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Nociceptin receptors in alcohol use disorders: a PET study using [¹¹C]NOP-1A

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Abstract

BACKGROUND—The neuropeptide transmitter nociceptin, which binds to the nociceptin/orphanin FQ peptide (NOP) receptor, is a core component of the brain's anti-stress system. Nociceptin exerts its anti-stress effect by counteracting the functions of corticotropin releasing factor, the primary stress-mediating neuropeptide in the brain. Basic investigations support a role for medications that target nociceptin receptors in the treatment of alcohol use disorders. Thus, it is of high interest to measure the in vivo status of NOP receptors in individuals with alcohol use disorders.

METHODS—Here, we used [¹¹C]NOP-1A and PET to measure the in vivo binding to NOP receptors in 15 DSM-IV alcohol-dependent humans and 15 healthy controls matched for age, gender, and smoking status. Alcohol-dependent individuals with no comorbid psychiatric, medical, or drug abuse disorders were scanned following two weeks of outpatient monitored abstinence (confirmed with 3×/week urine alcohol metabolite testing). [¹¹C]NOP-1A distribution volume (V_T) in regions of interest (including the amygdala, hippocampus, midbrain, striatal, and prefrontal cortical subdivisions) were measured with kinetic analysis using the arterial input function.

RESULTS—Regional [¹¹C]NOP-1A V_T in alcohol-dependence was not significantly different compared to healthy controls. No relationship between [¹¹C]NOP-1A V_T and other clinical measures (including duration and severity of alcohol abuse, craving, anxiety or depressive symptoms) were significant after correction for the multiple hypotheses tested.

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DISCLOSURES

The authors report no biomedical financial interests or potential conflicts of interest.

CONCLUSIONS—The results of this study do not support alterations in the binding to NOP receptors in alcohol dependence. However, this finding does not necessarily rule out alterations in nociceptin transmission in alcohol dependence.

Keywords

[¹¹C]NOP-1A; positron emission tomography; brain; anti-stress; alcohol dependence; nociceptin/orphanin FQ peptide receptors

INTRODUCTION

Alcohol dependence is a chronic disorder characterized by numerous relapses and periods of abstinence. The three phases of addiction are intoxication, withdrawal and craving—in which the earlier phase is dominated by impulsive behaviors and the later phase is dominated by compulsive behaviors (1). The transition from impulsive to compulsive behaviors in addiction has been linked to a shift in highly dopamine-dependent positive reinforcement (e.g., repeated reward from alcohol) to negative reinforcement (e.g., alcohol use repeatedly removes aversive withdrawal symptoms) motivated behaviors (2). Rodent investigations attribute negative reinforcement driven compulsive behaviors to an imbalance between neurotransmitters in the brain stress (corticotropin releasing factor, norepinephrine, orexin, vasopressin and dynorphin) and anti-stress system (nociceptin and neuropeptide Y) (1). Nociceptin, which binds to the nociceptin/orphanin FQ peptide (NOP) receptor, is one such neuropeptide transmitter that exerts anti-stress effects by counteracting the functional effects of endogenous corticotropin releasing factor (CRF) in the brain (3, 4). Based on this, a therapeutic role for NOP agonists is to *increase nociceptin transmission* to counteract an overactive CRF system and thereby block negative reinforcement driven relapse in alcohol use disorders has been postulated (5). Consistent with this notion, nociceptin has been shown in behavioral studies to block both the rewarding properties of alcohol and reduce alcohol consumption in the conditioned place preference behavioral paradigm (6). Nociceptin is also effective in preventing the somatic and affective signs of alcohol withdrawal in alcohol-dependent rodents during both acute and protracted withdrawal (7). Highly relevant to the clinic is the ability of nociceptin to block cue- and stress-induced alcohol reinstatement following prolonged abstinence in rodent models of alcohol use disorders (8, 9). Studies have also shown that decreased nociceptin levels in the amygdala are associated with increased alcohol intake (10), and an injection of nociceptin into the central nucleus of the amygdala suppresses alcohol self-administration (11). Further, consistent with this notion is a human postmortem study in which a 70% decrease in pronociceptin (the precursor protein of nociceptin) gene expression (mRNA) was observed in the hippocampus of alcohol dependent subjects compared to controls (12). However, no such decreases in pronociceptin mRNA was observed in other brain regions; such as, the central nucleus of the amygdala and prefrontal cortex both of which have been implicated in rodents. In the same postmortem brain tissue study, there was a 40% reduction of NOP receptor mRNA in the central nucleus of the amygdala of alcohol dependent subjects compared to controls. No such reductions in NOP mRNA were noted in the hippocampus and prefrontal cortex. Despite the fact that this human study in postmortem brains suggests decreased nociceptin transmission and NOP receptors in the hippocampus and amygdala respectively, the lack of

a difference in several other brain regions examined raise concerns as to the robustness of these findings. In addition, confounds inherent to the design of a postmortem human study (such as: inability to exclude alcohol use disorders with comorbid medical, psychiatric and drug/nicotine abuse history, within-subject differences in the cause of death, inability to control for the presence or absence of alcohol at the time of death that may lead to mix of subjects with intoxication, short-term withdrawal and long-term abstinence, etc.) and difficulties in extrapolating receptor density (Bmax) from measurements of mRNA cannot be excluded as factors influencing these results. Thus, it was important to use in vivo imaging methods to measure binding to NOP receptors in living humans with alcohol use disorders.

