009) and, although no significant correlation exists between anandamide concentrations in cerebrospinal fluid and serum (Koethe et al., 2009), it can be speculated that the CB $_1$ receptor upregulation in AD is a compensatory mechanism for a decrease in eCB tone during early abstinence. This change may represent a vulnerability factor for the development of craving and relapse. It is unclear, however, if these alterations are a state marker of early abstinence or represent a trait characteristic for AD. Animal models would suggest that we describe herein a state marker for early abstinence because CB $_1$ receptor function (Ortiz et al., 2004) as well as expression (Basavarajappa et al., 1998; Basavarajappa and Hungund, 2005) were reduced immediately after chronic alcohol intake. Future PET studies in actively drinking AD patients will answer this important question.

Our study does not answer if elevated CB $_1$ receptors are a pre-existing vulnerability factor for AD per se or result from alterations in other neurochemical systems that have been linked to the etiology of AD. For example, interactive effects between reduced striatal dopamine 2 and elevated CB $_1$ receptor availability were reported recently in animal models of AD (Thanos et al., 2011) and were substantiated with our human *in vivo* data showing elevated binding in the putamen suggesting complex system interactions moderating the reinforcing and addictive effects of alcohol in AD. Future longitudinal PET studies need to validate these animal models clarifying if changes of CB $_1$ receptor-mediated G-protein

signaling change in different phases of alcohol intake and abstinence as well as if altered CB₁ receptor expression in AD represents a change per se in the presence and absence of alterations in other neurochemical systems linked to AD.

In addition, it needs to be determined if women with AD show similar changes in CB₁ receptor binding, importantly because dopamine and CB₁ receptor interactions have only been studied in male mice to date (Thanos et al., 2011) and sex-dependent differences in eCB and dopaminergic functions do not allow to necessarily generalize our results for both sexes.

In conclusion, we report for the first time elevated CB₁ receptor binding in AD. Chronic alcohol use is seen as persistent challenges to the organism, leading to an altered set-point across multiple systems. This hypothesis is consistent with evidence of adaptations in brain reward and stress circuits that could contribute to important aspects of addictive processes like craving or urges, decreases in self-control, and a compulsive engagement in unhealthy behaviors, all of which characterize patients with AD. Our study directly implicates the CB₁ receptor in AD and suggests a mechanism for novel treatment development for AD (Bailey and Neumeister, 2011).

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References

- Bailey CR, Neumeister A. Cb1 receptor-mediated signaling emerges as a novel lead to evidence-based treatment development for stress-related psychopathology. *Neurosci Lett*. 2011; 502(1):1–4. [PubMed: 21787837]
- Basavarajappa BS, Cooper TB, Hungund BL. Chronic ethanol administration down-regulates cannabinoid receptors in mouse brain synaptic plasma membrane. *Brain Res*. 1998; 793(1–2):212–8. [PubMed: 9630633]
- Basavarajappa BS, Hungund BL. Role of the endocannabinoid system in the development of tolerance to alcohol. *Alcohol Alcohol*. 2005; 40(1):15–24. [PubMed: 15550443]
- Brown SA, Vik PW, McQuaid JR, Patterson TL, Irwin MR, Grant I. Severity of psychosocial stress and outcome of alcoholism treatment. *J Abnorm Psychol*. 1990; 99(4):344–8. [PubMed: 2266207]
- Brown SA, Vik PW, Patterson TL, Grant I, Schuckit MA. Stress, vulnerability and adult alcohol relapse. *J Stud Alcohol*. 1995; 56(5):538–45. [PubMed: 7475034]
- Burns HD, Van Laere K, Sanabria-Bohorquez S, Hamill TG, Bormans G, Eng WS, Gibson R, Ryan C, Connolly B, Patel S, Krause S, Vanko A, Van Hecken A, Dupont P, De Lepeleire I, Rothenberg P, Stoch SA, Cote J, Hagmann WK, Jewell JP, Lin LS, Liu P, Goulet MT, Gottesdiener K, Wagner JA, de Hoon J, Mortelmans L, Fong TM, Hargreaves RJ. [18F]MK-9470, a positron emission tomography (PET) tracer for in vivo human PET brain imaging of the cannabinoid-1 receptor. *Proc Natl Acad Sci U S A*. 2007; 104(23):9800–5. [PubMed: 17535893]
- Carson, RE.; Barker, WC.; Liow, J-S.; Adler, S.; Johnson, CA. Design of a motion-compensation OSEM List-mode Algorithm for Resolution-Recovery Reconstruction of the HRRT. Paper

