IMPORTANCE  Serotonin is involved in negative affect, but whether anxiety syndromes, such as social anxiety disorder (SAD), are characterized by an overactive or underactive serotonin system has not been established. Serotonin 1A autoreceptors, which inhibit serotonin synthesis and release, are downregulated in SAD, and serotonin transporter availability might be increased; however, presynaptic serotonin activity has not been evaluated extensively.

OBJECTIVE  To examine the serotonin synthesis rate and serotonin transporter availability in patients with SAD and healthy control individuals using positron emission tomography (PET) with the radioligands 5-hydroxytryptophan labeled with carbon 11 ([11C]5-HTP) and [11C]labeled 3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile ([11C]DASB).

DESIGN, SETTING, AND PARTICIPANTS  We performed a cross-sectional study at an academic clinical research center. Eighteen patients with SAD (9 men and 9 women; mean [SD] age, 32.6 [8.2] years) and 18 sex- and age-matched healthy controls (9 men and 9 women; mean [SD] age, 34.7 [9.2] years) underwent [11C]5-HTP PET imaging. We acquired [11C]DASB PET images for 26 additional patients with SAD (14 men and 12 women; mean [SD] age, 35.2 [10.7] years) and the same 18 sex- and age-matched healthy controls. Participants were recruited through newspaper advertisements. Data were acquired from March 12, 2002, through March 5, 2012, and analyzed from March 28, 2013, through August 29, 2014.

MAIN OUTCOMES AND MEASURES  The influx rate of [11C]5-HTP as a measure of serotonin synthesis rate capacity and [11C]DASB binding potential as an index of serotonin transporter availability were acquired during rest. We used the Liebowitz Social Anxiety Scale to measure severity of social anxiety symptoms.

RESULTS  The PET data were not available for analysis in 1 control for each scan. Increased [11C]5-HTP influx rate was observed in the amygdala, raphe nuclei region, caudate nucleus, putamen, hippocampus, and anterior cingulate cortex of patients with SAD compared with healthy controls (P < .05 corrected), supporting an enhanced serotonin synthesis rate. Increased serotonin transporter availability in the patients with SAD relative to healthy controls was reflected by elevated [11C]DASB binding potential in the raphe nuclei region, caudate nucleus, putamen, thalamus, and insula cortex (P < .05 corrected).

CONCLUSIONS AND RELEVANCE  Neurotransmission in SAD is characterized by an overactive presynaptic serotonin system, with increased serotonin synthesis and transporter availability. Our findings could provide important new insights into the etiology of anxiety disorders.
Anxiety disorders are debilitating psychiatric conditions that impose a considerable burden on patients and society, and social anxiety disorder (SAD) is one of the most common of these conditions. The neural underpinnings of excessive social anxiety are not fully characterized, although serotonin (5-hydroxytryptamine) has been suggested to be involved etiologically.

However, only a few studies have used molecular neuroimaging to examine serotonin dysfunction in SAD directly. A single-photon emission tomography study found increased serotonin transporter availability in the thalamus, but not in the raphe nuclei, in patients with SAD relative to healthy control individuals. Also, a positron emission tomography (PET) study showed that SAD is associated with reduced serotonin 1A receptor binding. Somatodendritic serotonin 1A autoreceptors in the raphe nuclei, which inhibit serotonin synthesis and release, and postsynaptic serotonin 1A heteroreceptors, which convey inhibitory signals in the amygdala, anterior cingulate cortex (ACC), and insula cortex, were downregulated. In addition, functional neuroimaging studies of SAD have demonstrated heightened, fear-induced neural reactivity in the amygdala, which is densely innervated by serotonin, with alterations in the hippocampus, ACC, insula cortex, and striatum. Moreover, the first line of pharmacologic treatment for SAD consists of selective serotonin reuptake inhibitors (SSRIs), which reduce excessive amygdala reactivity, restore initially suppressed ventromedial prefrontal cortex response to emotional challenge, and attenuate resting brain perfusion in the ACC and insula. Thus, findings from molecular and functional neuroimaging and treatment studies indicate that serotonergic neurotransmission in the amygdala, raphe nuclei, striatum, thalamus, hippocampus, insula cortex, and ACC may be compromised in SAD.

Given the inhibitory role of serotonin 1A autoreceptors on serotonin synthesis, previous findings of decreased autoreceptor binding in SAD may indicate increased serotonin formation and enhanced serotonergic activity. On the other hand, blocking the serotonin reuptake with SSRIs attenuates social anxiety symptoms and because posttreatment dietary depletion of the serotonin precursor tryptophan reverses the anxiolytic effects of SSRIs, the notion that increased serotonin availability is pivotal for anxiety reduction also has support. Indeed, whether anxiety conditions such as SAD are best characterized by serotonin overactivity or underactivity remains a matter of debate.

