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Nociceptin receptors are upregulated in cocaine use disorder: a positron emission tomography imaging study using [^{11}C]NOP-1A

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Abstract

OBJECTIVE: Nociceptin (N/OFQ) is an anti-stress neuropeptide transmitter in the brain that counteracts corticotrophin-releasing factor (CRF)-mediated stress and anxiety symptoms during drug and alcohol withdrawal. It also inhibits the release of a wide array of neurotransmitters including dopamine and glutamate, which allows for it to block the rewarding properties of cocaine. Chronic cocaine administration in rodents has been shown to decrease N/OFQ and increase nociceptive opioid peptide receptors (NOP) in the nucleus accumbens. No previous studies have reported on the in vivo status of NOP in chronic cocaine-abusing humans.

METHODS: [^{11}C]NOP-1A and PET were used to measure in vivo NOP binding in 24 cocaine use disorder (CUD) and 25 healthy control subjects matched for age, sex, and smoking status. CUD subjects with no comorbid psychiatric or medical disorders were scanned following two weeks of outpatient monitored abstinence. [^{11}C]NOP-1A distribution volume (V_T) were measured with kinetic analysis using the arterial input function in brain regions that mediate reward and stress behaviors. CUD subjects were also followed for 12-weeks after the PET scans to document relapse and relate it to V_T.

RESULTS: We observed a significant increase in [^{11}C]NOP-1A V_T in CUD compared to healthy controls. This increase, which was generalized across all the regions of interest (~10%), was most prominent in the midbrain, ventral striatum and cerebellum. However, increased V_T in these regions did not predict relapse.

CONCLUSION: Increased NOP in CUD suggests an adaptive response to decreased N/OFQ, or increased CRF transmission, or both. Future studies should examine the interactions between CRF and NOP to elucidate their role in negative reinforcement and relapse. NOP agonist medications to enhance N/OFQ should be explored as a therapeutic to treat CUD.

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DISCLOSURES

None

Keywords

[^{11}C]NOP-1A; positron emission tomography (PET); cocaine use disorders nociceptin/orphanin FQ peptide receptors (NOP)

INTRODUCTION

The endogenous neuropeptide nociceptin (N/OFQ) and its target nociceptive opioid peptide receptor (NOP) are structurally similar to dynorphin A and its target kappa opioid receptors (1, 2). However, N/OFQ has no affinity for the mu, kappa, or delta opioid receptors; and endogenous opioids such as endorphins, enkephalins and dynorphin do not bind to NOP. N/OFQ is an anti-stress/resilience mediating neuropeptide in the brain that counteracts stress and anxiety, which is primarily mediated by corticotrophin releasing factor (CRF), norepinephrine, orexin, vasopressin and dynorphin (3). N/OFQ blocks stress-induced analgesia, anorexia, and anxious behaviors in rodents (4–6). N/OFQ knockout mice show increased anxiety-like behaviors compared to wild-type mice (7). N/OFQ also blocks the rewarding properties of cocaine in behavioral paradigms including the acquisition of conditioned place preference (8, 9). In agreement with this, increases in the rewarding properties of cocaine have been observed in NOP knock-out mice (10). Studies have also shown that N/OFQ can reverse the psychomotor sensitization response that develops following chronic exposure to cocaine (considered a rodent model for drug craving) (9, 11, 12). N/OFQ also reduces cocaine-induced dopamine release in the nucleus accumbens (12–14). Two previous studies in rodents have evaluated the effects of chronic cocaine on N/OFQ and NOP. In the first report that used both radioimmunoassay and immuno-autoradiographic techniques, animals chronically administered cocaine showed decreased N/OFQ in the nucleus accumbens, substantia nigra/ventral tegmental area, and medial (but not lateral) caudate-putamen compared to controls (15). In a replication of this observation the expression of N/OFQ precursor protein (pro-N/OFQ) was found to be decreased in the nucleus accumbens and the lateral (but not medial) caudate-putamen in chronic cocaine rodents (16). NOP gene expression (mRNA) examined in the same chronic cocaine animals were significantly increased in the nucleus accumbens (~ 50%) but decreased in the caudate-putamen (~ 50–100%) relative to controls. In summary, several basic investigations suggest that N/OFQ and NOP alterations modulate drug-induced reward in cocaine use disorders (CUD). They also suggest a role for N/OFQ in balancing the functions of the stress-mediating neuropeptides such as CRF that promote negative reinforcement and lead to relapse (3).

