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ORIGINAL ARTICLE

Overlapping expression of serotonin transporters and neurokinin-1 receptors in posttraumatic stress disorder: a multi-tracer PET study

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The brain serotonergic system is colocalized and interacts with the neuropeptidergic substance P/neurokinin-1 (SP/NK1) system. Both these neurochemical systems have independently been implicated in stress and anxiety, but interactions between them might be crucial for human anxiety conditions. Here, we examined the serotonin and substance P/neurokinin-1 (SP/NK1) systems individually as well as their overlapping expression in 16 patients with posttraumatic stress disorder (PTSD) and 16 healthy controls. Participants were imaged with the highly selective radiotracers [^{11}C]-3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile (DASB) and [^{11}C]GR205171 assessing serotonin transporter (SERT) and NK1 receptor availability, respectively. Voxel-wise analyses in the amygdala, our *a priori*-defined region of interest, revealed increased number of NK1 receptors, but not SERT in the PTSD group. Symptom severity, as indexed by the Clinician-administered PTSD Scale, was negatively related to SERT availability in the amygdala, and NK1 receptor levels moderated this relationship. Exploratory, voxel-wise whole-brain analyses revealed increased SERT availability in the precentral gyrus and posterior cingulate cortex of PTSD patients. Patients, relative to controls, displayed lower degree of overlapping expression between SERT and NK1 receptors in the putamen, thalamus, insula and lateral orbitofrontal gyrus, lower overlap being associated with higher PTSD symptom severity. Expression overlap also explained more of the symptomatology than did either system individually, underscoring the importance of taking interactions between the neurochemical systems into account. Thus, our results suggest that aberrant serotonergic-SP/NK1 couplings contribute to the pathophysiology of PTSD and, consequently, that normalization of these couplings may be therapeutically important.

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INTRODUCTION

Posttraumatic stress disorder (PTSD)[1] is prevalent,[2] often chronic[3] and causes great individual suffering as well as high societal costs.[4] Following a traumatic event, patients with PTSD experience persistent and disabling symptoms including hyperarousal, intrusive memories and avoidance of stimuli associated with the event.[1]

Emerging research is beginning to elucidate neurochemical alterations in PTSD.[5–7] Given the defined onset by pairing of previously neutral cues with intense negative affect, fear conditioning has been suggested to be an important etiological mechanism.[8,9] Accordingly, several lines of evidence suggest similar neurochemical underpinnings of PTSD and fear conditioning, including serotonergic modulation. Patients with PTSD, and individuals prone to fear-conditioning processes, display exaggerated serotonin-modulated amygdala activity[10–13] and reduced serotonin transporter (SERT) availability.[14,15] Moreover, the low-expressing short variant of the SERT-linked polymorphic region is a risk factor for PTSD[16] and facilitates fear conditioning.[17] Also, treatment with selective serotonin reuptake inhibitors reduces PTSD symptoms[18] and disrupts fear conditioning.[19,20] Thus, there is evidence for involvement of SERT in PTSD but very few imaging studies have assessed this directly. The first aim of the present study was therefore to further investigate SERT availability in PTSD,[15] using positron emission tomography (PET) with radiolabelled DASB ([^{11}C]-3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile).[21]

Besides serotonergic alterations, pharmacological and emotional challenge studies have related the substance P/neurokinin-1 (SP/NK1) system to PTSD.[22,23] The NK1 receptor[24,25] and its preferred endogenous neuropeptide ligand SP are abundantly expressed in the amygdala[26] and suggested to be involved in anxiety and stress-related disorders.[22,27–29] This is based partly on studies showing that SP in the amygdala modulates stress and anxiety through its action on the NK1 receptor.[27,29–32] In line with this, symptom provocation in patients with PTSD increases amygdala activity[10] and heightens already elevated cerebrospinal SP concentrations.[22] Blocking NK1 receptors, on the other hand, attenuates PTSD hyperarousal symptoms[23] and increases activity in the ventromedial prefrontal cortex,[33] that is, an area modulating fear-related amygdala activity[34,35] reported to be hypoactive in PTSD.[36] Collectively, findings both in animals, using fear-conditioning models of PTSD, and in patients support SP involvement, acting through NK1 receptors, in PTSD symptomatology. However, direct *in vivo* assessment of the brain SP/NK1 system has not been performed in PTSD patients. Consequently, the

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second aim of the study was to assess NK1 receptor availability in patients with PTSD with the PET radiotracer [^{11}C]GR205171.[37]

The serotonin and SP/NK1 systems are extensively colocalized in cortical and subcortical regions.[38,39] For example, NK1 receptors are co-expressed with serotonin 5-HT_{1A} receptors in the amygdala,[39] and blocking SERT reduces substance P levels.[40] A recent multi-tracer PET study in humans used voxel-wise correlational analyses to index overlapping expression of neurotransmitter systems and reported high degree of overlap between the serotonergic and opioid systems in areas relevant for anxiety, including the amygdala.[41] However, to our knowledge there are no multi-tracer PET investigations performed on the joint expression of the serotonergic and SP/NK1 systems in any anxiety disorder. Thus, the third aim was to adopt a multi-system approach to examine overlapping expression of the serotonergic and SP/NK1 systems in patients with PTSD relative to controls, and to evaluate if the two systems modulate PTSD symptomatology independently or interactively.

