Archival Report

Decreased Nociceptin Receptors Are Related to Resilience and Recovery in College Women Who Have Experienced Sexual Violence: Therapeutic Implications for Posttraumatic Stress Disorder

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ABSTRACT

BACKGROUND: Posttraumatic stress disorder (PTSD) is a stress disorder that develops in only some individuals following a traumatic event. Data suggest that a substantial fraction of women recover after sexual violence. Thus, the investigation of stress and antistress neuropeptides in this sample has the potential to inform the neurochemistry of resilience following trauma. Nociceptin is an antistress neuropeptide in the brain that promotes resilience in animal models of PTSD.

METHODS: [^{11}C]NOP-1A positron emission tomography was used to measure the in vivo binding to nociceptin receptors in 18 college women who had experienced sexual violence irrespective of whether they met DSM-5 diagnostic criteria for PTSD. [^{11}C]NOP-1A data from 18 healthy control subjects were also included to provide a contrast with the sexual violence group. [^{11}C]NOP-1A total distribution volume (V_T) in the regions of interest were measured with kinetic analysis using the arterial input function. The relationships between regional V_T and Clinician-Administered PTSD Scale for DSM-5 total symptom and subscale severity were examined using correlational analyses.

RESULTS: No differences in [^{11}C]NOP-1A V_T were noted between the sexual violence and control groups. V_T in the midbrain and cerebellum were positively correlated with PTSD total symptom severity in the past month before positron emission tomography. Intrusion/re-experiencing and avoidance subscale symptoms drove this relationship. Stratification of subjects by a DSM-5 PTSD diagnosis and contrasting their V_T with that in control subjects showed no group differences.

CONCLUSIONS: Decreased midbrain and cerebellum nociceptin receptors are associated with less severe PTSD symptoms. Medications that target nociceptin should be explored to prevent and treat PTSD.

Keywords: [^{11}C]NOP-1A, Nociceptin/orphanin FQ peptide receptors, NOPs, PET, Positron emission tomography, Posttraumatic stress disorder, Resilience, Sexual violence

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Posttraumatic stress disorder (PTSD) is a stress disorder characterized by altered fear conditioning and memory reconsolidation following a traumatic event (1). The traumatic event most commonly preceding PTSD in women is sexual violence. A substantial fraction of women will experience sexual violence in adolescence or adulthood. An estimated 30% to 80% of these women will be diagnosed with PTSD during their lifetime, and current PTSD prevalence in this group approaches 15% (2–5). In contrast, the lifetime and current prevalence rates for PTSD (both genders) from all trauma is 6.8% and 3.6%, respectively (6). These trends suggest both higher prevalence of and greater recovery from PTSD following sexual violence as compared with other traumas. Understanding the neurochemistry of recovery in women who have experienced sexual

violence has the potential to inform therapeutic strategies to prevent and treat the symptoms of PTSD.

Ross *et al.* outline a framework for PTSD in which the three core DSM-5 symptom clusters of intrusive recollection, avoidance, and increased arousal can be viewed as abnormalities in classical fear conditioning, negative reinforcement, and sympathetic nervous system/hypothalamic-pituitary-adrenal axis stress response, respectively (1). This allows for the conceptualization of PTSD primarily as a learning and memory disorder in which abnormal trauma memory reconsolidation/extinction leads to intrusive thoughts and subsequent avoidance of trauma-related memories (i.e., DSM-5 PTSD Criteria B and C symptoms). Other PTSD symptoms including negative alterations in mood and cognition and

increased arousal/reactivity (i.e., Criteria D and E symptoms) are secondary manifestations. Food and Drug Administration–approved medications to treat PTSD such as sertraline and paroxetine are effective for the most part in treating these secondary symptoms and do not address the primary intrusion symptoms (7,8). The results of recent clinical trials with medications such as prazosin and propranolol targeting the primary symptoms in PTSD have been mixed (9–11). Linking novel neurochemical targets with PTSD symptom clusters may allow for the identification of new medications to treat the primary symptoms of PTSD.