No previous in vivo studies have characterized the N/OFQ system in chronic cocaine abusing humans due to the lack of a radiotracer to image NOP. [^{11}C]NOP-1A, first radiolabeled and validated by the NIMH Molecular Imaging Branch, has been successfully used to measure NOP in humans (17–19). Here, we used [^{11}C]NOP-1A and PET to measure the in vivo binding to NOP in 24 recently abstinent subjects with CUD and 25 healthy comparison subjects (HC). Following the [^{11}C]NOP-1A PET scan, CUD subjects were enrolled into a follow-up protocol in which they were monitored three times per week for 12-weeks using a contingency management approach. The objective of this follow-up was to

document relapse and relate it with NOP. We hypothesized an increase in [^{11}C]NOP-1A binding in CUD compared to HC in brain regions that mediate reward and stress behaviors. Such a finding would support the notion that NOP is upregulated in response to decreased N/OFQ in CUD.

MATERIALS AND METHODS

Human subjects

The University of Pittsburgh Human Research Protection Office and Radioactive Drug Research Committee approved the study. All subjects provided written informed consent. CUD subjects interested in abstaining from cocaine were recruited through advertisements displayed at addiction clinics, buses, newspapers and websites. Subjects were also recruited via a research registry administered by the University of Pittsburgh (Pitt+Me).

Study criteria for CUD subjects were [1] males or females between 18 and 50 years old; [2] fulfill DSM-5 criteria for CUD as assessed by the Structured Clinical Interview for DSM-5 (20) (SCID-5); [3] no other current psychiatric or drug/alcohol use disorder diagnosis except CUD (tobacco use disorder and recreational cannabis/alcohol use were not exclusionary); [4] no medical or neurological illness; [5] not currently taking any medical or psychotropic medications; [6] not currently pregnant; [7] no recent research or occupational radioactivity exposure; and [8] no contraindications for magnetic resonance imaging (MRI). Study criteria for healthy controls were: [1] males of females between 18 and 50 years old; [2] absence of present and past psychiatric and addictive disorders as assessed by SCID-5; and criteria 4 to 8 above.

Demographic, substance use and family history of addiction data were all recorded as recommended by NIDA in the Substance abuse and addiction assessments core PhenX Toolkit (https://www.phenxtoolkit.org). Clinical assessments performed included the Substance Use Inventory, Tiffany Cocaine Craving Questionnaire (CCQ), Fagerstrom Test for Nicotine Dependence (FTND), Perceived Stress Scale (PSS), Hamilton Anxiety Rating Scale (HAM-A), and Barratt Simplified Measure of Social Status (BSMSS). CUD subjects were monitored three times per week in the outpatient setting for abstinence from cocaine for a minimum of ten days before the PET scans. CUD subjects were only scanned if a urine drug screen confirmed the absence of all drugs of abuse (including cannabis) on scan day. Subjects were not allowed to use any nicotine products following arrival to the PET facility, i.e., their last cigarette was a minimum of 2 hours prior to the PET scan. Female subjects were scanned irrespective of menstrual cycle phase or hormonal contraceptive use

PET image acquisition and analysis

Before PET imaging, a magnetization prepared rapid gradient echo structural MRI scan was obtained using a Siemens 3T Trio scanner for region determination. [^{11}C]NOP-1A PET imaging sessions were conducted with the Siemens Biograph64 mCT scanner using methods previously described (17, 21–24). Following a low-dose CT scan of the brain that was acquired for attenuation correction, subjects received an intravenous bolus injection of [^{11}C]NOP-1A, and emission data was collected for 70 minutes. Metabolite-corrected arterial

input function and plasma free fraction (f_P) measurements were performed in all subjects. PET data were reconstructed by filtered back projection using the camera's built-in software. The image analysis software PMOD, version 3.802 (PMOD Technologies LLC, Zurich, Switzerland) was used to conduct frame-to-frame motion correction for head movement. The MR-PET image alignment was performed using a normalized mutual information algorithm. Regions of interest were generated for each subject using the built-in brain parcellation work-flow within PMOD's PNEURO Tool (25). Region generation was based off the AAL-VOIs atlas (26, 27). Regions of interest included the amygdala, hippocampus, midbrain, cerebellum, striatum (ventral striatum, caudate and putamen) and prefrontal cortex (specifically the dorsolateral, orbital, medial, and anterior cingulate) subdivisions. These regions that have been a focus of N/OFQ and NOP basic investigations are the same regions we examined in a prior study in alcoholics (24). All regions generated by the brain parcellation tool were visually inspected and adjusted as deemed necessary by an image analyst trained in manual region drawing. Regional volumes and time activity curves were also generated in PMOD. 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 implemented in MATLAB (21, 22, 28). V_T, which includes both the receptor-bound specific and non-specific binding was used as the outcome measure (as opposed to the binding potential relative to non-displaceable uptake, BP_{ND}) because there is no brain region in the brain that is devoid of specific binding to NOP (23).