In a major advancement, the imaging group at NIMH recently radiolabeled and successfully validated a NOP receptor antagonist radiotracer [^{11}C] (S)-3-(2'-fluoro-6',7'-dihydrospiro[piperidine-4,4'-thieno[3,2-c]pyran]-1-yl)-2-(2-fluorobenzyl)-N-methylpropanamide (NOP-1A) for use in humans (13). [^{11}C]NOP-1A binds with relatively high affinity to NOP receptors (K_D , 0.15 nM). [^{11}C]NOP-1A total uptake in various brain regions (V_T) is consistent with the known distribution of NOP receptors in human and primate brain (relatively high density in amygdala, striatum and cerebral cortex; and moderate density in cerebellum and midbrain) (14–17). Blocking studies in primates with the specific NOP receptor antagonist SB-612111 (K_D 0.33 nM, 1.0 mg/kg intravenously) indicate that 50–70% of [^{11}C]NOP-1A V_T in brain regions represents specific binding to NOP receptors (16). Consistent with this are studies in humans that have demonstrated >80% of the in vivo binding of [^{11}C]NOP-1A is displaceable by the NOP antagonist LY2940094 (18). [^{11}C]NOP-1A demonstrates desirable pharmacokinetics in humans for the following reasons: brain uptake peaks relatively quickly (~ 10 min) and washes out rapidly, brain data is modeled well by a 2-tissue compartment kinetic analysis, V_T values in brain regions range from 7 to 15, V_T remains stable following 70 min of data acquisition, and the test-retest variability for V_T is an acceptable 15% in all brain regions (17, 19). Here, we used [^{11}C]NOP-1A PET to measure the in vivo binding to NOP receptors in 15 recently abstinent subjects with alcohol dependence and 15 healthy comparison subjects. Based on the post-mortem human study, we hypothesized that individuals with alcohol dependence will have *lower* [^{11}C]NOP-1A V_T compared to healthy controls in brain regions that are involved in stress-response (amygdala and hippocampus) and addictive behaviors (midbrain, striatal, and prefrontal cortical subdivisions). Such a finding would suggest decreased NOP receptor mediated transmission in alcohol dependence. Alcohol dependent subjects were scanned early in abstinence (14+ days after last drink of alcohol) as opposed to when they were withdrawing from alcohol (3–5 days). The rationale for this was based on the fact that a decrease in NOP receptor levels that persists past acute withdrawal will be predictive of the neurobiology that underlies relapse in alcohol use disorders. The demonstration of such enduring and persistent changes in NOP that need to be corrected has the potential to spur the development of medications that target NOP as a treatment for alcohol use disorders. In contrast, transient abnormalities in NOP receptor levels witnessed during withdrawal that revert back to normal early in abstinence would not support the pursuit of NOP medications for relapse prevention.

MATERIALS AND METHODS

Human Subjects

Fifty-three alcohol dependent subjects and 26 healthy controls were enrolled in the study to arrive at 15 completers/group. The study was conducted following the approvals of the University of Pittsburgh Institutional Review Board and Radioactive Drug Research Committee. All subjects provided written informed consent. Alcohol dependent subjects and healthy controls were largely recruited through advertisements displayed at local community centers, buses, newspapers, and websites. In addition, addiction medicine clinics and hospital emergency rooms in the community referred alcohol dependent subjects. Study criteria for alcohol dependence were [1] males or females between 21 and 55 years old of all ethnic and racial origins; [2] fulfill DSM-IV criteria for alcohol dependence as assessed by SCID-IV; [3] no other DSM-IV Axis-I disorder other than alcohol abuse or dependence, including abuse or dependence to other recreational drugs (nicotine dependence was allowed); [4] no current use of cocaine, opiates, cannabis, sedative-hypnotics, amphetamines, 3,4-methylenedioxy-N-methylamphetamine, and phencyclidine, as confirmed by urine drug screen at screening; [5] not currently on any prescription or over the counter medications (other than oral contraceptive pills); [6] no medical or neurological disorders (including chronic respiratory disorders, renal, or liver disorders) as assessed by a complete physical exam and labs; [7] not currently pregnant; [8] no history of radioactivity exposure from nuclear medicine studies or occupation; and [9] no metallic objects in the body that are contraindicated for magnetic resonance imaging (MRI).