Nociceptin/orphanin FQ (N/OFQ), which binds to the nociceptive opioid peptide (NOP) receptor, is an antistress/resilience-regulating neuropeptide (12). Recent positron emission tomography (PET) studies with [^{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) allow for the investigation of NOP (13–17). N/OFQ stimulation of NOP receptors inhibits calcium and activates potassium ion channels (18). This allows NOP receptors to regulate the in vivo release of multiple neurotransmitters, including glutamate, gamma-aminobutyric acid, dopamine, serotonin, and acetylcholine (19). This mechanism may be of value in targeting the neurochemical abnormalities in PTSD, which involve excitatory, inhibitory, and monoamine transmission (20). Studies in control animals show a reduction in the number of amygdala NOP receptors during fear conditioning and expression (21). However, in animals with dysregulated fear, either an upregulation or no change in NOP receptors is observed in regions such as the amygdala, hippocampus, and midbrain (21,22). These findings have been interpreted variously as representing either an increase or decrease in N/OFQ signaling in PTSD (21,22). Intriguingly, these contradictory interpretations are supported by studies showing that both an NOP receptor antagonist and an NOP receptor agonist are effective in alleviating fear, anxiety, and pain in rodent models of PTSD (21,22). In summary, the preclinical literature is mixed with respect to the status of N/OFQ and NOP in animal models of PTSD. In a first step to examine NOP receptors in subjects with a history of trauma, we used PET to measure the in vivo binding of [^{11}C]NOP-1A in college women exposed to sexual violence. Consistent with the National Institute of Mental Health Research Domain Criteria initiative, we scanned subjects irrespective of whether they met the full DSM-5 diagnostic criteria for PTSD. We focused on sexual violence in adolescence and early adulthood to minimize the impact of childhood trauma on NOP.

METHODS AND MATERIALS

Human Subjects

The University of Pittsburgh Institutional Research Board approved the study. All subjects provided written informed consent. Subjects were recruited via advertisements in college newspapers, online ads, a university research registry, and college campus sexual assault survivor support groups.

Study criteria for women who have experienced sexual violence were women 1) 18 to 25 years old, 2) who had experienced sexual violence as a teenager or young adult (14–25 years

of age), excluding subjects with acute trauma (within the past 30 days), 3) with no history of childhood physical or sexual abuse, 4) with no history of DSM-5 psychiatric or addictive disorders other than PTSD, 5) with no current use of any drugs of abuse, 6) who were not currently on psychotropic medication, 7) with no medical or neurological illnesses, 8) who were not currently pregnant or breastfeeding, 9) with no significant prior exposure to radiation, and 10) with no contraindications for magnetic resonance imaging.

Study criteria for control subjects were women 1) 18 to 25 years of age, 2) with no history of exposure to actual or threatened sexual violence, 3) with no history of childhood physical or sexual abuse, and 4) with no history of DSM-5 disorders; and criteria 5 to 10 as listed previously.

Clinical assessments performed included the Structured Clinical Interview for DSM-5 to exclude any psychiatric and addictive disorders other than PTSD (23) and Clinician-Administered PTSD Scale for DSM-5 (CAPS-5) to assess PTSD symptom severity. This scale measured the severity of PTSD symptoms as experienced by the subject in the past month before PET (past month) and in the worst month after sexual violence (worst month). CAPS-5 scores for the worst month were obtained via the subjects' recall of the symptoms suffered months or years ago. CAPS-5 total symptom severity scores were calculated by summing severity scores of the individual symptoms in all four clusters: intrusion symptoms (cluster B), avoidance symptoms (cluster C), cognitive and mood symptoms (cluster D), and arousal and reactivity symptoms (cluster E) (24). Other clinical assessments used were the Life Stressor Checklist-Revised, to exclude any childhood physical or sexual abuse (25); Hamilton Anxiety Rating Scale (HAM-A) and Hamilton Depression Rating Scale (HDRS) (26,27); and Alcohol Use Disorders Identification Test (AUDIT), to quantify alcohol abuse (28).

Image Acquisition and Analysis

Before PET imaging, a structural magnetic resonance imaging scan was obtained using a Siemens 3T Trio scanner (Siemens Healthineers, Erlangen, Germany) for brain region of interest (ROI) determination. The synthesis of [^{11}C]NOP-1A was carried out as previously described (13). PET imaging sessions were conducted with the Siemens Biograph64 mCT scanner (Siemens Healthineers). The injected dose and mass of [^{11}C]NOP-1A were restricted to 12 mCi and ≤4.2 μg (29). Following a low-dose computed tomography scan of the brain acquired for attenuation correction, subjects received an intravenous bolus injection of [^{11}C]NOP-1A, and emission data were collected for 70 minutes (17). Metabolite-corrected arterial input function measurements were performed and analyzed as described previously (13,16,17,29,30). Free fraction was determined in plasma and in a saline buffer (to determine filter retention of the free tracer) using ultrafiltration (31,32).