Presynaptic serotonin activity, including synthesis and reuptake, are major contributors to serotonin neurotransmission. In vivo measurement of the serotonin synthesis rate can be accomplished by targeting the second enzymatic step of serotonin synthesis using PET and the tracer 5-hydroxytryptophan labeled with carbon 11 ([11C]5-HTP) as the marker. The serotonin transporter, blocked by SSRIs, is an important regulator of serotonin neurotransmission that influences responsivity of the neural fear circuitry and can be assessed successfully using PET with the tracer [11C]-labeled 3-amino-4-(2-dimethylaminomethylphenylsulfanyl)benzonitrile ([11C]DASB).

Herein, we used PET imaging with the radioligands [11C]5-HTP and [11C]DASB to evaluate the rate of serotonin synthesis in the brain and serotonin transporter availability, respectively, in patients with SAD and healthy controls. Based on earlier findings of reduced raphe nuclei serotonin 1A autoreceptor density and enhanced serotonin transporter availability in the thalamus, we could predict that SAD is associated with an increased rate of serotonin synthesis and increased transporter binding, that is, an overactive, presynaptic, serotonergic system. On the other hand, because SSRIs increase serotonin availability and reduce SAD symptoms, a reduced rate of serotonin synthesis is possible. Furthermore, because attenuated serotonin transporter binding has been reported in other anxiety disorders (although findings are mixed) and because genetically modified serotonin transporter knockout mice show increased anxiety levels, SAD could also be characterized by reduced transporter availability. Thus, although we predicted abnormalities in presynaptic serotonin functioning in SAD, we did not have a priori hypotheses regarding the direction.

Methods

Participants

Eighteen patients with SAD (9 men and 9 women; mean [SD] age, 32.6 [8.2] years) and 18 healthy controls (9 men and 9 women; mean [SD] age, 34.7 [9.2] years) underwent [11C]5-HTP PET imaging. Technical problems during PET image acquisition prevented analysis of data from 1 control, leaving 17 with data available for analysis (8 men and 9 women). The groups did not differ by age or sex distribution (P > .63). The [11C]DASB PET images were acquired for another 26 patients with SAD (14 men and 12 women; mean [SD] age, 35.2 [10.7] years) and the same 18 controls. Technical problems prevented analysis of 1 [11C]DASB PET scan from the control group, leaving data from 17 controls available for analysis (9 men and 8 women). Again, these groups did not differ by age or sex distribution (P > .72). The 2 SAD groups did not differ with respect to age, sex distribution, severity of social anxiety, number of individuals with generalized SAD, previous use of psychotropic medication, or number of individuals with current or a history of psychiatric comorbidity (P > .12) (eTable 1 in the Supplement details participant characteristics). Data were acquired from March 12, 2002, through March 5, 2012. The study was approved by the regional ethics committee in Uppsala and the Radiation Safety Committee for [11C]DASB PET and by the ethical review board of the Uppsala University Medical Faculty and the Uppsala University Isotope Committee for [11C]5-HTP PET. All participants gave written informed consent before the start of the study and were reimbursed for participation.

Participants were recruited through newspaper advertising. Main exclusion criteria were any other primary major psychiatric or neurologic disorder, somatic disease, ongoing or discontinued (within 2 months) psychological or psychotropic medication treatment, long-term use of prescribed medication, current drug or alcohol abuse or dependency, previous PET examination, pregnancy, or menopause.
Patients meeting the initial screening criteria for social phobia from the Social Phobia Screening Questionnaire\textsuperscript{33} and who did not fulfill any exclusion criteria were subsequently administered the Mini-International Neuropsychiatric Interview\textsuperscript{34} and the Structured Clinical Diagnostic Interview for the DSM-IV-TR\textsuperscript{35} to ascertain that they fulfilled the DSM-IV-TR criteria for SAD (social phobia).

All patients had a primary diagnosis of SAD. Severity of social anxiety symptoms was evaluated with the self-reported version of the Liebowitz Social Anxiety Scale (LSAS)\textsuperscript{37} for the patients who underwent $[^{11}C]$DASB PET imaging and with the clinician-administered version of the LSAS\textsuperscript{37} for the patients who underwent $[^{11}C]$DASB PET imaging. Scores on the LSAS range from 0 to 144, with higher scores indicating greater severity of symptoms. The self-reported and clinician-administered versions of the LSAS are highly correlated.\textsuperscript{37} Finally, a medical examination was performed.

The controls underwent similar assessments. All participants were appraised as healthy, and none of the controls fulfilled criteria for any current psychiatric disorder as assessed with the Mini-International Neuropsychiatric Interview\textsuperscript{34} or had any lifetime history of such disorders.