Relapse monitoring protocol for CUD subjects

In order to document relapse, CUD subjects were enrolled in a 12-week follow-up protocol after the [^{11}C]NOP-1A PET scan. This follow-up protocol was modeled after the contingency management protocol used in a previous [^{11}C]raclopride-amphetamine PET study (29, 30). Briefly, CUD met with the research team three times per week for urine drug screens (UDS) and earned voucher points on an escalating schedule for each negative UDS. Subjects earned bonus points for every three-consecutive cocaine-free UDS (one week of abstinence). Missed appointments reset the voucher points to a value that was lower by 10 points. Subjects had the potential to earn a maximum of $1197.00 for providing cocaine-free UDS on all of the scheduled monitoring visits. The money earned was disbursed to them on a weekly basis via a debit card. Subjects were terminated from the follow-up protocol: for testing positive for cocaine three times (i.e., were allowed only three distinct relapses); for missing three consecutive scheduled appointments (i.e., were lost to follow-up for a week); and for initiating any medications that influence N/OFQ (for example, buprenorphine, etc.). Subjects were also monitored once a week for depression and suicidal ideation, and de-briefed on their week's progress concerning abstinence.

Statistical analysis

All statistical analyses were conducted using IBM SPSS v.25. Normality of data was examined using Kolmgorov-Smirnov and Shapiro-Wilkes tests. Group demographic and baseline scan parameter (such as injected dose, mass, plasma clearance) comparisons were performed with unpaired t-tests. The primary analyses examined overall group differences in [^{11}C]NOP-1A V_T with a linear mixed model analysis (LMM) performed with regions of

interest as a repeated measure and diagnostic group (CUD vs. HC) as the fixed factor. The effect of sex and smoking status on V_T and diagnostic group were subsequently examined in a second level LMM analyses by including them as fixed factors in the model. Post-hoc unpaired t-tests in the individual regions of interest were also conducted. A two-tailed probability value of $p \leq 0.05$ was selected as the significance level for the primary analyses (LMM that included all the regions of interest). A Benjamini-Hochberg false discovery rate (FDR) correction with $\alpha = 0.05$ was applied to correct for multiple comparisons in the individual regions of interest in the post-hoc analyses.

Group comparison of regional V_T in CUD who abstained, relapsed and drop-out were evaluated using a LMM (as described previously). Studies in CUD have previously shown that early treatment response and motivation as reflected by clinic attendance in the first 2–3 weeks predicts sustained abstinence in CUD subjects' during 12-weeks of contingency management treatment (29, 31). Based on this we also used a LMM to compare V_T in CUD subjects who relapsed and/or dropped-out within the first 2 weeks (out of a 12-week follow-up) to that in those who did not relapse and/or continued in the protocol past 2 weeks. The amount of voucher money earned, which reflects abstinence was used as a secondary outcome measure in a Pearson product moment correlation (only regions that survived FDR correction were used for all correlational analyses). Lastly, a Cox proportional hazards model was used to determine the association between time to relapse and regional V_T in the CUD group. Time to relapse was defined as the time between the date of [^{11}C]NOP-1A PET scan and the date of the first relapse to cocaine (positive cocaine UDS) over the 12-week follow-up period. Data were censored if the subjects did not experience relapse by the end of the follow-up period, including those lost to follow-up. Models were run with and without adjustment for potential confounding factors (such as sex, PSS, CCQ). The relationship between regional V_T and other clinical measures of stress (PSS), anxiety (HAM-A), craving (CCQ) and severity (cocaine use duration in years, frequency in days and amount spent in dollars) of cocaine abuse in CUD were assessed by Pearson product moment correlation. A two-tailed probability value of $p \leq 0.05$ was selected as the significance level for all the analyses that involved clinical measures.

RESULTS

Twenty-four individuals with CUD were matched with 25 HC on age, sex, ethnicity and nicotine status as closely as possible. Table 1 lists the demographics variables and clinical characteristics of the study sample.