PET data were reconstructed using filtered back-projection. The image analysis software PMOD, version 3.802 (PMOD Technologies LLC, Zurich, Switzerland), was used to conduct frame-to-frame motion correction and magnetic resonance–PET coregistration. ROIs were generated for each subject using the built-in brain parcellation work-flow within PMOD's Neuro Tool (PNEURO). Region generation was based on the AAL-VOIs atlas (33,34). ROIs included the amygdala,

hippocampus, insula, midbrain, cerebellum, striatum (ventral striatum, caudate, and putamen), and prefrontal cortex (specifically the dorsolateral, orbital, medial, and anterior cingulate cortex) subdivisions (30). All regions generated 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 ROIs was performed using a two-tissue compartment kinetic analysis using the arterial input function implemented in MATLAB version R2016b (The MathWorks, Inc., Natick, MA) (17,29,35). V_T, which includes both the receptor-bound specific and nonspecific binding, was used as the outcome measure (16).

Statistical Analysis

All statistical analyses were conducted using SPSS, version 25 (IBM Corp., Armonk, NY). Comparisons between the sexual violence and healthy control groups on the demographic variables and baseline scan parameters (such as injected dose, mass, plasma clearance) were performed with unpaired t tests. The primary analyses conducted were correlational in nature because not all subjects who had experienced sexual violence met the DSM-5 diagnostic criteria for PTSD. Normality of the data used were confirmed using Shapiro-Wilk's tests prior to correlations. The relationship between regional [^{11}C]NOP-1A V_T and the severity of CAPS-5 PTSD symptoms (past and worst months) were examined with Pearson's product moment correlation coefficient. The same test was also used to explore relationships between regional [^{11}C]NOP-1A V_T and other clinical rating scales, such as HAM-A, HDRS, and AUDIT. DSM-5 PTSD diagnosis–based group differences in [^{11}C]NOP-1A V_T were also explored with a linear mixed model (LMM) analysis performed with ROI as a repeated measure and diagnosis as the fixed factor. Regions and diagnosis-by-region interaction were included in the model as explanatory variables. A two-tailed probability value of $p < .05$ was selected as the significance level for all analyses.

RESULTS

Eighteen women who had experienced sexual violence were matched with 18 control subjects on age, ethnicity, and nicotine status. Nine of 18 subjects in the sexual violence group met CAPS-5 PTSD diagnostic criteria in the past month (PTSD PM); an additional 5 subjects met the CAPS-5 PTSD diagnostic criteria in their worst month (PTSD WM) but not in the past month; and 4 subjects did not meet the CAPS-5 PTSD diagnostic criteria in their worst or past month (Resilient). Table 1 lists demographic variables and clinical characteristics of the study sample. Sexual violence subjects had significantly higher anxiety, depression, and life stressors (other Life Stressor Checklist-Revised traumatic events reported by the sexual violence subjects are included in Supplemental Table S1) compared with control subjects. No significant group differences were observed in the AUDIT scores.

[^{11}C]NOP-1A PET Scan Data

Tables 2 and 3 show [^{11}C]NOP-1A scan parameters and regional V_T in women who have experienced sexual violence and control subjects. No significant between-group differences were noted