MATERIALS AND METHODS

Participants

Eighteen PTSD patients and 18 healthy controls (HC) underwent [^{11}C]DASB and [^{11}C]GR205171 PET imaging. Owing to technical problems during PET image acquisition, analyses for two patients and two HCs were not possible, leaving 16 patients (8 men, mean ± s.d. age 38.7 ± 13.0 years) and 16 HCs (8 men, mean ± s.d. age 34.0 ± 9.7 years) in the final sample. All participants were right-handed and the groups did not differ in age or sex (P's >0.26) (see Supplementary Table 1 for participant characteristics).

Participants were recruited through the Department of Psychiatry and the Department of Obstetrics and Gynecology at Uppsala University Hospital. Besides a clinical psychiatric evaluation using DSM-IV criteria for PTSD,[1] a medical examination was performed. All patients had a primary PTSD diagnosis with index trauma exposure consisting of combat ($n=8$), traffic accident ($n=3$), witnessed suicide ($n=2$), sexual assault ($n=2$) or domestic violence ($n=1$). Symptom severity was evaluated with the Clinician-administered PTSD Scale (CAPS),[42] and depressive symptomatology with the Montgomery-Åsberg Depression Rating Scale.[43] The Mini International Neuropsychiatric Interview[44] was used to assess psychiatric co-morbidity. Main exclusion criteria were any other major psychiatric illness (for example. schizophrenia), except depression or another anxiety disorder, or neurological disorder, somatic disease, ongoing or discontinued psychological treatment within the last 2 months, treatment with psychotropic medication, chronic use of prescribed medication, current drug or alcohol abuse/dependency, previous PET-examination, pregnancy or menopause.

Similar assessments were made for the HCs, excluding the clinical symptom scales. All HC participants were healthy and none fulfilled criteria for any current psychiatric disorder, as assessed with the Mini International Neuropsychiatric Interview,[44] nor did they have a life-time history of such disorders.

Image acquisition

PET images were acquired at rest using a 32-ring ECAT EXACT HR+ camera (Siemens/CTI, Knoxville, TN, USA), which enables the acquisition of 63 contiguous planes of data with a slice thickness of 2.46 mm, resulting in a total axial field of view of 155 mm. Subjects fasted 3 h, and refrained from tobacco, alcohol and caffeine 12 h, before PET investigations. A venous catheter for tracer injections was inserted in the arm of the subject. For each PET investigation, subjects were positioned supine in the scanner with the head gently fixated and a 10-min transmission scan was performed using three retractable germanium (^{68}Ge) rotating line sources.

[^{11}C]DASB. Coinciding with start of the emission scan, subjects were injected intravenously with on average 390 ± 35.1 MBq [^{11}C]-3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile ([^{11}C]DASB)[21] as a rapid bolus injection and 22 frames of data were acquired in three-dimensional mode during 60 min (1 × 60 s, 4 × 30 s, 3 × 60 s, 4 × 120 s, 2 × 180 s, 8 × 300 s).

[^{11}C]GR205171. For the [^{11}C]GR205171[37] PET assessments, a similar procedure was used. After the transmission scan, 361 ± 68.9 MBq of [^{11}C]GR205171 was injected intravenously as a fast bolus simultaneously with the start of the emission scan. Data were acquired in three-dimensional mode and consisted of 17 frames (4 × 60 s, 3 × 120 s, 10 × 300 s) with a total duration of 60 min.

Image analysis

Dynamic images were reconstructed using ordered subset expectation maximization with six iterations and eight subsets and a 4 mm Hanning post-filter. Parametric [^{11}C]DASB and [^{11}C]GR205171 images were calculated for each voxel as an index of SERT and NK1 receptor availability, respectively. For [^{11}C]DASB, the reference Logan method[45,46] was performed on a time interval of 30–60 min and binding potential (BP_{ND})[47] was estimated as the distribution volume ratio-1 relative to cerebellum. Parametric images showing influx rate K_i (ml cm^{-3} min^{-1}) of [^{11}C]GR205171, that is, an index of NK1 receptor availability, were calculated using a modified reference Patlak method[29,48] and the time interval of 30–60 min. The cerebellum was chosen as reference region for both tracers as it is assumed to have no specific binding of [^{11}C]DASB[46,49] or [^{11}C]GR205171.[37,50,51] The reference region was defined using the PVElab software,[52] an observer-independent approach for automatic generation of volumes of interest. For both tracers, the volumes of interest template was applied to each participant's PET [^{11}C]DASB BP_{ND} image summed over all 22 frames.[53]

The [^{11}C]DASB BP_{ND} and [^{11}C]GR205171 K_i images were co-registered to the summed [^{11}C]DASB image for each subject. The summation image was then normalized to the PET template from Statistical Parametric Mapping 8 (SPM8; (Wellcome Department of Cognitive Neurology, University College London, www.fil.ion.ucl.ac.uk)), and the calculated transformation parameters applied to the parametric images, resulting in images normalized to the Montreal Neurological Institute (MNI) standard space with isotropic 4 × 4 × 4 mm^3 voxels. The MNI normalized [^{11}C]DASB BP_{ND} and [^{11}C]GR205171 K_i images were subsequently smoothed with an 8-mm isotropic Gaussian kernel.