[^{11}C]NOP-1A scan parameters

Table 2 shows no significant group differences between CUD and HC in any of the baseline scan parameters. Consistent with that reported previously, [^{11}C]NOP-1A plasma free fraction (f_P) measurements were not reliable because the tracer showed relatively high retention to the filter in the saline buffer solution condition (24). No significant group differences in the regions of interest volume determined from MRI scans were noted (data not shown).

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

Regional [^{11}C]NOP-1A V_T was significantly higher in CUD compared to HC (LMM, effect of diagnosis, F (1, 47) = 4.39, p = 0.042; effect of region, F (10, 470) = 520.20, p < 0.001; region * diagnosis interaction, F (10, 470) = 1.45, p = 0.156).

[^{11}C]NOP-1A V_T in the regions of interest were on average 7 to 12% lower in females compared to males (Figure 1). The inclusion of sex as a factor in the LMM did not alter the significance of the above described result (effect of diagnosis, F (1, 45) = 4.65, p = 0.037; effect of sex, F (1, 45) = 4.45, p = 0.041; sex * diagnosis interaction, F (1, 45) = 0.25, p = 0.622). However, the effect of diagnosis fell short of significance when tobacco use status was included as an additional factor in the LMM (effect of diagnosis, F=3.88, df=1, 43, p=0.055; effect of smoking, F=0.03, df=1, 43, p=0.86; smoking * diagnosis interaction, F=0.11, df=1, 43, p=0.74). Excluding the four CUD subjects who tested positive for cannabis during the outpatient monitoring prior to PET did not change the results (data from n = 20 CUD and n = 25 HC; LMM, effect of diagnosis, F (1, 43) = 5.65, p =0.022; effect of region, F (10, 430) = 500.02, p < 0.001; region * diagnosis interaction, F (10, 430 = 1.97, p = 0.035).

Unpaired t-tests conducted at the level of the individual regions of interest were significant in four of the eleven regions of interest (see Table 3). The comparisons in the ventral striatum and cerebellum survived the FDR correction.

Relationship between V_T and relapse to cocaine during follow up

There were no significant differences in V_T between CUD who abstained vs. relapsed vs. drop-out during the follow-up period (LMM, effect of final outcome status, F (2, 21) = 0.13, p = 0.88; effect of region, F (10, 210) = 230.65, p < 0.001; region * diagnosis interaction, F (20, 210) = 0.49, p = 0.97, see Figure 2). Stratification of the CUD subjects based on whether they relapsed and/or dropped-out within the first two weeks (n = 9 CUD < 2-weeks; n = 15 > 2 weeks) also revealed no group differences in [^{11}C]NOP-1A V_T (LMM, effect of duration, F (1, 22) = 1.24, p = 0.28; effect of region, F (10, 220) = 223.81, p < 0.001; time * region interaction, F (10, 220) = 0.65, p = 0.77. There were also no significant correlations between V_T and the amount of voucher money earned during follow-up (midbrain, r = 0.19, p = 0.36; ventral striatum, r = 0.18, p = 0.41; and cerebellum r = 0.18, p = 0.40). Lastly, V_T in the regions of interest were also not predictive of time to relapse to cocaine (midbrain, Exp (B) = 1.0, p = 0.99; ventral striatum, Exp (B) = 0.97, p = 0.78; and cerebellum, Exp (B) = 1.02, p = 0.95). These results were unchanged irrespective of whether the models were adjusted for potential confounding factors such as sex, PSS and CCQ (data not shown).

Higher levels of perceived stress on PSS were predictive of less time to relapse (Exp (B) = 1.08, p = 0.05). No such relationship was observed between cocaine cravings (CCQ) and time to relapse (Exp (B) = 1.00, p = 0.88).

Relationships between V_T and clinical measures

No significant relationships were observed between regional [^{11}C]NOP-1A V_T and any of the other clinical measures (including cocaine use frequency, duration and amount of money spent, perceived stress, anxiety and cocaine craving) in CUD.

Relationship between V_T and age

The negative relationship between age and V_T fell short of significance in the medial prefrontal ($r = -0.39$, $p = 0.06$) and dorsolateral prefrontal cortex ($r = -0.36$, $p = 0.08$), but not other regions (data not shown, $p > 0.1$) in healthy controls. --REMOVE LINE ABOUT MULTIPLE CORRECTION-- No such relationship between age and V_T were noted in any of the regions in CUD.

DISCUSSION

In this [^{11}C]NOP-1A PET study, we found that: (1) cocaine abuse in humans is associated with a generalized ~10% increase in binding to NOP in brain regions that mediate reward and stress behaviors (the four regions that were significant included the midbrain, ventral striatum hippocampus and cerebellum); (2) females have less NOP binding compared to males; and (3) increased stress during cocaine withdrawal predicts an early relapse. An important negative result is the lack of a relationship between V_T and relapse.