**Image Acquisition**

The PET images were acquired using a 32-ring high-resolution scanner (ECAT EXACT HR+; Siemens/CTI), which enables the acquisition of 63 contiguous planes of data with a section thickness of 2.46 mm, resulting in a total axial field of view of 155 mm. Participants fasted for 3 hours and refrained from using tobacco, alcohol, and caffeine 12 hours before the PET investigations. A venous catheter for tracer injections was inserted in the arm of the participant. For each PET investigation, participants were positioned in the scanner with their head gently fixed, and a 10-minute transmission scan was performed using 3 retracted germanium 68 rotating line sources.

$[^{11}C]$5-HTP PET Assessments

For the $[^{11}C]$5-HTP PET assessments, the tracer $[^{11}C]$5-HTP\textsuperscript{21,38} was injected as a rapid bolus (mean [SD], 7.41 [2.68] mCi; to convert to megabecquerels, multiply by 37), whereupon the tracer $[^{11}C]$5-HTP and $[^{11}C]$DASB images were entered into separate analyses in SPM8. We examined group differences between patients with SAD and controls using 2-sample t tests. To explore the relationship between severity of social anxiety symptoms and serotonin synthesis or reuptake, parametric $[^{11}C]$5-HTP and $[^{11}C]$DASB images were entered into separate regression models with the LSAS total score as the predictor. The statistical threshold for significance was set at $p < 0.05$.

**Image Analysis**

Data were analyzed from March 28, 2013, through August 29, 2014. We calculated parametric images using graphic methods with a reference region input, showing an influx rate ($K_i$, in minutes$^{-1}$) of $[^{11}C]$5-HTP, that is, an index of serotonin synthesis rate, and binding potential (BP$_{ND}$)\textsuperscript{39} of $[^{11}C]$DASB, that is, an index of serotonin transporter availability, for each voxel. For $[^{11}C]$5-HTP, a modified reference Patlak method\textsuperscript{22,40,41} correcting for binding of $[^{11}C]$5-HTP in the cerebellum, was used during an interval of 30 to 60 minutes\textsuperscript{22,23} to estimate $K_i$. For $[^{11}C]$DASB, the reference Logan method\textsuperscript{42} was used during an interval of 30 to 60 minutes, and BP$_{ND}$ was estimated as the distribution volume ratio $- 1$ relative to the cerebellum. The cerebellum was used as the reference region because it is assumed to have no specific binding of $[^{11}C]$DASB.\textsuperscript{43}

The reference region was defined using the PVElab software,\textsuperscript{44} an observer-independent approach for automatic generation of volumes of interest. For $[^{11}C]$5-HTP, a coregistered $^{15}$O-water PET scan of each patient was used for volume-of-interest definition\textsuperscript{44}; for $[^{11}C]$DASB, the volume-of-interest template was applied to a PET image summed for all 22 frames.\textsuperscript{45}

Each individual’s $[^{11}C]$5-HTP $K_i$ image was coregistered to their $^{15}$O-water summation image using affine transformation. The $^{15}$O-water summation image was then normalized to the PET template from Statistical Parametric Mapping 8 software (SPM8; Wellcome Department of Cognitive Neurology, University College London [http://www.fil.ion.ucl.ac.uk]), and the calculated transformation parameters were applied to the $[^{11}C]$5-HTP $K_i$ image, resulting in $[^{11}C]$5-HTP $K_i$ images normalized to the Montreal Neurological Institute (MNI) standard space. The MNI-normalized $[^{11}C]$5-HTP $K_i$ images were subsequently smoothed with a 12-mm isotropic gaussian kernel.

The $[^{11}C]$DASB BP$_{ND}$ images were coregistered to the summation image of all 22 $[^{11}C]$DASB frames for each participant. The summation images were then normalized to the SPM8 PET template, and the transformation parameters were applied to the BP$_{ND}$ images. The resulting MNI-normalized $[^{11}C]$DASB BP$_{ND}$ images were subsequently smoothed with a 12-mm isotropic gaussian kernel.

**Statistical Analysis**

A priori anatomic regions of interest (ROIs) were chosen based on earlier functional, structural, and neurochemical findings in SAD and areas rich in serotonin synthesis and reuptake (eFigure in the Supplement).\textsuperscript{6,7,46–50} The ROIs included the amygdala, raphe nuclei, caudate nucleus, putamen, thalamus, hippocampus, insula cortex, and ACC, defined using the Automated Anatomical Labeling library from the Wake Forest University Pickatlas\textsuperscript{46} except for the raphe nuclei ROI, which was defined from the PVElab software.\textsuperscript{44}

We entered parametric $[^{11}C]$5-HTP and $[^{11}C]$DASB images into separate analyses in SPM8. We examined group differences between patients with SAD and controls using 2-sample t tests. To explore the relationship between severity of social anxiety symptoms and serotonin synthesis or reuptake, parametric $[^{11}C]$5-HTP and $[^{11}C]$DASB images were entered into separate regression models with the LSAS total score as the predictor. The statistical threshold for significance was set at
Behavioral data and participant characteristics were analyzed using publicly available software (R, version 3.1.0; R Foundation for Statistical Computing).