Table 1. Demographic and Clinical Characteristics of the Sample

Variable	Experienced Sexual Violence Group ($n = 18$)	Healthy Control Group ($n = 18$)
Age, Years	22 ± 2	22 ± 2
Ethnicity		
African American	2	1
Asian	2	3
Hispanic	0	1
Caucasian	13	12
More than one ethnicity	1	1
Nicotine Use	2	2
Sex Hormone Status		
Follicular phase	0	4
Luteal phase	5	1
Hormone based contraception	11	10
Unable to determine	2	3
Exposure to Sexual Violence (Single/Multiple)	10/8	–
Trauma Window (i.e., Time Between Initial Trauma and PET), Months	36 ± 22	–
Clinician-Administered PTSD Scale for DSM-5		
Worst month		–
PTSD diagnosis criteria met	14	
PTSD total symptoms score (0–80)	48 ± 22	–
Past month		
PTSD diagnosis criteria met	9	–
PTSD total symptoms score (0–80)	22 ± 15	–
Subscale scores		
Cluster B: intrusion symptoms score (0–20)	5 ± 4	–
Cluster C: avoidance symptoms score (0–8)	3 ± 2	–
Cluster D: cognitions and mood symptoms score (0–28)	8 ± 6	–
Cluster E: arousal and reactivity symptoms score (0–24)	6 ± 5	–
Dissociative symptoms score (0–8)	1 ± 1	–
Life Stressor Checklist-Revised (0–30)[a]	6 ± 3	2 ± 1[b]
Hamilton Anxiety Rating Scale (0–56)	7 ± 4	2 ± 2[b]
Hamilton Depression Rating Scale (0–68)[a]	8 ± 3	3 ± 2[b]
Alcohol Use Disorders Identification Test (0–40)[a]	6 ± 6	4 ± 2

Values are mean $\pm$ SD or n.

PET, positron emission tomography; PTSD, posttraumatic stress disorder.

[a]Data were available for only $n = 12$ of 18 healthy control subjects.

[b]$p \leq .05$, unpaired t test.

in any of the scan parameters (see Table 2), regional volumes (data not shown), and V_T (LMM effect of group [$F_{1,34} = 0.15$, $p = .70$], effect of region [$F_{11,374} = 537.07$, $p < .001$], and region-by-diagnosis interaction [$F_{11,374} = 0.48$, $p = .92$]) (see data in Table 3).

Relationship Between V_T and CAPS-5 Symptoms

In the Past Month Prior to PET. In the past month before PET, there were significant positive correlations between V_T

Table 2. [¹¹C]NOP-1A PET Scan Parameters

Parameter	Experienced Sexual Violence Group ($n = 18$)	Healthy Control Group ($n = 18$)
Injected Dose, mCi	12.4 ± 0.7	12.3 ± 0.8
Specific Activity, Ci/mmol	2118 ± 1022	2396 ± 785
Injected Mass, μg	2.9 ± 1.0	2.4 ± 0.8
Plasma Free Fraction, %	14.1 ± 2.3	14.4 ± 2.3
Saline Buffer Free, %	66.5 ± 12.7	73.7 ± 11.0
Clearance, L/hour	149.1 ± 33.9	129.0 ± 27.5

Values are mean ± SD.
PET, positron emission tomography.

Table 3. Regional [¹¹C]NOP-1A V_T in Women Who Have Experienced Sexual Violence and Control Subjects

Region of Interest	Experienced Sexual Violence Group ($n = 18$)	Healthy Control Group ($n = 18$)
Amygdala	13.9 ± 2.2	14.3 ± 1.9
Hippocampus	10.5 ± 1.3	10.6 ± 2.0
Midbrain	8.6 ± 1.2	8.7 ± 1.0
Ventral Striatum	13.6 ± 1.8	13.9 ± 1.7
Caudate	11.2 ± 1.6	11.2 ± 1.4
Putamen	12.6 ± 1.8	12.7 ± 1.5
Dorsolateral Prefrontal Cortex	12.8 ± 1.8	13.0 ± 1.6
Orbital Frontal Cortex	13.2 ± 1.6	13.6 ± 1.7
Medial Prefrontal Cortex	12.7 ± 1.8	12.9 ± 1.6
Anterior Cingulate Cortex	12.9 ± 1.7	13.2 ± 1.8
Insula	14.8 ± 2.0	14.9 ± 1.9
Cerebellum	7.5 ± 1.0	7.6 ± 0.8

Values are mean ± SD.
V_T, total distribution volume.

and past-month CAPS-5 total symptom severity scores (bivariate). This relationship was significant in the midbrain and cerebellum (Figure 1) but not in the other ROIs (data not shown). These relationships remained significant with the use of nonparametric Spearman's rank-order correlation tests (see Supplement). These relationships were also significant when examined with partial correlations that controlled for the effect of HDRS scores (midbrain [$r = .77$, $p = .0003$] and cerebellum [$r = .79$, $p = .00015$]). Although there was no relationship between HDRS scores and V_T (see Supplement), this was done because the spread of the HDRS scores in the sexual violence group was relatively large (5–17 subjects; with 6 of 18 sexual violence subjects with HDRS scores ≥10). These partial but not bivariate correlations survived the Bonferroni correction (12 regions × 2 sets of CAPS-5 scores, past month and worst month; $p = .05/24$ [≤ .002]).