Increased [^{11}C]NOP-1A binding in cocaine users suggests a compensatory upregulation of NOP in response to decreased N/OFQ in the brain. This is supported by studies in chronic cocaine rodents that have shown decreases in N/OFQ in the nucleus accumbens, caudate-putamen and substantia nigra/ventral tegmental area (15, 16). However, NOP's regulatory response to decreased N/OFQ in these rodents has been shown to be variable at the level of the striatum, i.e., increased in the nucleus accumbens (ventral striatum) and decreased in the caudate-putamen(16). The PET data in CUD, which showed an increase in NOP in the ventral striatum and putamen and no change in the caudate, is only partially consistent with these rodent data. The lack of an increase in NOP in the caudate, which is observed in both the humans and rodent studies, is still noteworthy because it might relate to the fact that the caudate is the only striatal subdivision in which dopamine transmission is not blunted in CUD (32). Studies examining the relationship between NOP V_T and amphetamine-induced dopamine release in CUD might clarify this relationship. Behavioral paradigms that mimic chronic stress in rodents consistently show upregulation of NOP in the limbic-brain regions (33–36). Thus, increased NOP in CUD might also reflect an adaptive response to balance the effects of CRF, which increases stress and promotes relapse. Supportive of this is a study in which an upregulation of NOP in the bed nucleus of stria terminalis and the amygdala were noted following increases in CRF (33, 37). Similar interactions between NOP and other stress-promoting neuropeptides such as dynorphin have also been described in chronic cocaine rodents (16). Future imaging studies with [^{11}C]NOP-1A investigating NOP-CRF and NOP-dynorphin interactions in CUD should be considered because they might clarify the interplay between stress and anti-stress neuropeptides in negative reinforcement and relapse. N/OFQ stimulates NOP to inhibit calcium channels and activate potassium channels (38). This allows for N/OFQ to inhibit the release of multiple neurotransmitters including

dopamine, serotonin, acetylcholine, glutamate, and GABA (39). Increased N/OFQ has been shown to inhibit cocaine-induced dopamine release in the nucleus accumbens (12–14). This mechanism has therapeutic potential because it can decrease the cocaine reward experience. It is likely that N/OFQ directly modulates midbrain dopamine neurons to impact cocaine-induced dopamine release because 50–90% of tyrosine hydroxylase positive cells (TH +) in the ventral tegmental area and substantia nigra express NOP (40, 41). However, an effect for pre-synaptic NOP on dopamine terminals cannot be ruled out because administration of N/OFQ into the nucleus accumbens also blocks cocaine-induced dopamine release (13). Increased N/OFQ has also been shown to decrease glutamate transmission in the reward- and stress-mediating brain regions such as the cortex, midbrain, amygdala and cerebellum (42–44). This mechanism also has therapeutic potential because increased glutamate in CUD have been linked to relapse and reinstatement (45). These mechanistic investigations and this PET study support clinical trials with a NOP agonist to increase N/OFQ transmission in CUD. The inability to link NOP V_T with relapse in this study cannot be viewed as less supportive of this conceptual model because it is not possible to quantitate endogenous N/OFQ levels from a baseline [^{11}C]NOP-1A scan. [^{11}C]NOP-1A imaging paradigms that measure the regulatory response of NOP to stress/CRF might be more successful in predicting relapse in CUD.

Sex-based differences have been reported for stress-mediating neuropeptides and their receptors including CRF and kappa-opioid receptors (46, 47). To our knowledge, decreased NOP in females relative to males is the first report of sex-based differences in an anti-stress system in the brain. This unreported observation was also present as a trend level finding in a separate set of fifteen healthy controls (5 females and 10 males; eleven regions of interest; LMM, effect of gender on V_T, $p = 0.055$, region, $p < 0.001$, gender * region interaction, $p = 0.012$) in our previous [^{11}C]NOP-1A alcohol study (19). Studies to understand the clinical relevance of decreased NOP in females are necessary because it may explain previously described behavioral and physiological phenomena, such as females reporting greater subjective levels of stress, showing increased sensitivity to CRF, etc. It may also provide clues as to the reasons why females are at higher risk for certain chronic stress disorders such as depression, anxiety, chronic pain, etc. Sex had no effect on the increased NOP in CUD, i.e., there was no sex * diagnosis interaction. However, the effect size of the difference in [^{11}C]NOP-1A V_T in CUD Vs. controls was much larger in females compared to males (Table 4). This is interesting as chronic cocaine rodent and human studies consistently report more negative affect, withdrawal symptoms and stress-induced relapse/reinstatement in females compared to males (48). It is tempting to speculate greater decreases in resilience-mediating N/OFQ and/or increases in stress-mediating CRF (both of which have been shown to upregulate NOP receptors in basic investigations) as reasons for more unpleasant symptoms and subsequent relapse in chronic cocaine abusing females. Future [^{11}C]NOP-1A PET studies should focus on these sex differences, and also control for the fluctuations in sex hormones associated with menstrual cycle and hormonal contraceptive use.