Alcohol dependent subjects completed a minimum of 14-days of outpatient abstinence monitored with witnessed urine toxicology. Subjects were monitored for alcohol and recreational drug use with urine alcohol metabolite (ethyl glucuronide and ethyl sulfate) and urine drug screens three times/week for two consecutive weeks. Since alcohol metabolites and common drugs of abuse can be detected for 2 to 3 days after use, subjects were informed that they should not use alcohol or street drugs for the 14 days prior to the PET study. In order to promote abstinence from alcohol during this two-week period, subjects were paid \$50 for each urine sample that was negative for ethyl glucuronide and ethyl sulfate. Alcohol dependent subjects were scheduled for the PET scans after successful completion of the abstinence monitoring protocol. Subjects who tested positive for ethyl glucuronide and ethyl sulfate were offered up to three attempts to re-start the abstinence monitoring protocol. This abstinence monitoring protocol ensured that all subjects were abstinent for a minimum of two weeks prior to the PET scan. Alcohol dependent subjects were also monitored for alcohol withdrawal signs and symptoms three times/week during the first week of abstinence using the Clinical Institute Withdrawal Assessment of Alcohol Scale, CIWA (20). Alcohol dependent subjects who were at risk of severe withdrawal, i.e., scored greater than 19 on the Clinical Institute Withdrawal Assessment of Alcohol Scale and had prior history of alcohol withdrawal seizures or delirium tremens, were excluded from the research protocol. Clinical assessments, including the Penn Alcohol Craving Scale, Hamilton Anxiety Rating Scale (HAM-A), and Center for Epidemiological Studies Depression Scale (CES-D), were performed once at baseline as part of the intake. These intake assessments were completed within the first week of enrollment in the study. The severity of alcohol dependence was

assessed in with the Michigan Alcohol Screening Test (21), Alcohol Dependence Scale (22) and Alcohol Use Disorders Identification Test (23). The craving to use alcohol was assessed with the Penn Alcohol Craving Scale (24). Nicotine dependence was assessed using the Fagerstrom Test for Nicotine Dependence (25). Anxiety and depressive symptoms were measured using the HAM-A (26) and the CES-D (27).

Healthy control subjects matched for age, gender, smoking status, no past or present neurological or psychiatric illnesses including substance abuse (confirmed by urine drug screen both at screening and the day of the PET scan). Healthy controls and alcohol dependent subjects underwent the PET scan as outpatients.

Image acquisition and analysis

Prior to PET imaging, a magnetization prepared rapid gradient echo structural MRI scan was obtained using a Siemens 3T Trio scanner for region determination. The synthesis of [^{11}C]NOP-1A was carried out as previously described (13). PET imaging sessions were conducted with the ECAT EXACT HR+ camera. The injected dose and mass of [^{11}C]NOP-1A were restricted to 12 mCi and 4.2 μg (19). Following a transmission scan, subjects received an intravenous bolus injection of [^{11}C]NOP-1A and emission data was collected for 70 minutes, which is the minimum scanning time necessary to arrive at stable V_T values for [^{11}C]NOP-1A (17). Input function measurements were performed to correspond with that reported in (16) and (17). Briefly, arterial blood samples were collected throughout the course of the scan (33 total samples with 20 over initial 2 min). Following centrifugation of the samples (2 min at 12,500 g), plasma was collected and activity measured in 200 μl aliquots on a gamma counter. To determine the plasma activity representing unmetabolized parent compound ([^{11}C]NOP-1A) seven samples (collected at 4, 8, 12, 20, 30, 40 and 60 min) were further processed using HPLC methods previously described (13). The seven measured parent fractions for [^{11}C]NOP-1A were fitted using a Hill model (28, 29) consistent with that reported in prior validation studies (17, 19). Plasma free fraction (f_p) was determined using ultrafiltration as previously described (30, 31). In addition, an aliquot of saline buffer solution mixed with the radiotracer was processed to determine the filter retention of the free tracer.

PET data were reconstructed and processed with the image analysis software MEDx (Sensor Systems, Inc., Sterling, Virginia) and SPM2 (www.fil.ion.ucl.ac.uk/spm) as described in (31). Frame-to-frame motion correction for head movement and MR-PET image alignment were performed using a mutual information algorithm implemented in SPM2. MRI segmentation was performed using the automated segmentation tool in Functional MRI of the Brain Software Library (32). Based on prior basic studies that have evaluated nociceptin and NOP, regions of interest (ROIs) were restricted to the amygdala, hippocampus, midbrain, striatum (ventral striatum, caudate and putamen) and prefrontal cortex (specifically the dorsolateral, orbital, medial, and anterior cingulate) subdivisions. In addition, the cerebellum was included as a region for which we had no specific hypothesis. Prefrontal cortical regions and subcortical regions of interest were defined on the MRI using a segmentation-based and direct identification method described in (31, 33, 34). Regional volumes and time activity curves were then generated in MEDx as outlined in (33, 35).

Derivation of [^{11}C]NOP-1A volume of distribution expressed relative to total plasma concentration (V_T) in the regions of interest were performed using a two-tissue compartment kinetic analysis using the arterial input function (17, 19, 36). V_T , which is the total radioligand in the region of interest, includes both the receptor-bound specific (V_S) and non-specific binding (V_{ND}). This was used as the primary outcome measure rather than V_S because [^{11}C]NOP-1A blocking studies in primates have shown that there is no region in the brain that is devoid of specific binding to NOP receptors (16). Thus, it was necessary to perform an arterial line in all subjects.