Removal of the four Resilient subjects who did not meet diagnostic criteria for PTSD in their worst or past month had no effect on the statistical significance of these correlations. Removal of the two subjects who used nicotine did not alter the correlation coefficient (r), but it changed the p values for the relationships between past month CAPS-5 total severity scores and V_T to trend-level (see Supplemental Table S3).

Midbrain and cerebellum V_T were positively correlated with past month clusters B (see Figure 2) and C (midbrain [$r = .53$, $p = .02$] and cerebellum [$r = .54$, $p = .02$]), but not D (midbrain [$r = .28$, $p = .25$] and cerebellum [$r = .28$, $p = .26$]) and E severity scores (midbrain [$r = .40$, $p = .10$] and cerebellum [$r = .37$, $p = .13$]).

In the Worst Month After Sexual Violence. In the worst month after sexual violence, there were no significant correlations between V_T and worst-month CAPS-5 total symptom (or cluster) severity scores.

Relationship Between V_T and Other Clinical Parameters

No significant relationships were noted between regional V_T and HAM-A, HDRS, and AUDIT scores in the control or sexual violence groups. There was also no relationship between V_T and the time that had elapsed since sexual violence (i.e., trauma window) (see Supplement).

DSM-5 PTSD Diagnosis and [¹¹C]NOP-1A V_T

No differences in [¹¹C]NOP-1A V_T were observed in subjects with a diagnosis of PTSD PM ($n = 9$ of 18 in sexual violence
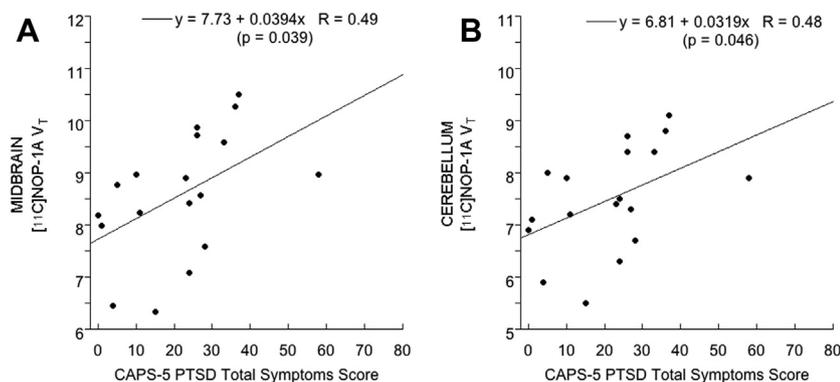

Figure 1. The relationship between [¹¹C]NOP-1A total distribution volume (V_T) and Clinician-Administered PTSD Scale for DSM-5 (CAPS-5) total symptoms score. Increased binding to nociceptive opioid peptide receptors in the **(A)** midbrain and **(B)** cerebellum in women who have experienced sexual violence is associated with more severe posttraumatic stress disorder (PTSD) symptoms.
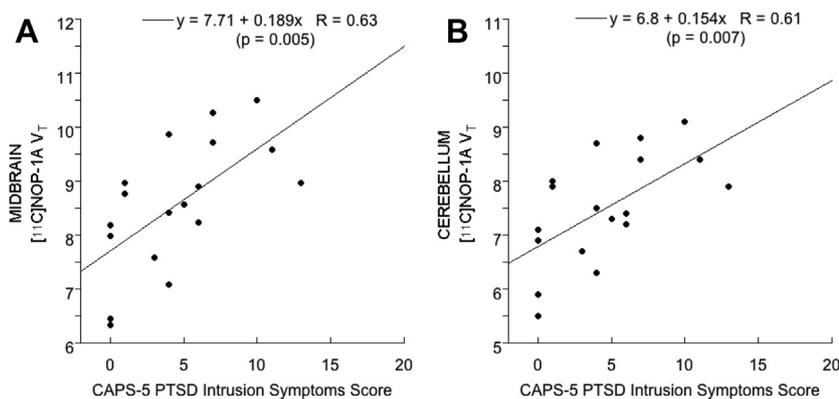
Figure 2. The relationship between **(A)** midbrain and **(B)** cerebellum total distribution volume (V_T) and Clinician-Administered PTSD Scale for DSM-5 (CAPS-5) intrusion (cluster B) symptoms score. Increased binding to nociceptive opioid peptide receptors in women who have experienced sexual violence is associated with more severe posttraumatic stress disorder (PTSD) intrusion symptoms.

group) compared with control subjects (LMM effect of diagnosis [$F_{1,25} = 0.27$, $p = .61$], effect of region [$F_{11,275} = 315.29$, $p < .001$], region-by-diagnosis interaction [$F_{11,275} = 0.42$, $p = .95$]).