The role of negative reinforcement in promoting stress/anxiety, and leading to relapse to drugs/alcohol is well established in rodent models of addiction (3). Consistent with this model, we found that increased stress during cocaine withdrawal is predictive of less time to relapse. However, we found no relationship between perceived stress/anxiety and the anti-

stress/resilience conferring NOP (V_T). Subjects were scanned after they abstained from cocaine for a minimum of ten days, which may have limited the ability to detect such a relationship. PET studies focusing on the neurochemistry of withdrawal are warranted because the behavioral symptoms experienced during this period predicts relapse.

We were unable to exclude differences in [^{11}C]NOP-1A non-specific binding (V_{ND}) and plasma free fraction (f_P) as contributors to V_T. Reassuringly, prior [^{11}C]NOP-1A blocking studies in humans suggest 50–75% of V_T represents specific binding to NOP (18). In summary, we demonstrated a significant increase in NOP in CUD. The data showed a trend towards regionally-selective increases in NOP that were convincingly evidenced in the midbrain, ventral striatum and cerebellum. Increased NOP in CUD may be indicative of an adaptive response to decreased N/OFQ, or increased CRF, or both. The clinical development of NOP agonists to enhance N/OFQ transmission should be explored as a treatment for CUD.

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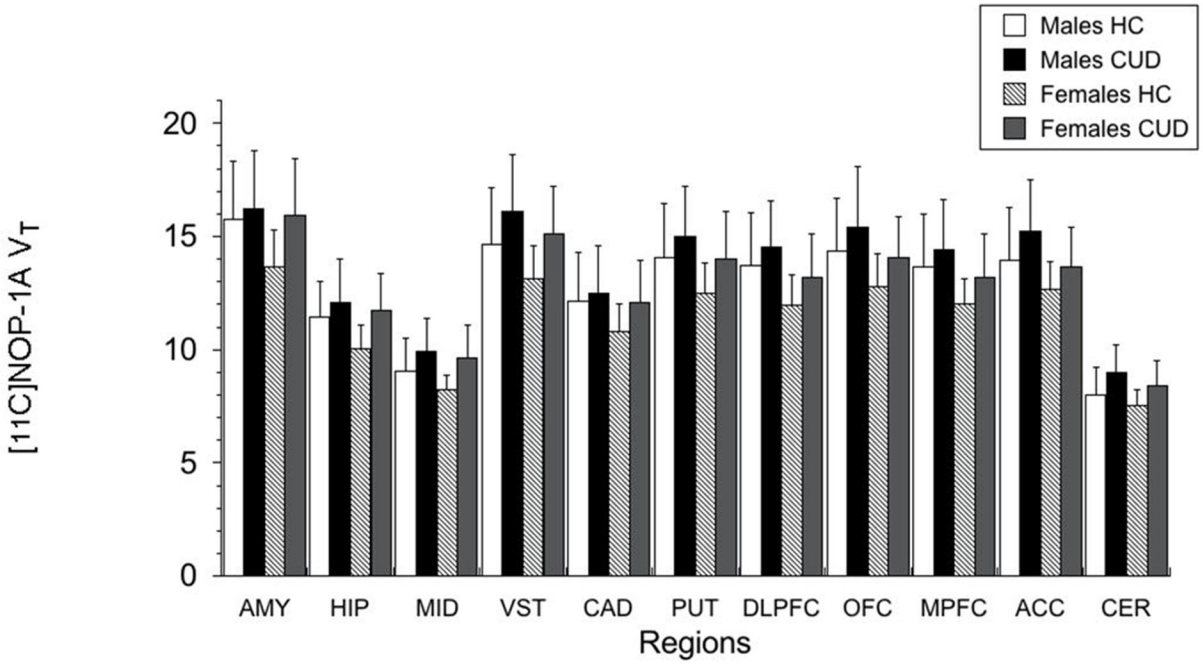

Figure 1.