Statistical analysis

Group demographic and baseline scan parameter (such as injected dose, mass, plasma clearance) comparisons were performed with unpaired t-tests. Group differences in [^{11}C]NOP-1A V_T were analyzed with a linear mixed model analysis performed with regions of interest as a repeated measure and diagnostic group (alcohol dependence vs. healthy controls) as the fixed factor (IBM SPSS Statistics). The relationship between PET data and clinical characteristics (days since last drink, number of drinking days/month, amount of drinks/day, scores on rating scales for alcohol, alcohol craving, anxiety and depression, etc.,) were explored by Pearson product moment correlation coefficient. A two-tailed probability value of $p < 0.05$ was selected as the significance level for all analyses.

RESULTS

Fifteen alcohol dependent subjects were matched with 15 healthy controls on age, gender, ethnicity and smoking status. Table 1 lists demographics variables and clinical characteristics of the study sample. Alcohol dependent subjects were more likely to have a first degree relative with alcohol use disorder and start using alcohol earlier than controls. They also consumed significantly more amounts of alcohol at an increased frequency compared to controls. When compared to controls, alcohol dependent subjects scored significantly higher on depressive and anxiety rating scales despite not meeting DSM-IV diagnostic criteria on SCID-IV disorders.

Baseline parameters

Table 2 shows the [^{11}C]NOP-1A scan parameters for healthy controls and alcohol dependent subjects. No significant group differences were noted in any of these scan parameters. The [^{11}C]NOP-1A plasma free fraction (fp) measurements were not reliable because the tracer showed a relatively high retention to the filter in the saline buffer solution condition. No significant between-group differences were noted in the regions of interest volumes determined from the MRI scans (data not shown). Thus, no partial volume correction (also known as atrophy correction) was performed on [^{11}C]NOP-1A V_T in regions of interest.

In vivo binding of [^{11}C]NOP-1A (V_T)

As shown in Figure 1, no differences in [^{11}C]NOP-1A V_T were observed in alcohol dependence compared to healthy controls (linear mixed model, effect of diagnosis, $F(1, 28) = 0.015$, $p = 0.90$; effect of region, $F(10, 280) = 341.88$, $p < 0.001$; region $\times$ diagnosis interaction, $F(10, 280) = 0.41$, $p = 0.94$). In addition, unpaired t-tests conducted at the level

of the individual regions of interest failed to show any significant differences between the two groups.

Clinical correlations

There was a significant negative correlation between [^{11}C]NOP-1A V_T in the orbital frontal cortex and cravings for alcohol as measured with the Penn Alcohol Craving Scale (Figure 2, $y = -0.20 + 16.66x$, $r^2 = 0.29$, $p = 0.04$). However, this relationship does not survive any correction for multiple hypotheses testing that accounts for the number of regions and clinical correlations examined. Trend level negative correlations with cravings for alcohol were also observed with V_T in the caudate, $r^2 = 0.23$, $p = 0.07$ and medial prefrontal cortex, $r^2 = 0.22$, $p = 0.08$. No other relationship between regional V_T and any of the clinical measures such as alcohol frequency, amount, duration of abuse, other alcohol-related scores, anxiety rating scales, and depression rating scales in alcohol dependent subjects were significant.

DISCUSSION

In this PET study, we found no differences in the in vivo binding of [^{11}C]NOP-1A in alcohol dependence relative to controls in critical brain regions that have been implicated in stress and addiction. It is unlikely that this PET study was underpowered to detect group differences in NOP-1A V_T . For example, in this study, [^{11}C]NOP-1A V_T in the amygdala (the region in which a significant decrease in NOP receptors was reported in postmortem studies) in alcohol dependent subjects and controls was 14.00 ± 2.32 and 13.99 ± 1.78 respectively. Using these data in a power calculation ($\beta = 0.8$) suggests that [^{11}C]NOP-1A PET scans will need to be acquired in $n = 671,142$ subjects/group to demonstrate statistically significant group differences. These data are convincing in showing no between-group difference in NOP-1A V_T , and increasing power is unlikely to alter this conclusion. The negative result in this study can be viewed as unexpected given the wealth of basic investigations that support a role for nociceptin in cue- and stress-induced relapse in rodent models of alcohol use disorders. However, they are largely consistent with the human postmortem studies, which have examined NOP mRNA in individuals with substance use disorders. In the initial postmortem study that reported a 40% reduction in NOP mRNA in the central amygdala in alcohol use disorders relative to controls ($n = 15/\text{group}$), no significant group differences were observed in the hippocampus or prefrontal cortex (12). Consistent with this are the results of a more recent postmortem study that examined NOP receptor mRNA in the anterior insula, medial dorsal thalamus, and dorsal anterior cingulate cortex as it pertains to depression, addictive disorders, and suicide. The results of this study, which included 27 individuals with substance use disorders (56% had only alcohol dependence, 22% had both alcohol and drug dependence, 22% had only drug dependence) and 53 controls, also failed to demonstrate differences in NOP mRNA in the three brain regions that were examined (37). It is necessary for future postmortem studies to both replicate, as well as confirm with radioligand binding methodology, the decrease in NOP reported in the amygdala in alcohol use disorders. Several methodological factors may have influenced the results of this PET study. The use of V_T as the outcome measure, which includes the receptor-specific and non-specific binding component, may have diminished the