Statistical analysis

The amygdala was chosen *a priori* as region of interest (ROI) based on earlier functional, and neurochemical findings indicating the region as a primary site of pathology in PTSD.[10,15,54–56] The Automated Anatomical Labeling library from the Wake Forest University Pickatlas[57] was used to define the amygdala bilaterally. All analyses included age and sex as covariates as there are reports of age and sex effects in both SERT[58] and NK1 receptor availability.[59,60]

Comparisons of patients vs controls in SERT and NK1 receptor availability. Two-sample *t*-tests in SPM8 were used to examine group differences in [^{11}C]DASB BP_{ND} and [^{11}C]GR205171 K_i between PTSD patients and HC participants. For the *a priori* amygdala, results were thresholded with $P < 0.05$ and cluster extent of 640 mm^3 to balance type I and type II errors, as the amygdala is strongly implicated in PTSD pathophysiology.[10,15,54,55] For whole-brain analyses, the statistical threshold for significance was set at combined height $P < 0.001$ and cluster extent of 640 mm^3.

Overlapping expression of SERTs and NK1 receptors. Analyses of overlapping expressions were performed by means of correlations between [^{11}C]DASB BP_{ND} and [^{11}C]GR205171 K_i. For each voxel, Pearson's product–moment correlation between [^{11}C]DASB BP_{ND} and [^{11}C]GR205171 K_i across individuals was determined, as an index of regional overlap between SERT and NK1 receptor availability. The overlap index ranged from −1 (lowest degree of overlapping expression) to +1 (highest degree of overlapping expression). The correlation coefficients were calculated separately for the PTSD patients and the HC participants and transformed using Fisher's r-to-z transformations to stabilize the variance of the correlation coefficients. The z-transformed correlation coefficients were used in subsequent voxel-wise group comparisons.

Relationships between neurotransmitter systems and symptom severity. Linear regression analyses within the PTSD group were performed using total CAPS score as outcome and [^{11}C]DASB BP_{ND}, [^{11}C]GR205171 K_i and the interaction between them ([^{11}C]DASB BP_{ND} × [^{11}C]GR205171 K_i) as predictors. Age and sex were also entered as predictors to safeguard against potential confound from these variables. One female patient did

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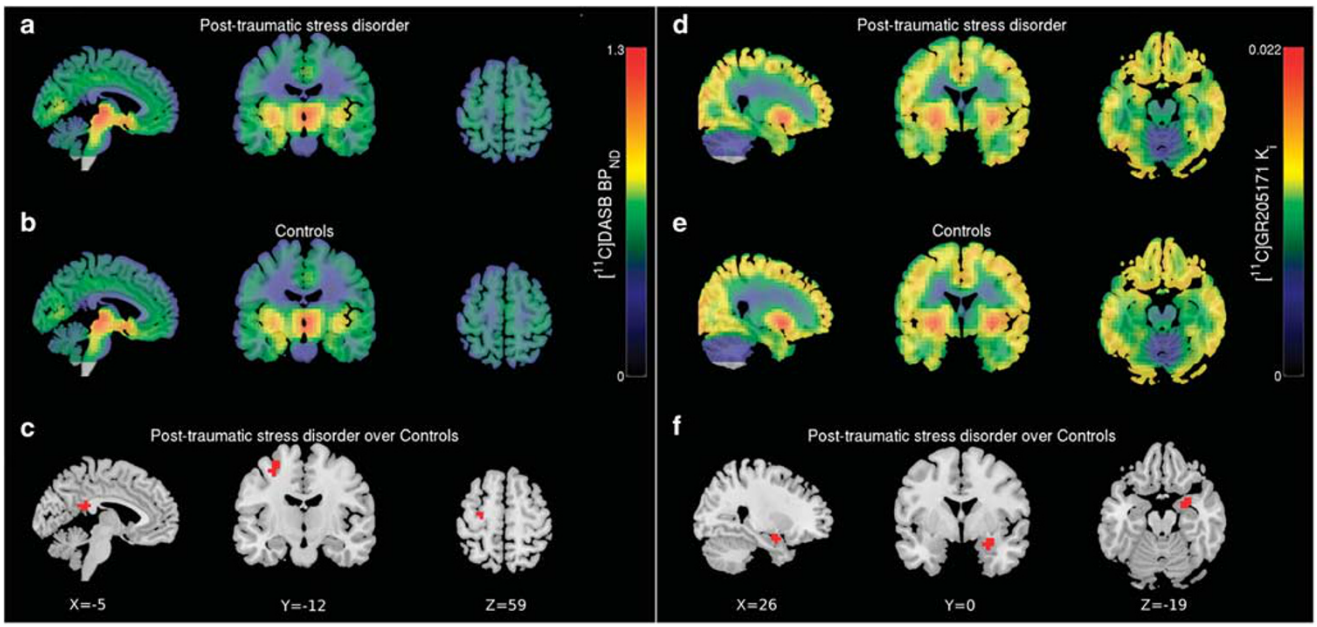