Female subjects when compared to male subjects had significantly lower V_T values in the regions of interest. Sex-based differences were not present with respect to the increase in V_T in CUD relative to HC **(sex * diagnosis interaction, p = 0.62)**. 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.

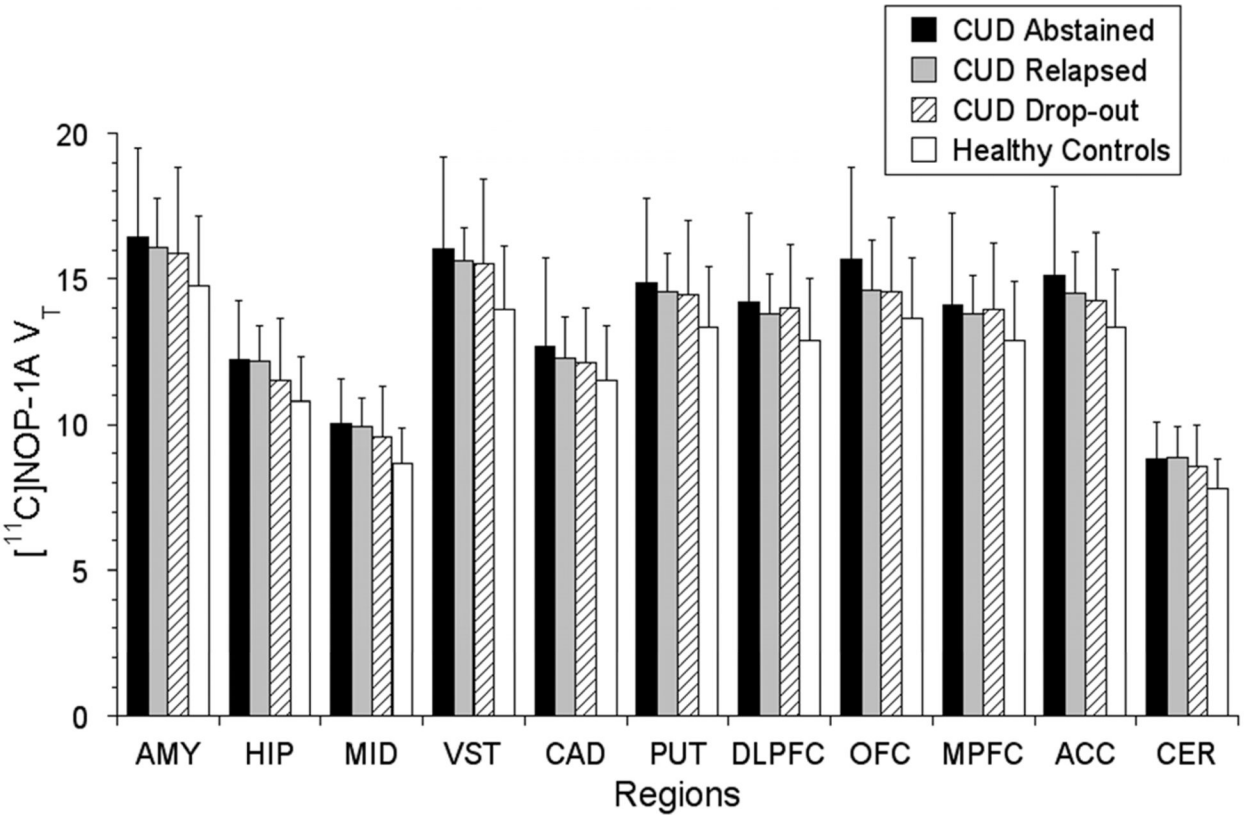

Figure 2.

CUD subjects who abstained (n = 6), relapsed (n = 9) and drop-out (n = 9) during the 12-week follow-up period demonstrated no significant differences in V_T. Also, included in the figure for a visual contrast is baseline V_T data from healthy controls (n = 25). Regions shown are 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.

Table 1.