- presented at the Conf Record IEEE Nuclear Science Symposium and Medical Imaging; Portland, OR. 2003.
- Colombo G, Serra S, Brunetti G, Gomez R, Melis S, Vacca G, Carai MM, Gessa L. Stimulation of voluntary ethanol intake by cannabinoid receptor agonists in ethanol-preferring sP rats. *Psychopharmacology (Berl)*. 2002; 159(2):181–7. [PubMed: 11862347]
- Coutts AA, Anavi-Goffer S, Ross RA, MacEwan DJ, Mackie K, Pertwee RG, Irving AJ. Agonist-induced internalization and trafficking of cannabinoid CB1 receptors in hippocampal neurons. *J Neurosci*. 2001; 21(7):2425–33. [PubMed: 11264316]
- Dom G, De Wilde B, Hulstijn W, van den Brink W, Sabbe B. Decision-making deficits in alcohol-dependent patients with and without comorbid personality disorder. *Alcohol Clin Exp Res*. 2006; 30(10):1670–7. [PubMed: 17010134]
- Femenia T, Garcia-Gutierrez MS, Manzanares J. CB1 receptor blockade decreases ethanol intake and associated neurochemical changes in fawn-hooded rats. *Alcohol Clin Exp Res*. 2010; 34(1):131–41. [PubMed: 19860799]
- First, MB.; Gibbon, M.; Spitzer, RL.; Williams, JBW. Biometrics Department, New York State Psychiatric Institute, New York. New York: 1996.
- Fox HC, Hong KI, Siedlarz KM, Bergquist K, Anderson G, Kreek MJ, Sinha R. Sex-specific dissociations in autonomic and HPA responses to stress and cues in alcohol-dependent patients with cocaine abuse. *Alcohol Alcohol*. 2009; 44(6):575–85. [PubMed: 19793926]
- Gallate JE, Saharov T, Mallet PE, McGregor IS. Increased motivation for beer in rats following administration of a cannabinoid CB1 receptor agonist. *Eur J Pharmacol*. 1999; 370(3):233–40. [PubMed: 10334497]
- Glass M, Dragunow M, Faull RL. Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience*. 1997; 77(2):299–318. [PubMed: 9472392]
- Goldstein RZ, Leskovan AC, Hoff AL, Hitzemann R, Bashan F, Khalsa SS, Wang GJ, Fowler JS, Volkow ND. Severity of neuropsychological impairment in cocaine and alcohol addiction: association with metabolism in the prefrontal cortex. *Neuropsychologia*. 2004; 42(11):1447–58. [PubMed: 15246283]
- Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR, Rice KC. Cannabinoid receptor localization in brain. *Proc Natl Acad Sci U S A*. 1990; 87(5):1932–6. [PubMed: 2308954]
- Hilton J, Yokoi F, Dannals RF, Ravert HT, Szabo Z, Wong DF. Column-switching HPLC for the analysis of plasma in PET imaging studies. *Nucl Med Biol*. 2000; 27(6):627–30. [PubMed: 11056380]
- Hirvonen J, Goodwin RS, Li CT, Terry GE, Zoghbi SS, Morse C, Pike VW, Volkow ND, Huestis MA, Innis RB. Reversible and regionally selective downregulation of brain cannabinoid CB1 receptors in chronic daily cannabis smokers. *Mol Psychiatry*.
- Horti AG, Fan H, Kuwabara H, Hilton J, Ravert HT, Holt DP, Alexander M, Kumar A, Rahmim A, Scheffel U, Wong DF, Dannals RF. 11C-JHU75528: a radiotracer for PET imaging of CB1 cannabinoid receptors. *J Nucl Med*. 2006; 47(10):1689–96. [PubMed: 17015906]
- Hungund BL, Szakall I, Adam A, Basavarajappa BS, Vadasz C. Cannabinoid CB1 receptor knockout mice exhibit markedly reduced voluntary alcohol consumption and lack alcohol-induced dopamine release in the nucleus accumbens. *J Neurochem*. 2003; 84(4):698–704. [PubMed: 12562514]
- Hunt WA, Barnett LW, Branch LG. Relapse rates in addiction programs. *J Clin Psychol*. 1971; 27(4):455–6. [PubMed: 5115648]
- Ichise M, Toyama H, Innis RB, Carson RE. Strategies to improve neuroreceptor parameter estimation by linear regression analysis. *J Cereb Blood Flow Metab*. 2002; 22(10):1271–81. [PubMed: 12368666]
- 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. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. *J Cereb Blood Flow Metab*. 2007; 27(9):1533–9. [PubMed: 17519979]