Figure 1. Left panel shows average serotonin transporter availability indexed by [^{11}C]-3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile (DASB) binding potential (BP_{ND}) in (**a**) patients with posttraumatic stress disorder (PTSD), (**b**) healthy controls and (**c**) clusters of significantly increased [^{11}C]DASB BP_{ND} in PTSD patients in the precentral gyrus and posterior cingulate cortex. All rows depict slices at Montreal Neurological Institute (MNI) coordinate (–5, –12, 59). The colorbar indicates [^{11}C]DASB BP_{ND} for the two top rows. Right panel shows average neurokinin-1 receptor availability ([^{11}C]GR205171 K_i) in (**d**) patients with PTSD, (**e**) healthy controls and (**f**) cluster of significantly increased [^{11}C]GR205171 K_i in PTSD patients in the amygdala. All rows depict slices at MNI coordinate (26, 0, –19). The colorbar indicates [^{11}C] GR205171 K_i for the two top rows.

not complete the CAPS interview, leaving 15 PTSD patients in the regression analyses. Both *a priori*-defined amygdala ROI and voxel-wise whole-brain regression analyses were performed. For the amygdala ROI analyses, mean BP_{ND} and K_i values were extracted and four linear regression models tested. The first two models included only [^{11}C]DASB BP_{ND} and [^{11}C]GR205171 K_i, respectively. In the third model, both [^{11}C] DASB BP_{ND} and [^{11}C]GR205171 K_i were entered. The fourth model added the interaction between [^{11}C]DASB BP_{ND} and [^{11}C]GR205171 K_i (both variables mean centered) to the third model. Positive interaction terms were taken to indicate that higher overlapping expression of SERT and NK1 receptors was related to greater PTSD symptom severity, whereas negative interaction terms were interpreted as a negative relationship between overlapping levels and PTSD symptom severity.

Voxel-wise correlation and regression analyses were performed with combined $P < 0.05$ and cluster extent of 640 mm^3, to balance type I and type II errors and as these novel analyses are considered exploratory. Only voxels showing specific binding were considered in the analyses, that is, where mean [^{11}C]DASB BP_{ND} and [^{11}C]GR205171 K_i exceeded 0.1 and 0.005, respectively. This resulted in ~10 000 voxels for DASB analyses and 25 000 voxels for GR205171 analyses.

Participant characteristics and the ROI regression analyses were analyzed using R 3.1.0 (R Foundation for Statistical Computing, Vienna, Austria).

Supplementary analyses

To safeguard against effects of potential outliers and extreme values, we complemented the Pearson's correlational analyses with Spearman's rank correlations, and the multiple regression analyses with robust and rank-based regression methods. For the sake of completeness, we conducted whole-brain regression analyses of relationships between the neurotransmitter systems and the three subscales of CAPS, that are arousal, avoidance and intrusion.

Ethical statement

The study was approved by the Regional Ethical Vetting Board, Uppsala and the Radiation Safety Committee, the Uppsala University Medical Faculty Ethical Review Board and the Uppsala University Isotope Committee. All study participants gave written informed consent before the study start and were reimbursed for their participation.

RESULTS

Comparisons of patients vs controls

SERT availability. No difference in SERT availability ([^{11}C]DASB BP_{ND}) in the amygdala was evident between the PTSD patients and the HC participants ($P > 0.05$, see Supplementary Figure 1). The PTSD patients relative to HCs had higher SERT availability in the left precentral gyrus (Brodmann area 6; MNI *x*, *y*, *z*: −26, −12, 62; $Z = 3.45$, $P < 0.001$, cluster = 768 mm^3) and posterior cingulate cortex (Brodmann area 23; MNI *x*, *y*, *z*: −10, −40, 22; $Z = 3.39$, $P < 0.001$, cluster = 768 mm^3), see Figure 1, left panel, c.

NK1 receptor availability. Compared with the HCs, the NK1 receptor availability ([^{11}C]GR205171 K_i) was elevated in PTSD patients in the right amygdala only (MNI *x*, *y*, z: 26, 0, −18; $Z = 2.21$, $P = 0.011$, cluster = 832 mm^3), see Figure 1, right panel, f.

Overlapping expression of SERTs and NK1 receptors. Voxel-wise analyses of overlapping expression, that is, between-system correlations, showed significant overlap between SERT and NK1 receptor expression over widespread brain areas in both groups, with relatively lower overlap in the PTSD group in the putamen, thalamus, insula and lateral orbitofrontal gyrus, and higher overlap within the inferior temporal gyrus, cuneus, middle cingulate cortex and medial orbitofrontal gyrus, see Table 1, Figure 2 and Supplementary Figure 2.