Demographic and clinical characteristics of cocaine use disorder and healthy control subjects

	Cocaine use disorder n = 24	**Healthy controls n = 25**
Age	40 ± 9	37 ± 9
Sex		
Females	10	11
Males	14	14
Ethnicity		
African American	10	5
Caucasian	12	18
Asian	0	1
Hispanic	1	0
More than one race	1	1
Barratt Simplified Measure of Social Status		
Education	14 ± 3	15 ± 4
Occupation	21 ± 10	27 ± 4*
Total	33 ± 13	42 ± 12*
Tobacco use	16	13
> 10 cigarettes per day	*7*	*4*
Cannabis use (recreational)	4	-
Hamilton Anxiety Rating Scale (range 0 to 56)	8.5 ± 7.5	2.1 ± 3.7*
Perceived Stress Scale (range 0 to 40)	15.2 ± 7.8	5.5 ± 5.3*
Cocaine Craving Questionnaire (range 0 to 70)	26 ± 15	
Age of first use of cocaine (years)	23 ± 8	
Duration of cocaine use (years)	15 ± 9	
Cocaine use frequency per week (days)	2 ± 2	
Amount spent on cocaine per week (dollars)	126 ± 64	
12-week follow-up protocol to monitor for relapse following PET scan		
Final Outcome		
Abstinent	6	

	Cocaine use disorder n = 24	Healthy controls n = 25
Relapsed (cocaine +)	9	
Drop-out	9	
Amount of money earned in vouchers (dollars)	415 ± 476	
Time to an event, i.e., first relapse or drop-out or completion (days)	35 ± 34	
Time to first relapse (days)	13 ± 14	
Duration in follow-up protocol (days)	43 ± 35	

* $p < 0.05$, unpaired t-tests for numerical variables and Chi-square test for categorical variables.

Table 2.

[^{11}C]NOP-1A scan parameters

Scan Parameter	Cocaine use disorder n = 24	Healthy controls n=25
Injected dose (mCi)	12.4 ± 0.7	12.3 ± 1.0
Specific Activity (Ci/mmoles)	2169 ± 975	2230 ± 558
Injected mass (μg)	2.8 ± 1.0	2.5 ± 0.6
Plasma free fraction (f_p, %) †	14.5% ± 3.2%	13.2% ± 2.3%
Plasma free fraction buffer (f_p, %) †	71.0% ± 15.7%	76.1% 11.5%
Clearance (L/h)	151.8 ± 38.9	155.1 ± 38.1

Values are mean ± SD in cocaine use disorder and healthy control subjects

SD - Standard deviation

† Data was only available from n=23 CUD and 22 HC due to problems with sample processing.

Table 3.

[^{11}C]NOP-1A volume of distribution expressed relative to total plasma concentration (V_T) in regions of interest

Regions of interest	Cocaine use disorder n = 24	Healthy controls n = 25	Uncorrected p-values
Amygdala	16.10 ± 2.48	14.86 ± 2.40	0.084
Hippocampus	11.93 ± 1.76	10.85 ± 1.53	0.027
Midbrain	9.81 ± 1.40	8.72 ± 1.22	0.0056
Ventral Striatum	15.70 ± 2.35	13.99 ± 2.21	0.0118 *
Caudate	12.32 ± 1.99	11.56 ± 1.90	0.177
Putamen	14.60 ± 2.18	13.37 ± 2.13	0.051
Dorsolateral Prefrontal Cortex	13.97 ± 2.09	12.98 ± 2.11	0.105
Orbital Frontal Cortex	14.85 ± 2.40	13.68 ± 2.15	0.078
Medial Prefrontal Cortex	13.93 ± 2.14	12.96 ± 2.05	0.115
Anterior Cingulate Cortex	14.57 ± 2.18	13.39 ± 1.99	0.053
Cerebellum	8.73 ± 1.21	7.81 ± 1.03	0.0062 *

Values are mean ± SD in cocaine use disorder and healthy control subjects

SD - Standard deviation

* Significant following multiple hypotheses correction with FDR

Table 4.

Effect size of the difference in [^{11}C]NOP-1A V_T in cocaine use disorder vs. healthy controls by sex

Regions of interest	Males (d)	Female (d)	Effect size ratio (female/male)
Amygdala	0.19	1.10	5.8
Hippocampus	0.37	1.27	3.4
Midbrain	0.62	1.33	2.1
Ventral Striatum	0.62	1.12	1.8
Caudate	0.16	0.85	5.3
Putamen	0.44	0.89	2.0
Dorsolateral Prefrontal Cortex	0.38	0.73	1.9
Orbital Frontal Cortex	0.45	0.77	1.7
Medial Prefrontal Cortex	0.35	0.77	2.2
Anterior Cingulate Cortex	0.57	0.70	1.2
Cerebellum	0.81	0.96	1.2

d is the Cohen's effect size maximum likelihood estimation of the difference between cocaine use disorder and healthy controls