ability to detect altered NOP in alcohol dependence. This would be the case if there were significant differences in [^{11}C]NOP-1A non-specific binding (V_{ND}) between alcohol dependence and controls. We were unable to measure V_{ND} to exclude this possibility, because [^{11}C]NOP-1A lacks a reference region in the brain and there are currently no FDA-approved NOP antagonists to block specific signal in humans. Recent studies with [^{11}C]NOP-1A and a proprietary NOP antagonist LY2940094 have shown that greater than 80% of the in vivo binding of [^{11}C]NOP-1A (V_{T}) is displaceable and specific to NOP receptors (18). This suggests that it is less likely for small to medium size between-group differences in non-specific binding (V_{ND}) to have influenced our results, because specific binding represents such a large fraction of [^{11}C]NOP-1A V_{T} . Another factor that may have contributed to the negative results relates to the time-point at which the alcohol dependent subjects were scanned relative to their last alcoholic drink. In this study, alcohol dependent subjects were monitored for abstinence for a minimum of 14-days prior to their [^{11}C]NOP-1A PET scan. This led to a mean duration of abstinence in alcohol dependent subjects of 28 ± 11 days (minimum of 16 to a maximum of 54 days) prior to the PET scans. No relationship between regional [^{11}C]NOP-1A V_{T} and the number of days participants were abstinent from alcohol prior to the PET were noted. Ethanol administration studies in rodents have shown that nociceptin tissue concentration in the brain is lower at 30 minutes and 5 days, but not at 21 days following the cessation of alcohol (38). Based on this it is tempting to speculate that lower nociceptin and NOP receptor levels in alcohol withdrawal (1–5 days) recovered back to control levels with prolonged abstinence (21+ days) in our study. Such a postulation is in line with the ability of nociceptin to ameliorate the withdrawal symptoms in rodent models of alcohol use disorders (7). A related issue that may explain the negative result in this study was the absence of significant withdrawal signs and symptoms as reported in the CIWA (range 0 to 67) in alcohol dependent subjects (CIWA average score in $n=15$ alcohol dependent subjects was 2.6 ± 3.3 on day 1 and 1.2 ± 2.8 on day 7 after abstaining from alcohol). This is likely due to the fact that alcohol dependent individuals in this study were not severely dependent on alcohol; for example, high scores on the CIWA were exclusionary, abstinence was monitored on an outpatient basis, alcohol abuse clinical ratings were only suggestive of mild to moderate dependence. Nevertheless, the relatively low CIWA scores in alcohol dependent subjects is somewhat contradictory to the higher anxiety and depression scores (vs. controls) reported in the HAM-A and CES-D, which were performed during the same time period. Future studies should better characterize acute and protracted withdrawal in alcohol dependent subjects in order to examine whether NOP is abnormal. Studies to investigate NOP in individuals with severe alcohol dependence deserve further consideration as well. Lastly, given the extensive basic science literature on the role of NOP in stress and negative reinforcement it is possible that NOP receptor binding is altered only in alcohol-dependent subjects who drink to alleviate stress/withdrawal (stress-relief drinkers) as opposed to those who drink to feel happy/high (reward-motivated drinkers). Such NOP studies that focus on the factors that motivate alcohol consumption could clarify whether there are differences in NOP receptor binding in alcohol dependent subjects based on their interaction with stressors and/or withdrawal symptoms, i.e., the drivers of negative reinforcement.

Correlational analyses showed that decreased [^{11}C]NOP-1A binding (V_T) in the orbital frontal cortex is associated with increased craving for alcohol (Figure 2). Koob and colleagues have demonstrated that increases in brain CRF, the primary stress mediating neuropeptide, underlies relapse in alcohol use disorders (2). Studies in rodents support increases in brain CRF levels and CRF1 receptors not only in acute but also in protracted withdrawal, i.e., weeks after detoxification from repeated cycles of alcohol intoxication/withdrawal (39, 40). One of the mechanisms by which the brain counteracts increased CRF/stress has been shown to involve an upregulation of the NOP receptors to enhance nociceptin transmission/anti-stress (41, 42). Thus, increased craving in alcohol dependence with lower [^{11}C]NOP-1A V_T may represent an inadequate compensatory response to increased CRF. [^{11}C]NOP-1A studies in alcohol use disorders with larger sample sizes need to replicate the relationship between V_T and craving, and link it to relapse to realize its translational potential. The lack of a relationship between [^{11}C]NOP-1A V_T and anxiety/mood symptoms in recently abstinent alcohol dependence was unexpected as rodent models of alcohol use disorder have linked nociceptin to the somatic/affective signs in early and prolonged withdrawal. Nevertheless, the lack of moderate to severe anxiety and depressive symptoms in alcohol dependence enrolled in this study may have led to the inability to detect such a relationship.