Relationships between neurotransmitter systems and symptom severity

Amygdala a priori analyses. In the *a priori*-defined ROI analyses of the amygdala, mean [^{11}C]DASB BP_{ND} and [^{11}C]GR205171 K_i in the amygdala were extracted bilaterally for each participant and

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Table 1. Overlapping expression of SERT; [^{11}C]DASB BP_{ND} and NK1R; [^{11}C]GR205171 influx rate, K_i in PTSD as compared with HC

	BA	*Hemisphere*	*MNI* x, y, z			*HC* r[a]	*PTSD* r[a]	*diff* r[b]	*diff* P-*value*	*Cluster volume*[c]
HC > PTSD										
Putamen	—	Right	26	8	−10	0.78	−0.47	1.25	< 0.001	2048
Thalamus	—	Left	−14	−20	6	0.73	−0.43	1.16	0.001	1088
Insula	13	Left	−38	−20	−2	0.51	−0.58	1.09	0.004	640
Orbitofrontal gyrus	10	Right	34	56	−2	0.75	−0.24	0.99	0.005	640
Insula	47	Left	−26	16	−14	0.69	−0.14	0.83	0.020	704
PTSD > HC										
Inferior temporal gyrus	37	Right	46	−52	−22	−0.70	0.58	−1.28	< 0.001	704
Cuneus	19	Left	−6	−84	30	−0.62	0.66	−1.28	< 0.001	3392
Middle cingulate cortex	24	Right	10	8	34	−0.29	0.82	−1.11	< 0.001	896
Orbitofrontal gyrus	11	Right	6	56	−10	−0.22	0.82	−1.04	0.001	832

Abbreviations: BA, Brodmann area; BP_{ND}, binding potential; HC, healthy control; MNI, Montreal Neurological Institute; NK1R, neurokinin-1 receptor; PSTD, posttraumatic stress disorder; SERT, serotonin transporters. [a]Overlapping expression indexed by Pearson's product–moment correlation coefficient, *r*. [b]Differences in Pearson's *r* correlation coefficient between groups: r_{HC}–r_{PTSD}, as an index of difference in overlapping expression. [c]Cluster volume in mm^3.

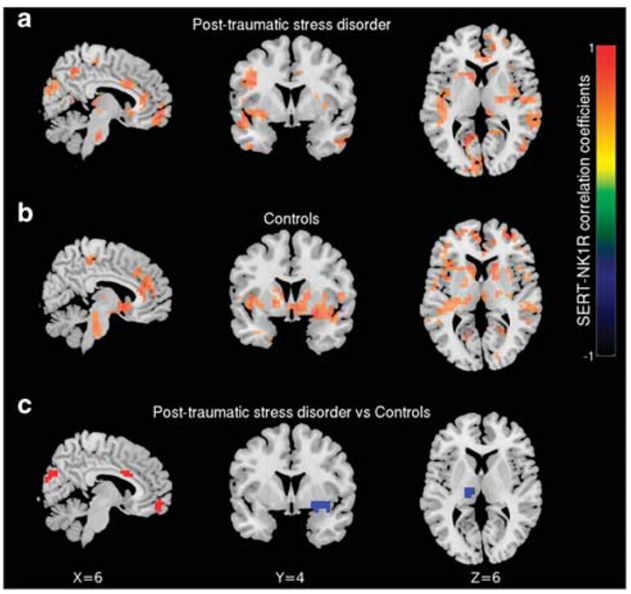

Figure 2. Overlapping expression of serotonin transporter (SERT; [^{11}C]-3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile (DASB) BP_{ND}) and neurokinin-1 receptor (NK1R; [^{11}C]GR205171 K_i) in (**a**) patients with posttraumatic stress disorder (PTSD), (**b**) healthy controls and (**c**) clusters of significantly altered overlap in PTSD. Red clusters indicate higher degree of overlap and blue clusters lower overlap in PTSD patients relative to controls. All rows depict slices at Montreal Neurological Institute coordinate (6, 4, 6). The colorbar indicates Pearson's correlation coefficients for correlations between [^{11}C]DASB BP_{ND} (SERT) and [^{11}C]GR205171 K_i (NK1 receptors) for the two top rows. Parametric images are overlaid on a standard MRI image.

entered into four separate linear regression models with CAPS score as outcome. In the first model, only [^{11}C]DASB BP_{ND} was entered in a simple linear regression model revealing significant negative contribution of SERT availability to symptom scores ($\beta = -0.67$, $P = 0.016$, see Supplementary Figure 3). Second, only [^{11}C]GR205171 K_i was entered into a simple regression model revealing no significant contribution of NK1 receptor availability to symptom scores ($\beta = -0.32$, $P = 0.212$, see Supplementary Figure 4). Third, both [^{11}C]DASB BP_{ND} and [^{11}C]GR205171 K_i were entered into a multiple linear regression model. For this model, there was a significant contribution of [^{11}C]DASB BP_{ND} ($\beta = -0.64$, $P = 0.046$), whereas the contribution of [^{11}C]GR205171 K_i remained non-significant ($\beta = -0.08$, $P = 0.736$). The fourth model added the interaction between [^{11}C]DASB BP_{ND} and [^{11}C]GR205171 K_i to the third model, significantly increasing the explained variance (adjusted R^2) in CAPS score from 53 to 75% ($F(2,9) = 9.67$, $P = 0.013$). Importantly, in this model only the interaction term ($\beta = -0.49$, $P = 0.013$), but not [^{11}C]DASB BP_{ND} ($\beta = -0.31$, $P = 0.195$) or [^{11}C]GR205171 K_i ($\beta = -0.08$, $P = 0.656$), significantly predicted symptom severity (see Figure 3, bottom panel). Notably, NK1 receptor availability moderated the relationship between SERT availability and symptom severity because low SERT levels were associated with relatively higher anxiety, irrespective of availability of NK1 receptors, whereas high SERT availability was associated with relatively higher anxiety only when NK1R levels were low.