- Koethe D, Giuffrida A, Schreiber D, Hellmich M, Schultze-Lutter F, Ruhrmann S, Klosterkötter J, Piomelli D, Leweke FM. Anandamide elevation in cerebrospinal fluid in initial prodromal states of psychosis. *Br J Psychiatry*. 2009; 194(4):371–2. [PubMed: 19336792]
- Maccioni P, Colombo G, Carai MA. Blockade of the cannabinoid CB1 receptor and alcohol dependence: preclinical evidence and preliminary clinical data. *CNS Neurol Disord Drug Targets*. 2010; 9(1):55–9. [PubMed: 20201816]
- Maccioni P, Pes D, Carai MA, Gessa GL, Colombo G. Suppression by the cannabinoid CB1 receptor antagonist, rimonabant, of the reinforcing and motivational properties of a chocolate-flavoured beverage in rats. *Behav Pharmacol*. 2008; 19(3):197–209. [PubMed: 18469537]
- Makris N, Oscar-Berman M, Jaffin SK, Hodge SM, Kennedy DN, Caviness VS, Marinkovic K, Breiter HC, Gasic GP, Harris GJ. Decreased volume of the brain reward system in alcoholism. *Biol Psychiatry*. 2008; 64(3):192–202. [PubMed: 18374900]
- Malinen H, Hyytiä P. Ethanol self-administration is regulated by CB1 receptors in the nucleus accumbens and ventral tegmental area in alcohol-preferring AA rats. *Alcohol Clin Exp Res*. 2008; 32(11):1976–83. [PubMed: 18782338]
- Mangieri RA, Hong KI, Piomelli D, Sinha R. An endocannabinoid signal associated with desire for alcohol is suppressed in recently abstinent alcoholics. *Psychopharmacology (Berl)*. 2009; 205(1):63–72. [PubMed: 19343330]
- Miller WR, Westerberg VS, Harris RJ, Tonigan JS. What predicts relapse? Prospective testing of antecedent models. *Addiction*. 1996; 91(Suppl):S155–72. [PubMed: 8997790]
- Naassila M, Pierrefiche O, Ledent C, Daoust M. Decreased alcohol self-administration and increased alcohol sensitivity and withdrawal in CB1 receptor knockout mice. *Neuropharmacology*. 2004; 46(2):243–53. [PubMed: 14680762]
- Nolen-Hoeksema S, Hilt L. Possible contributors to the gender differences in alcohol use and problems. *J Gen Psychol*. 2006; 133(4):357–74. [PubMed: 17128956]
- Normandin MD, Weinzimmer DP, Ropchan J, Labaree D, Lin K-S, Mason NS, Carson RE, Neumeister A, Huang Y. Modeling analysis of the CB1 PET tracer [11C]OMAR in rhesus monkeys and humans. *Neuroimage*. 2010a; 52(10):S13.
- Normandin MD, Zheng M-Q, Ropchan JR, Labaree D, Najafzadeh S, Hull R, Qian W, Henry S, Williams WA, Carson RE, Huang Y, Neumeister A. Imaging the cannabinoid CB1 receptor in humans with [11C]OMAR: test-retest reproducibility and gender differences. *Neuroimage*. 2010b; 52(10):S82–S83.
- O'Brien CP, Childress AR, Ehrman R, Robbins SJ. Conditioning factors in drug abuse: can they explain compulsion? *J Psychopharmacol*. 1998; 12(1):15–22. [PubMed: 9584964]
- Ortiz S, Oliva JM, Perez-Rial S, Palomo T, Manzanares J. Differences in basal cannabinoid CB1 receptor function in selective brain areas and vulnerability to voluntary alcohol consumption in Fawn Hooded and Wistar rats. *Alcohol Alcohol*. 2004; 39(4):297–302. [PubMed: 15208160]
- Poncelet M, Maruani J, Calassi R, Soubrie P. Overeating, alcohol and sucrose consumption decrease in CB1 receptor deleted mice. *Neurosci Lett*. 2003; 343(3):216–8. [PubMed: 12770700]
- Schuckit MA, Smith TL. An 8-year follow-up of 450 sons of alcoholic and control subjects. *Arch Gen Psychiatry*. 1996; 53(3):202–10. [PubMed: 8611056]
- Serrano A, Parsons LH. Endocannabinoid influence in drug reinforcement, dependence and addiction-related behaviors. *Pharmacol Ther*. 2011; 132(3):215–41. [PubMed: 21798285]
- Sinha R. How does stress increase risk of drug abuse and relapse? *Psychopharmacology (Berl)*. 2001; 158(4):343–59. [PubMed: 11797055]
- Sullivan EV, Pfefferbaum A. Neurocircuitry in alcoholism: a substrate of disruption and repair. *Psychopharmacology (Berl)*. 2005; 180(4):583–94. [PubMed: 15834536]
- Terry GE, Hirvonen J, Liow JS, Zoghbi SS, Gladding R, Tauscher JT, Schaus JM, Phebus L, Felder CC, Morse CL, Donohue SR, Pike VW, Halldin C, Innis RB. Imaging and quantitation of cannabinoid CB1 receptors in human and monkey brains using (18)F-labeled inverse agonist radioligands. *J Nucl Med*. 51(1):112–20. [PubMed: 20008988]
- Terry GE, Hirvonen J, Liow JS, Zoghbi SS, Gladding R, Tauscher JT, Schaus JM, Phebus L, Felder CC, Morse CL, Donohue SR, Pike VW, Halldin C, Innis RB. Imaging and quantitation of