No differences in [^{11}C]NOP-1A V_T were observed in subjects with a diagnosis of PTSD WM ($n = 14$ of 18 in sexual violence group) compared with control subjects (LMM effect of diagnosis [$F_{1,30} = 0.08$, $p = .78$], effect of region [$F_{11,330} = 434.95$, $p < .001$], region-by-diagnosis interaction [$F_{11,330} = 0.55$, $p = .87$]).

DISCUSSION

In this [^{11}C]NOP-1A PET imaging study, we scanned college women who had experienced sexual violence irrespective of whether they met the DSM-5 diagnostic criteria for PTSD in the past or worst month. The results of this study show that increased midbrain and cerebellum V_T is related to greater PTSD symptoms. This relationship with V_T was significant for PTSD symptoms experienced in the recent past, but not in the worst month since sexual violence. This suggests that increased NOP receptors in women who have experienced sexual violence are an adaptive response to ongoing as opposed to historical PTSD symptoms. However, these PET data do not exclude the possibility that women with increased NOP receptors (e.g., genetically determined) may be at greater risk to develop long-lasting PTSD symptoms. This interpretation is supported by the inclusion of women who were resilient and recovered from PTSD in this study. The intrusion, re-experiencing, and avoidance symptoms presently considered primary features of PTSD were strongly associated with V_T (1). No such associations were noted with the secondary mood, cognition, arousal, and reactivity symptoms of PTSD. DSM-5–based stratification of the sexual violence subjects into groups (PTSD PM, PTSD WM, and Resilient) and contrasting them with control subjects revealed no differences in V_T.

The positive association observed between PTSD symptom severity and [^{11}C]NOP-1A V_T in the midbrain and cerebellum suggest a role for N/OFQ and NOP in recovery following trauma. An increase in NOP messenger RNA in the limbic-related brain regions has been reported following restraint stress (36), social defeat stress (37), social crowding (38), and the single-

prolonged stress exposure paradigm (22). Increases in N/OFQ signaling could be accomplished via either increased N/OFQ release or an upregulation of NOP receptors. Studies have reported mixed results and lack consensus with respect to whether N/OFQ levels increase in the brain following stress (36,39–41). This has led to the postulation that an upregulation of NOP receptors in response to stress is an adaptive physiological response to enhance N/OFQ signaling in the brain (36). Further supportive of this is a study in which an upregulation of NOP, but not of N/OFQ messenger RNA), was observed in the bed nucleus of the stria terminalis following an acute increase in the stress-mediating neuropeptide corticotropin-releasing factor (42). Increased NOP in more severe PTSD might also reflect lower levels of the endogenous neurotransmitter N/OFQ. Such lines of reasoning would support a therapeutic role for NOP
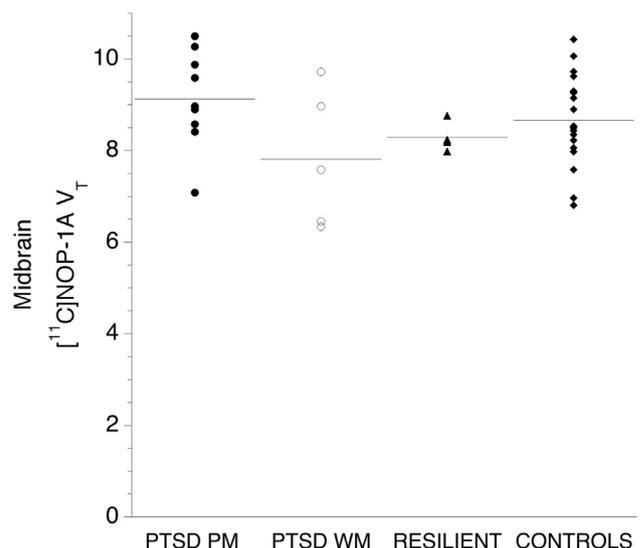