Whole-brain analyses. In whole-brain, voxel-wise exploratory analyses, both [^{11}C]DASB BP_{ND} and [^{11}C]GR205171 K_i in large parts of the brain were significantly related to CAPS score in simple voxel-wise regression analyses (see Figure 3, top panel). The regression coefficients (β) were largely negative for both tracers. Voxel-wise multiple linear regression analyses including simultaneously [^{11}C]DASB BP_{ND}, [^{11}C]GR205171 K_i and their interaction revealed that the [^{11}C]DASB $BP_{ND} \times$ [^{11}C]GR205171 K_i interaction term was significantly related to CAPS, measuring PTSD symptom severity. Except for a few regions, a negative relationship was found between the interaction term and symptom severity, indicating that more severe PTSD symptoms were associated with lower overlap between SERT and NK1 receptor expression (see Table 2 and Figure 3, top panel, c). Restricting the regression analyses only to clusters (> 1 voxel) with an altered overlap in expression in PTSD relative to controls, yielded significant negative interaction effects only in areas with lower overlap, that is, in the insula (MNI *x*, *y*, *z*: −26, 16, −10; $\beta = -1.01$, $P = 0.049$, cluster = 192 mm^3), thalamus (MNI *x*, *y*, *z*: −10, −16,6; $\beta = -0.76$, $P = 0.017$, cluster = 128 mm^3) and putamen (MNI *x*, *y*, *z*: 26,8, −14; $\beta = -0.63$, $P = 0.009$, cluster = 128 mm^3), indicating a negative relationship between PTSD symptom severity and levels of overlapping SERT-NK1 receptor expression.

Supplementary analyses

When safeguarding against outliers and extreme values by substituting the original Pearson's correlations with Spearman's rank-order correlations, the difference in SERT-NK1 receptor correlations between patients and controls remained (Supplementary Table 2). Robust and rank-based regressions

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revealed similar results as the ordinary least square method in the analyses of symptom severity and receptor systems (Supplementary Table 3). The CAPS arousal, avoidance and intrusion subscales were significantly related to availability of SERT and NK1 receptors, as well as to their overlap in cortical and subcortical regions, see Supplementary Tables 4, 5, 6 and Supplementary Figures 5, 6 and 7.