In conclusion, we were unable to detect alterations in the in vivo binding of [^{11}C]NOP-1A in recently abstinent alcohol dependence compared to healthy controls. This result is partially consistent with human postmortem studies that have examined NOP in addictive disorders. However, it is largely incongruent with basic investigations that implicate NOP in stress, negative reinforcement, and relapse in rodent models of alcohol use disorder. The failure to detect between-group differences in NOP receptor binding does not rule out the presence of decreased nociceptin transmission in alcohol dependence. [^{11}C]NOP-1A studies that incorporate a challenge drug or task to alter endogenous nociceptin tone are required to further investigate this issue in alcohol dependence.

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References

1. Koob GF. Theoretical frameworks and mechanistic aspects of alcohol addiction: alcohol addiction as a reward deficit disorder. *Curr Top Behav Neurosci*. 2013; 13:3–30. [PubMed: 21744309]
2. Koob GF. A role for brain stress systems in addiction. *Neuron*. 2008; 59:11–34. [PubMed: 18614026]
3. Ciccocioppo R, Martin-Fardon R, Weiss F, Massi M. Nociceptin/orphanin FQ inhibits stress- and CRF-induced anorexia in rats. *Neuroreport*. 2001; 12:1145–1149. [PubMed: 11338181]
4. Cruz MT, Herman MA, Kallupi M, Roberto M. Nociceptin/orphanin FQ blockade of corticotropin-releasing factor-induced gamma-aminobutyric acid release in central amygdala is enhanced after chronic ethanol exposure. *Biol Psychiatry*. 2012; 71:666–676. [PubMed: 22153590]

5. Witkin JM, Statnick MA, Rorick-Kehn LM, Pintar JE, Ansonoff M, Chen Y, et al. The biology of Nociceptin/Orphanin FQ (N/OFQ) related to obesity, stress, anxiety, mood, and drug dependence. *Pharmacol Ther.* 2014; 141:283–299. [PubMed: 24189487]
6. Ciccocioppo R, Panocka I, Polidori C, Regoli D, Massi M. Effect of nociceptin on alcohol intake in alcohol-preferring rats. *Psychopharmacology (Berl).* 1999; 141:220–224. [PubMed: 9952048]
7. Economidou D, Cippitelli A, Stopponi S, Braconi S, Clementi S, Ubaldi M, et al. Activation of brain NOP receptors attenuates acute and protracted alcohol withdrawal symptoms in the rat. *Alcohol Clin Exp Res.* 2011; 35:747–755. [PubMed: 21223310]
8. Ciccocioppo R, Economidou D, Fedeli A, Angeletti S, Weiss F, Heilig M, et al. Attenuation of ethanol self-administration and of conditioned reinstatement of alcohol-seeking behaviour by the antioioid peptide nociceptin/orphanin FQ in alcohol-preferring rats. *Psychopharmacology (Berl).* 2004; 172:170–178. [PubMed: 14624331]
9. Martin-Fardon R, Ciccocioppo R, Massi M, Weiss F. Nociceptin prevents stress-induced ethanol-but not cocaine-seeking behavior in rats. *Neuroreport.* 2000; 11:1939–1943. [PubMed: 10884047]
10. Gustafsson L, Ploj K, Nylander I. Effects of maternal separation on voluntary ethanol intake and brain peptide systems in female Wistar rats. *Pharmacol Biochem Behav.* 2005; 81:506–516. [PubMed: 15927243]
11. Economidou D, Hansson AC, Weiss F, Terasmaa A, Sommer WH, Cippitelli A, et al. Dysregulation of nociceptin/orphanin FQ activity in the amygdala is linked to excessive alcohol drinking in the rat. *Biol Psychiatry.* 2008; 64:211–218. [PubMed: 18367152]
12. Kuzmin A, Bazov I, Sheedy D, Garrick T, Harper C, Bakalkin G. Expression of pronociceptin and its receptor is downregulated in the brain of human alcoholics. *Brain Res.* 2009; 1305(Suppl):S80–85. [PubMed: 19501074]
13. Pike VW, Rash KS, Chen Z, Pedregal C, Statnick MA, Kimura Y, et al. Synthesis and evaluation of radioligands for imaging brain nociceptin/orphanin FQ peptide (NOP) receptors with positron emission tomography. *J Med Chem.* 2011; 54:2687–2700. [PubMed: 21438532]
14. Berthele A, Platzer S, Dworzak D, Schadrack J, Mahal B, Buttner A, et al. [3H]-nociceptin ligand-binding and nociceptin opioid receptor mRNA expression in the human brain. *Neuroscience.* 2003; 121:629–640. [PubMed: 14568023]
15. Bridge KE, Wainwright A, Reilly K, Oliver KR. Autoradiographic localization of (125)I[Tyr(14)] nociceptin/orphanin FQ binding sites in macaque primate CNS. *Neuroscience.* 2003; 118:513–523. [PubMed: 12699786]
16. Kimura Y, Fujita M, Hong J, Lohith TG, Gladding RL, Zoghbi SS, et al. Brain and whole-body imaging in rhesus monkeys of 11C-NOP-1A, a promising PET radioligand for nociceptin/orphanin FQ peptide receptors. *J Nucl Med.* 2011; 52:1638–1645. [PubMed: 21880575]
17. Lohith TG, Zoghbi SS, Morse CL, Araneta MF, Barth VN, Goebel NA, et al. Brain and whole-body imaging of nociceptin/orphanin FQ peptide receptor in humans using the PET ligand 11C-NOP-1A. *J Nucl Med.* 2012; 53:385–392. [PubMed: 22312136]
18. Raddad E, Chappell A, Meyer J, Wilson A, Ruegg CE, Tauscher J, et al. Occupancy of Nociceptin/Orphanin FQ Peptide Receptors by the Antagonist LY2940094 in Rats and Healthy Human Subjects. *Drug Metab Dispos.* 2016; 44:1536–1542. [PubMed: 27353045]
19. Lohith TG, Zoghbi SS, Morse CL, Araneta MD, Barth VN, Goebel NA, et al. Retest imaging of [11C]NOP-1A binding to nociceptin/orphanin FQ peptide (NOP) receptors in the brain of healthy humans. *Neuroimage.* 2014; 87:89–95. [PubMed: 24225488]
20. Sullivan JT, Sykora K, Schneiderman J, Naranjo CA, Sellers EM. Assessment of alcohol withdrawal: the revised clinical institute withdrawal assessment for alcohol scale (CIWA-Ar). *Br J Addict.* 1989; 84:1353–1357. [PubMed: 2597811]
21. Selzer ML. The Michigan Alcoholism Screening Test: the quest for a new diagnostic instrument. *Am J Psychiatry.* 1976; 127:1653–1658.
22. Skinner HA, Allen BA. Alcohol dependence syndrome: measurement and validation. *J Abnorm Psychol.* 1982; 91:199–209. [PubMed: 7096790]
23. Saunders JB, Aasland OG, Babor TF, de la Fuente JR, Grant M. Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption--II. *Addiction.* 1993; 88:791–804. [PubMed: 8329970]