- cannabinoid CB1 receptors in human and monkey brains using (18)F-labeled inverse agonist radioligands. *J Nucl Med*. 2010; 51(1):112–20. [PubMed: 20008988]
- Terry GE, Liow JS, Zoghbi SS, Hirvonen J, Farris AG, Lerner A, Tauscher JT, Schaus JM, Phebus L, Felder CC, Morse CL, Hong JS, Pike VW, Halldin C, Innis RB. Quantitation of cannabinoid CB1 receptors in healthy human brain using positron emission tomography and an inverse agonist radioligand. *Neuroimage*. 2009; 48(2):362–70. [PubMed: 19573609]
- Thanos PK, Dimitrakakis ES, Rice O, Gifford A, Volkow ND. Ethanol self-administration and ethanol conditioned place preference are reduced in mice lacking cannabinoid CB1 receptors. *Behav Brain Res*. 2005; 164(2):206–13. [PubMed: 16140402]
- Thanos PK, Gopez V, Delis F, Michaelides M, Grandy DK, Wang GJ, Kunos G, Volkow ND. Upregulation of cannabinoid type 1 receptors in dopamine D2 receptor knockout mice is reversed by chronic forced ethanol consumption. *Alcohol Clin Exp Res*. 2011; 35(1):19–27. [PubMed: 20958329]
- Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Mazoyer B, Joliot M. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage*. 2002; 15(1):273–89. [PubMed: 11771995]
- Van Laere K, Goffin K, Casteels C, Dupont P, Mortelmans L, de Hoon J, Bormans G. Gender-dependent increases with healthy aging of the human cerebral cannabinoid-type 1 receptor binding using [(18)F]MK-9470 PET. *Neuroimage*. 2008; 39(4):1533–41. [PubMed: 18077184]
- Vinod KY, Hungund BL. Cannabinoid-1 receptor: a novel target for the treatment of neuropsychiatric disorders. *Expert Opin Ther Targets*. 2006; 10(2):203–10. [PubMed: 16548770]
- Vinod KY, Yalamanchili R, Thanos PK, Vadasz C, Cooper TB, Volkow ND, Hungund BL. Genetic and pharmacological manipulations of the CB(1) receptor alter ethanol preference and dependence in ethanol preferring and nonpreferring mice. *Synapse*. 2008; 62(8):574–81. [PubMed: 18509854]
- Vinod KY, Yalamanchili R, Xie S, Cooper TB, Hungund BL. Effect of chronic ethanol exposure and its withdrawal on the endocannabinoid system. *Neurochem Int*. 2006; 49(6):619–25. [PubMed: 16822589]
- Wong DF, Kuwabara H, Horti AG, Raymont V, Brasic J, Guevara M, Ye W, Dannals RF, Ravert HT, Nandi A, Rahmim A, Ming JE, Grachev I, Roy C, Cascella N. Quantification of cerebral cannabinoid receptors subtype 1 (CB1) in healthy subjects and schizophrenia by the novel PET radioligand [11C]OMAR. *Neuroimage*. 2010; 52(4):1505–13. [PubMed: 20406692]

Figure 1.