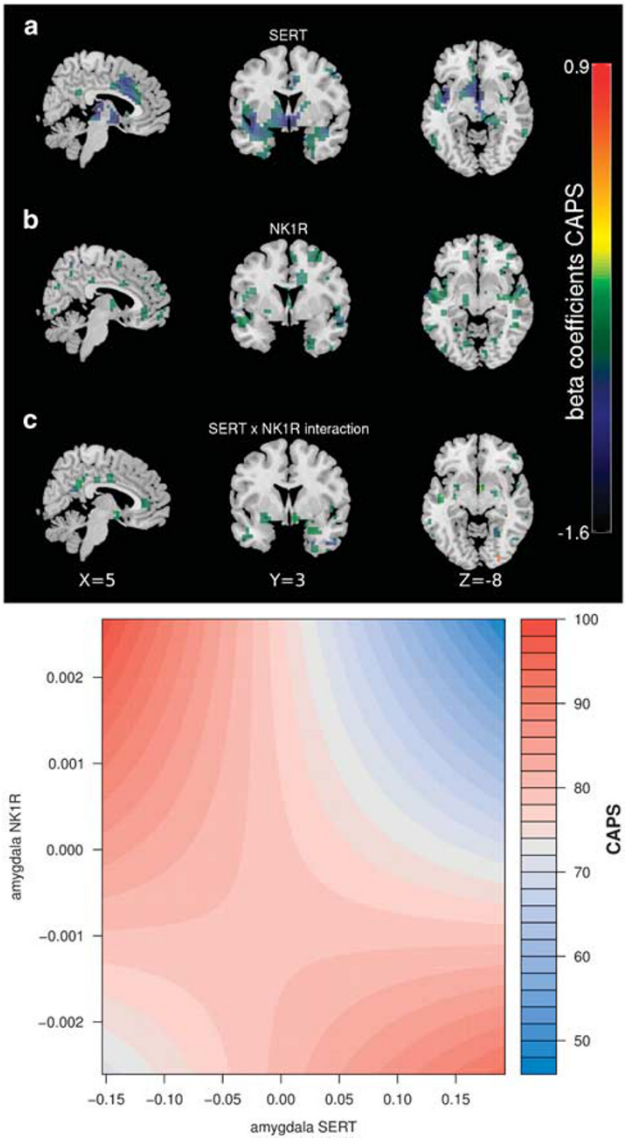

Figure 3. Neurotransmitter system expression overlap and posttraumatic stress disorder (PTSD) symptom severity, indexed by the Clinician-administered PTSD Scale (CAPS). Top panel shows beta weights for relationship between total CAPS score for separate simple regression models including (**a**) serotonin transporter (SERT) availability ([^{11}C]-3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile (DASB) BP_{ND}), (**b**) neurokinin-1 receptor (NK1R) availability ([^{11}C]GR205171 K_i), respectively, and (**c**) for the interaction between SERT and NK1R availability in a multiple regression model including both SERT and NK1R availability and the interaction. Beta coefficient images are overlaid on a standard MRI image. All rows depict slices at MNI coordinate (5, 3, −8). The colorbar indicates regression coefficients (beta). Bottom panel shows an interaction plot, illustrating the relationship between neurotransmitter system expression overlap in the amygdala and PTSD symptom severity as measured by CAPS. The amount of NK1R modulated the relationship between SERT availability and symptom severity because low SERT levels were associated with relatively higher anxiety, irrespective of number of NK1 receptors, whereas high SERT availability was associated with relatively higher anxiety only when NK1R levels were low. Predictor variables are mean centered. The scale on the right denotes CAPS score.

DISCUSSION

In this multi-tracer PET study, we observed elevated brain SERT and NK1 receptor availability as well as altered correlations between them in patients with PTSD. SERT BP_{ND} in various brain regions including the amygdala was negatively related to PTSD symptom severity. This relationship was modulated by NK1 receptor availability, such that low SERT availability was associated with high anxiety, regardless of NK1 receptor levels, whereas high SERT availability was linked to high anxiety only when NK1 receptor levels were low, supporting that the function of these systems is better evaluated interactively than independently.

The relation between SERT and NK1 receptors were altered in PTSD patients, with both higher and lower degree of overlapping expression in several brain regions. Lower degree of overlap in the *a priori* ROI amygdala predicted greater symptom severity. Exploratory analyses revealed the same pattern in the insula, thalamus and putamen. The alterations in the joint structure of the serotonergic and SP/NK1 systems may indicate a functional imbalance between these systems in PTSD. Given that SP is a neuropeptide backup system for serotonin during periods of high stress, we speculate that the modulatory role of SP is disrupted in patients with PTSD and that this relates to symptomatology.

We did not replicate a recent report of reduced SERT BP_{ND} in the amygdala in PTSD patients,[15] instead we found increased SERT availability in the precentral gyrus and posterior cingulate cortex. Consistent with Murrough *et al.*,[15] who reported a negative correlation between anxiety scores and amygdala SERT availability in PTSD, we also found a negative relationship between PTSD symptoms and SERT in the amygdala, as well as in other brain territories. These findings are in agreement with a recent report of an inverse association between number of amygdala SERT and fear learning.[14] The apparent discrepancy between elevated SERT availability in the PTSD group and a negative association between SERT and PTSD symptoms may suggest regionally specific effects of SERT availability on symptoms or a non-linear relationship. The finding may also reflect conscious coping or more automated balancing processes within the serotonergic system in the brain, as suggested by Pietrzak *et al.*[61] for the noradrenergic system in PTSD. Because symptom severity in patients with social anxiety disorder also is inversely related to SERT in fear-expressing anterior cingulate cortex areas,[62] as well as positively related to serotonin synthesis in the amygdala, we suggest that serotonin in fear coding and expressing[55] brain areas in patients with anxiety disorders facilitates anxiety. This suggestion is also consistent with recent evidence for anxiogenic effects of serotonin in the amygdala in anxious rat strains.[63]

Consistent with reports of upregulated NK1 receptors after prolonged stress,[64] the PTSD group had increased NK1 receptor availability in the amygdala. In addition, SP acting through NK1 receptors in the amygdala is linked to anxiety-like behavior in animals.[30,65–67] Our results are in agreement with findings of elevated SP concentrations in cerebrospinal fluid of PTSD patients.[22] Furthermore, increased NK1 receptor levels may be associated with reduced cortisol levels in PTSD patients,[68] as SP inhibits activity in the hypothalamic–pituitary–adrenocortical axis.[69] Moreover, social anxiety disorder is associated with a similar increase in NK1 receptor availability in the amygdala,[70] whereas patients with panic disorder exhibit widespread reduction of NK1 receptors across the brain. Taken together, the findings suggest a role for the SP/NK1 system in various anxiety disorders, consistent with animal studies.[27] It should be noted, however, that pharmacological alterations of the SP/NK1 system

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Table 2. Relationship between symptom severity CAPS Total score and the overlapping expression between neurotransmitter systems, SERT availability (BP_{ND}) and NK1R (K_i), PTSD