Figure 3. The lack of DSM-5–based diagnostic group differences in [^{11}C]NOP-1A midbrain binding between posttraumatic stress disorder in past month (PTSD PM) vs. PTSD in worst month (PTSD WM) vs. no PTSD in the past and/or worst month (Resilient) vs. healthy control subjects with no prior history of sexual violence (Controls). V_T, total distribution volume.

receptor agonists in promoting both resilience and recovery in PTSD (21,43). Intriguingly, a retrospective study found that the weak NOP receptor agonist buprenorphine was more effective than opioid agonists, which have no NOP affinity, in improving PTSD symptoms in veterans with chronic pain and opioid use disorders (44). However, animal studies also support a role for NOP receptor antagonists in treating PTSD (22,45). The correlational results from this PET study implicate NOP but do not necessarily inform the field as to whether a NOP receptor agonist or antagonist will be successful in treating PTSD. Future clinical trials with NOP compounds are necessary to clarify their therapeutic role, if any, in PTSD.

The correlational findings in this study involved the midbrain (substantia nigra, raphe nucleus, ventral tegmental area, and red nucleus) and cerebellum. The involvement of the midbrain and cerebellum in a cerebellar-limbic-thalamo-cortical network that functions as an innate alarm system in response to a threat is consistent with emerging imaging literature in PTSD (46). In addition to the established role of the midbrain in regulating startle, hypervigilance, and escape, these studies suggest that the midbrain and cerebellum process subconscious fear- and trauma-related cues in PTSD (47,48). The periaqueductal gray matter, a part of the midbrain, elicits adaptive behaviors in response to a threat (49). The cerebellum, which receives teaching signals via climbing fibers from the olivary nucleus, is involved in the learning of complex cognitive processes including emotions (49). Studies also suggest that the cerebellum plays a role similar to the amygdala in the consolidation of fear-conditioned memories and fear expression (50). Basic studies have also demonstrated direct and indirect (via inferior olivary nucleus) connections between the periaqueductal gray matter and the cerebellum (49). The periaqueductal gray matter gates sensory information to the cerebellum via these connections. It also influences the motor output from cerebellar nuclei, which when combined with its own control over spinal motor reflex pathways allows an animal to freeze, get ready, and escape danger (49,51). In summary, basic data suggest that the clinical correlations involving the midbrain and cerebellum are relevant. However, replication of these correlational findings in a larger sample of individuals with more diverse traumatic experiences is necessary to confirm them.

Numerous investigations have implicated N/OFQ and NOP in animal models of learning and memory [reviewed in Andero (43)]. N/OFQ impairs fear acquisition and memory consolidation in a range of behavioral paradigms, including the contextual/auditory fear conditioning, object recognition, passive avoidance learning, and water maze tests. It also disrupts the retrieval and reconsolidation of previously consolidated traumatic memories (52). N/OFQ inhibits K+ stimulated glutamate release by ~38% in rodent cerebellum and midbrain slices—the exact same regions in which we observed clinical correlations between NOP and intrusive symptoms (53). The mechanism by which N/OFQ disrupts trauma-related memory consolidation involves its ability to inhibit glutamate release and interrupt N-methyl-D-aspartic acid–mediated long-term potentiation (43,54). N/OFQ also inhibits the release of monoamine neurotransmitters such as dopamine, acetylcholine, serotonin, and norepinephrine, all of which are

involved in cue-induced and context-dependent learning and are likely relevant to its ability to disrupt the formation of trauma-related memories (43). Increased NOP receptors in women with intrusive trauma-related memories after sexual violence might be reflective of a continued effort by the brain to enhance N/OFQ signaling to reduce glutamate and monoamine transmission. Imaging studies examining the interactions between NOP and glutamate and between NOP and dopamine, and linking them with intrusive traumatic memories and fear expression in PTSD, are necessary to clarify this mechanism. The lack of a relationship between [^{11}C]NOP-1A V_T and anxiety/depressive symptoms is inconsistent with basic reports (55). The relatively low level of anxiety and depressive symptoms (HAM-A score = 7 ± 3, HDRS score = 8 ± 3) in subjects who had experienced sexual violence may have contributed to the inability to detect a relationship with V_T. However, the failure to observe this relationship is consistent with what we have recently reported with [^{11}C]NOP-1A PET in individuals with addictive disorders (30). There were no DSM-5 PTSD diagnosis–based group differences in V_T compared with healthy control subjects (see Figure 3). It is tempting to ascribe this to an insufficient number of subjects with a diagnosis of PTSD in this study. However, the effect size to detect group differences between individuals with PTSD in the past month versus control subjects is a modest 0.46 (when derived using data from the midbrain, the region in which we found the strongest clinical correlation) (see Figure 3). Power calculations using this effect size suggest a need to enroll 124 subjects/group to detect between-group differences when using a two-tailed unpaired t test with a p value <.05. Future [^{11}C]NOP-1A studies in psychiatric and addictive disorders should not only focus on demonstrating group differences, but also examine the relationship between NOP V_T and clinical symptoms.