24. Flannery BA, Volpicelli JR, Pettinati HM. Psychometric properties of the Penn Alcohol Craving Scale. *Alcohol Clin Exp Res*. 1999; 23:1289–1295. [PubMed: 10470970]
25. Fagerstrom K, Heatherton TF, Koslowski T. Nicotine addiction and its assessment. *Ear Nose Throat J*. 1991; 69:763–765.
26. Hamilton M. The assessment of anxiety scales by rating. *Br J Med Psychol*. 1959; 32
27. Radloff LS. The use of the Center for Epidemiologic Studies Depression Scale in adolescents and young adults. *J Youth Adolesc*. 1991; 20:149–166. [PubMed: 24265004]
28. Hill AV. The possible effects of the aggregation of the molecules of haemoglobin on its disassociation curves. *Phys Med Biol*. 1910; 1991:749–761.
29. Gunn RN, Sargent PA, Bench CJ, Rabiner EA, Osman S, Pike VW, et al. Tracer kinetic modeling of the 5-HT_{1A} receptor ligand [carbonyl-¹¹C]WAY- 100635 for PET. *Neuroimage*. 1998; 8:426–440. [PubMed: 9811559]
30. Gandelman MS, Baldwin RM, Zoghbi SS, Zea-Ponce Y, Innis RB. Evaluation of ultrafiltration for the free fraction determination of single photon emission computerized tomography (SPECT) radiotracers: b-CIT, IBF and iomazenil. *J Pharmaceutical Sci*. 1994; 83:1014–1019.
31. Narendran R, Frankle WG, Mason NS, Rabiner EA, Gunn RN, Searle GE, et al. Positron emission tomography imaging of amphetamine-induced dopamine release in the human cortex: a comparative evaluation of the high affinity dopamine D_{2/3} radiotracers [¹¹C]FLB 457 and [¹¹C]fallypride. *Synapse*. 2009; 63:447–461. [PubMed: 19217025]
32. Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, et al. Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage*. 2004; 23(Suppl 1):S208–219. [PubMed: 15501092]
33. Abi-Dargham A, Mawlawi O, Lombardo I, Gil R, Martinez D, Huang Y, et al. Prefrontal dopamine D₁ receptors and working memory in schizophrenia. *J Neurosci*. 2002; 22:3708–3719. [PubMed: 11978847]
34. Narendran R, Mason NS, Laymon C, Lopresti B, Velasquez N, May M, et al. A comparative evaluation of the dopamine D_{2/3} agonist radiotracer [¹¹C]NPA and antagonist [¹¹C]raclopride to measure amphetamine-induced dopamine release in the human striatum. *Journal of Pharmacology and Experimental Therapeutics*. 2010; 63:574–584.
35. Abi-Dargham A, Martinez D, Mawlawi O, Simpson N, Hwang DR, Slifstein M, et al. Measurement of striatal and extrastriatal dopamine D₁ receptor binding potential with [¹¹C]NNC 112 in humans: validation and reproducibility. *J Cereb Blood Flow Metab*. 2000; 20:225–243. [PubMed: 10698059]
36. Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN, et al. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. *J Cereb Blood Flow Metab*. 2007; 27:1533–1539. [PubMed: 17519979]
37. Lutz PE, Zhou Y, Labbe A, Mechawar N, Turecki G. Decreased expression of nociceptin/orphanin FQ in the dorsal anterior cingulate cortex of suicides. *Eur Neuropsychopharmacol*. 2015; 25:2008–2014. [PubMed: 26349406]
38. Lindholm S, Ploj K, Franck J, Nylander I. Nociceptin/orphanin FQ tissue concentration in the rat brain. Effects of repeated ethanol administration at various post-treatment intervals. *Prog Neuropsychopharmacol Biol Psychiatry*. 2002; 26:303–306. [PubMed: 11817508]
39. Zorrilla EP, Valdez GR, Weiss F. Changes in levels of regional CRF-like-immunoreactivity and plasma corticosterone during protracted drug withdrawal in dependent rats. *Psychopharmacology (Berl)*. 2001; 158:374–381. [PubMed: 11797058]
40. Sommer WH, Rimondini R, Hansson AC, Hippskind PA, Gehlert DR, Barr CS, et al. Upregulation of voluntary alcohol intake, behavioral sensitivity to stress, and amygdala crhr1 expression following a history of dependence. *Biol Psychiatry*. 2008; 63:139–145. [PubMed: 17585886]
41. Ciccocioppo R, de Guglielmo G, Hansson AC, Ubaldi M, Kallupi M, Cruz MT, et al. Restraint stress alters nociceptin/orphanin FQ and CRF systems in the rat central amygdala: significance for anxiety-like behaviors. *J Neurosci*. 2014; 34:363–372. [PubMed: 24403138]
42. Rodi D, Zucchini S, Simonato M, Cifani C, Massi M, Polidori C. Functional antagonism between nociceptin/orphanin FQ (N/OFQ) and corticotropin-releasing factor (CRF) in the rat brain:

evidence for involvement of the bed nucleus of the stria terminalis. *Psychopharmacology (Berl)*. 2008; 196:523–531. [PubMed: 17989958]

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Figure 1.

Shows the lack of difference in $[^{11}\text{C}]\text{NOP-1A } V_T$ in regions of interest in alcohol dependent subjects (black bars) compared to healthy controls (white bars). AMY: amygdala, HIP: hippocampus, MID: midbrain, VST: ventral striatum, CAD: caudate, PUT: putamen, DLPFC: dorsolateral prefrontal cortex, OFC: orbital frontal cortex, MPFC: medial prefrontal cortex, ACC: anterior cingulate cortex, CER: cerebellum

Figure 2.

Shows the relationship between [¹¹C]NOP-1A V_T in orbital frontal cortex and craving for alcohol as measured with the Penn Alcohol Craving Scale in alcohol dependent subjects. Decreased NOP receptor availability in alcohol dependent subjects was associated with increased cravings for alcohol in abstinence.

Table 1

Demographic and clinical parameters for Healthy controls and Alcohol dependent subjects (n= 15/group)

	Healthy Controls		Alcohol dependence	
	Mean	SD	Mean	SD
Gender				
<i>Male</i>	10		10	
<i>Female</i>	5		5	
Ethnicity				
<i>African American</i>	2		1	
<i>More than one race</i>	1		1	
<i>Caucasian</i>	12		13	
Nicotine use	9		9	
Fagerstrom test for nicotine dependence: high/moderate/low	0/5/4		1/5/3	
30+ / 10–20 / < 10 cigarettes	2/6/1		1/5/3	
Positive family history for alcoholism in first degree relative	0		10 *	
Age	36	10	37	10
Weight (Kg)	86	21	88	25
Age of first use of alcohol	18	2	15 *	2
Alcohol frequency (days/month)	2	3	20 *	7
Alcohol amount (standard drinks/day)	1	1	10 *	5
Abstinence before scans (days)	-	-	28	11
Michigan Alcohol Screening Test (range 0 to 22)	-	-	12	4
Alcohol dependence scale (range 0 to 47)	-	-	20	8
Alcohol use disorders identification test (range 0 to 40)	-	-	24	6
Penn alcohol craving scale (range 0 to 30)	-	-	19	6
Hamilton anxiety rating scale (range 0 to 56) *	2	3	14 *	8
Center for epidemiologic studies depression scale (range 0 to 60)	5	4	17 *	9

* p 0.05, unpaired t-tests

Table 2

[11C]NOP-1A PET scan parameters

		Injected dose (mCi)	Specific activity (Ci mmol ⁻¹)	Injected mass (ug)	Free Fraction in plasma (%)	Free Fraction in buffer (%)
Healthy Controls	Mean	12.6	3309	2.2	14.0	70.2
	SD	0.6	1898	1.2	3.0	13.1
Alcohol Dependence	Mean	12.3	3153	1.9	14.3	65.9
	SD	0.5	1177	0.8	2.5	10.6

Values are mean and standard deviation (SD), n = 15 per group

* p 0.05, paired t-tests