	BA	*Hemisphere*	*MNI* x, y, z			β^a	P-*value*	*Cluster volume*[b]
Negative relationship with CAPS total score								
Middle frontal gyrus	10	Right	34	40	30	−1.17	0.004	832
Inferior frontal trigeminal gyrus	46	Right	46	24	22	−1.11	0.026	2304
Inferior occipital gyrus	19	Left	−50	−64	−18	−1.04	< 0.001	2880
Inferior temporal gyrus	20	Right	46	0	−38	−1.04	0.010	2304
Middle temporal gyrus	37	Right	46	−68	6	−1.02	< 0.001	896
Insula	47	Left	−26	16	−10	−1.01	0.049	4416
Parahippocampal gyrus	35	Right	22	−12	−30	−0.92	0.003	960
Lingual gyrus	19	Right	22	−56	−6	−0.91	0.012	1152
Anterior cingulate cortex	24	Left	−2	36	6	−0.88	0.009	2944
Precentral gyrus	6	Right	38	−8	54	−0.87	0.022	1152
Precuneus	23	Right	6	−52	22	−0.84	0.002	1984
Precuneus	7	Right	2	−64	30	−0.84	0.019	1856
Middle frontal gyrus	9	Right	46	20	38	−0.80	0.002	768
Orbitofrontal gyrus	47	Right	26	8	−18	−0.78	0.008	4800
Middle temporal gyrus	22	Right	62	−44	2	−0.77	< 0.001	2688
Middle cingulate cortex	24	Right	2	−4	34	−0.74	0.033	768
Middle cingulate cortex	31	Left	−10	−28	38	−0.74	0.028	896
Superior temporal pole	38	Right	50	4	−10	−0.72	0.004	1472
Middle frontal gyrus	10	Right	34	52	10	−0.71	0.039	896
Rolandic operculum	22	Left	−46	−8	14	−0.54	0.039	1024
Positive relationship with CAPS total score								
Fusiform gyrus	36	Right	30	−36	−14	0.95	0.011	1088
Inferior occipital gyrus	18	Right	26	−92	−6	0.75	0.017	640

Abbreviations: BA, Brodmann area; BP_{ND}, binding potential; CAPS, Clinician-administered PTSD scale; HC, healthy control; MNI, Montreal Neurological Institute; NK1R, neurokinin-1 receptor; PSTD, posttraumatic stress disorder; SERT, serotonin transporter. [a]Regression coefficient. [b]Cluster volume in mm^3.

do not invariably affect anxiety, as initial positive clinical trials of NK1 antagonists for PTSD and other affective disorders[23,71–73] have been difficult to replicate.[33,50,74] The reason for this is debated, and has been suggested to reflect that the necessary receptor occupancy needed for effective treatment was not obtained in the larger trials.[75] Notably, because anxiety patients have increased levels of NK1 receptors, receptor occupancy studies in HC may underestimate the doses needed to achieve adequate occupancy. Moreover, NK1 antagonists could be selectively effective for state-dependent high-stress symptoms by reducing neural amygdala activity, such as symptom provocation, not readily assessed in clinical trials.[71] Indeed, only hyperarousal symptoms were improved in a recent trial of the NK1 antagonist GR205171 for PTSD.[23] Consistently, in the present study, the arousal subscale of the CAPS was negatively related to NK1 receptor availability within various brain regions, including the amygdala.

The study limitations include a relatively small sample size, although typical for a PET study. The small sample may be sensitive for outliers and extreme values causing spurious correlations and interactions. Here, the results remained when complementing our original analyses with rank-based correlations and regressions as well as robust regressions, making us conclude that the results are not caused by outliers or extreme values. As this is the first study evaluating the overlap in expression patterns between neurotransmitter systems *in vivo* in an anxiety or stress-related disorder, liberal statistical thresholds were chosen to avoid type II errors. To mitigate spurious findings, we combined statistical level and extent criteria. Results should, however, be regarded tentative until replicated. However, it should also be noted that our conservative *a priori* amygdala ROI analyses supported the voxel-wise findings. Furthermore, the lack of a non-PTSD trauma control group prevented us from discerning the effects of trauma exposure. However, although trauma in itself may have profound acute effects, it seems unlikely that a trauma that did not result in any long-lasting symptoms that would have any effects on PET scans more than a decade later. To minimize confounding factors, we excluded severe psychiatric co-morbidity such as psychotic disorders and substance use disorders, which may hamper the generalizability of the results to PTSD patients with more severe co-morbidity. It should also be noted that substance use disorders were assessed by self-report only. The limited spatial resolution of PET imaging also precludes conclusions regarding overlap between SERT and NK1 receptors at the cellular level. Our results should therefore be thought of as co-existence of SERT and NK1 receptors in the same brain region, here defined by the voxel.

In summary, the current results indicate that overlapping expression of SERT and NK1 receptors is altered in PTSD and that lower overlap is related to worse symptoms. In the amygdala, a negative relationship between SERT availability and PTSD symptom severity was moderated by NK1 receptor levels, such that lower degree of overlap in expression was related to more severe PTSD symptoms. Overlapping expression of SERT and NK1 receptors explained more of the symptomatology than either system individually. Studying interactions between several neurotransmitter systems in a multimodal imaging framework represents a new path in neurobiological anxiety research and may potentially serve to guide future therapeutics.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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