A limitation of the study is that it is unclear whether factors such as the phase of menstrual cycle, hormonal contraceptive use, and nicotine use influenced the correlational findings observed in this study. No studies have examined the effects of menstrual cycle phases on NOP receptor expression in brain regions such as the midbrain and cerebellum. However, recent investigations in the spinal cord trigeminal neurons and hypothalamus (a unique brain region, which regulates the synthesis of sex steroids) suggest that the progesterone to estrogen ratio, which fluctuates during the menstrual cycle, influences NOP receptor expression and binding. These studies have reported increases in NOP in the hypothalamic nuclei following both estrogen and estrogen + progesterone treatment and decreases in NOP in the trigeminal neurons during the proestrous (high estrogen/low progesterone) compared with diestrous (low estrogen/high progesterone) phase of the rodent menstrual cycle (56,57). It is unclear as to whether and how menstrual cycle phase may have influenced [^{11}C]NOP-1A V_T measurements in the midbrain and cerebellum in this imaging study. A retrospective evaluation of the phase of menstrual cycle based on the subjects' self-reported last menstrual period before the scan shows that the majority of women with a history of sexual violence were scanned either in the luteal phase or when on a hormone-based contraceptive (n = 16 of 18) (see Table 1). This is reassuring, as it suggests relative stability in estrogen and progesterone levels at the time

of the PET scan in women who experienced sexual violence, despite higher levels in luteal phase ($n = 5$) and lower levels on hormonal contraceptives ($n = 11$) (58,59). Furthermore, no significant differences in V_T in the midbrain and cerebellum in women scanned in the follicular phase or luteal phase, or on hormone-based contraceptives, also suggest a limited effect for sex-hormone ratio on NOP receptor binding (Supplemental Table S2). Nevertheless, it seems prudent for future [^{11}C]NOP-1A PET studies in women to measure serum estrogen and progesterone levels at time of the PET scan to exclude the effects of sex hormones on V_T. With respect to another confound, the use of nicotine, only 2 of 18 subjects who experienced sexual violence were smokers. Excluding these individuals from the analysis did not change correlation coefficient (r), but it changed the p value to a trend (from significant) for the relationship between V_T and CAPS-5 total symptom severity (this was not the case for the relationship between CAPS-5 intrusion symptoms and V_T). This is likely attributable to a loss of power as opposed to the influence of smoking status on the relationship. This interpretation is supported by our legacy [^{11}C]NOP-1A data in healthy control subjects in which we find no significant differences in V_T based on smoking status (Supplemental Table S4). Based on this, we conclude that the impact of smoking is minimal on the findings reported in this study. Other limitations of the study are the exclusion of a clinically representative sample of PTSD with more diverse trauma and severe symptoms because of the concerns of comorbidity and psychiatric medications. The inability to exclude individual differences in [^{11}C]NOP-1A nonspecific binding as a contributor to V_T is also a concern for which there was no technical solution. This concern is somewhat alleviated by prior blocking studies in humans that have demonstrated that 50% to 75% of [^{11}C]NOP-1A V_T represents specific binding to NOP receptors (60). In summary, we showed a relationship between [^{11}C]NOP-1A V_T and the severity of posttraumatic stress symptoms in college women who had experienced sexual violence during adolescence/ young adulthood. These correlational data were also supportive of a role for NOP in mediating trauma-related intrusion and avoidance symptoms. The approach used in this study highlights the continued need to investigate the pathophysiology of psychiatric disorders using a DSM-5 agnostic approach as recommended in the National Institute of Mental Health Research Domain Criteria initiative.

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