Upper panel: box plots showing significantly different [^{11}C]OMAR volume of distribution (V_T) values between alcohol dependent patients (ETOH) and healthy control subjects (HC) in individual regions of interest (ROI) constituting a circuit that has been implicated in the etiology of alcohol dependence. ACC indicates anterior cingulate cortex, PCC indicates posterior cingulate cortex, OFC indicates orbitofrontal cortex.

Lower panel: [^{11}C]OMAR positron emission tomography (PET) images (coronal view) illustrate elevated amygdala [^{11}C]OMAR binding in a single patient with alcohol dependence (right) relative to a healthy control subject (left).

Table 1

Study Participant Demographic, Clinical and Positron Emission Tomography Procedural Characteristics

	Alcohol Dependent (N=8)	Healthy Control (N=8)	P Value
Demographics			
Age, years	37.4 ± 7.9	32.5 ± 6.9	0.20
Age Range, years	25–49	24–45	-
Race/Ethnicity	3C, 5AA, 0H	4C, 2AA, 2H	-
BMI	26.0 ± 4.0	26.4 ± 2.7	0.83
Smoking status (Y/N)	5/3	0/8	0.007
Age at First Drink, years	15.8 ± 2.4	21.9 ± 3.7	0.002
Injection Parameters			
Injected Dose (MBQ)	669 ± 15.4	633 ± 96	0.33
Specific Activity (MBQ/nmol)	107 ± 44.3	125 ± 59.3	0.52
Injected Mass (μg)	3.82 ± 2.40	3.27 ± 2.22	0.64

Data presented in mean ± standard deviation, unless otherwise indicated. P values determined by independent sample t-tests for continuous variables or by chi-square for dichotomous variables.

AA, African-American; AS, Asian-American; BMI, body mass index; C, Caucasian; CIWA-Ar, Clinical Institute Withdrawal Assessment of Alcohol Scale, Revised; F, female; H, Hispanic; M, male; N, nonsmoker; S, smoker.

Table 2
Regional [¹¹C]OMAR Volumes of Distribution (V_T) Values in Men with Alcohol Dependence and Healthy Control Subjects

Brain Region	Alcohol Dependence (n=8)	Healthy Control (n=8)	Mean Difference	% Difference	Post Hoc Analysis of Variance (ANOVA)
Amygdala	1.37 ± 0.21	1.13 ± 0.17	0.24	21.2%	F(1,14)= 6.78, p= 0.021
ACC	1.44 ± 0.23	1.23 ± 0.12	0.21	17.1%	F(1,14)= 5.50, p= 0.034
PCC	1.02 ± 0.20	0.83 ± 0.05	0.19	22.9%	F(1,14)= 6.95, p= 0.020
OFC	1.28 ± 0.18	1.07 ± 0.11	0.21	19.6%	F(1,14)= 7.66, p= 0.015
Insula	1.27 ± 0.24	1.05 ± 0.14	0.22	20.1%	F(1,14)= 5.40, p= 0.036
Hippocampus	1.38 ± 0.22	1.17 ± 0.13	0.21	17.9%	F(1,14)= 5.07, p= 0.041
Putamen	1.45 ± 0.22	1.25 ± 0.13	0.20	16%	F(1,14)= 4.71, p= 0.048

Mean ± standard deviation [¹¹C]OMAR V_T values in seven *a priori* regions of interest in alcohol dependent compared to healthy control subjects. Statistics in table represent between-subject post-hoc ANOVA results with regional [¹¹C]OMAR V_T as dependent variable and group as predictor variable. ACC indicates anterior cingulate cortex, PCC indicates posterior cingulate cortex, OFC indicates orbitofrontal cortex