Supplementary Analyses
Because SAD is more prevalent in women than in men and because earlier studies have found sex differences in serotonin synthesis and serotonin transporter availability in healthy participants and patients with anxiety disorders, we evaluated the effects of sex on serotonin synthesis rate and serotonin transporter availability with the interaction between sex and group (ie, SAD or control). Parametric [11C]5-HTP and [11C]DASB images were entered into separate 2-way, between-subject (sex × group), voxel-wise analyses of variance in SPM8 (eAppendix 1 in the Supplement).}

Results
Serotonin Synthesis
Statistical parametric mapping within the a priori ROIs revealed an increased rate of serotonin synthesis in patients with SAD compared with controls in the amygdala, brainstem corresponding to the raphe nuclei region, caudate nucleus, putamen, hippocampus, and dorsal ACC (Figure 1 and Table). No additional significant effect of diagnosis on [11C]5-HTP Ki was found in an exploratory whole-brain analysis. Within the SAD group, social anxiety symptom scores correlated positively with [11C]5-HTP Ki in the right amygdala (MNI coordinates: 24, 4, −16; z = 3.29; cluster size, 1056 mm3; P = .001, FWE) (Figure 2).

Serotonin Transporter Availability
Within the predefined ROIs, increased serotonin transporter availability in patients with SAD compared with controls was found in the brainstem corresponding to the raphe nuclei region, caudate nucleus, putamen, thalamus, and insula (Figure 3 and Table) and at a more lenient statistical threshold in the amygdala (right: MNI coordinates: 24, 4, −16; z = 2.61; cluster size, 656 mm3; P = .005 uncorrected; left: MNI coordinates: −18, 0, −12; z = 2.04; cluster size, 168 mm3; P = .02 uncorrected). A whole-brain exploratory analysis likewise revealed augmented [11C]DASB BPND in patients with SAD compared with controls in the right putamen (MNI coordinates: 26, −12, 12; z = 5.11; cluster size, 2624 mm3; P = .003, FWE) and left thalamus (MNI coordinates: −10, −12, 8; z = 4.66; cluster size, 296 mm3; P = .02, FWE), with no additional regional differences. Within the SAD group, we found a significant negative correlation between social anxiety symptom severity scores (LSAS) and [11C]DASB BPND in the left dorsal ACC (MNI coordinates: −10, 32, 26; z = 3.44; cluster size, 256 mm3; P = .02, FWE) (Figure 2).
Supplementary Analyses

Women had a lower serotonin synthesis rate and higher serotonin transporter availability than men. Also, increased [11C]5-HTP Ki and [11C]DASB BPND were found in patients with SAD compared with controls in men and women during separate analyses, without any significant interactions between sex and group for either tracer (eAppendix 2 and 3 and eTables 2-5 in the Supplement). No significant differences were observed when comparing patients with and without concurrent or previous psychiatric comorbidity or history of psychotropic medication use.

Discussion

Using PET, we found higher rates of serotonin synthesis and/or transporter availability in patients with SAD than controls in the amygdala, raphe nuclei region, caudate nucleus, putamen, thalamus, hippocampus, insula cortex, and ACC. Although this study is, to our knowledge, the first imaging study of serotonin synthesis in SAD, we replicate and extend previous single-photon emission tomography findings of increased serotonin transporter binding in the thalamus of patients with SAD.6 These findings are consistent with an overactive presynaptic serotonin system in socially anxious individuals, which may be important for the pathogenesis of anxiety.

Notably, within the SAD group, the rate of serotonin synthesis in the dorsal amygdala correlated positively with severity of social anxiety symptoms. Although the spatial resolution of PET imaging is limited, the location of the amygdala cluster is consistent with the central nucleus,89 that is, the major output region of the amygdala, which mediates fear and anxiety.57 Because anxious rat strains are hyperserotonergic58,59 and because results of imaging studies in healthy individuals indicate a positive relationship between extracellular serotonin and reactivity in the amygdala,25,60 our [11C]5-HTP results suggest that increased extracellular serotonin concentration underlies elevated anxiety and amygdala responsivity9,47,61 in SAD. Further supporting this notion, in animals, decreased serotonin concentrations after inhibition of serotonin synthesis with parachlorophenylalanine are associated with reduced levels of anxiety,62 whereas increased serotonin concentrations after acute SSRI63,64 or fenfluramine65 administration are associated with increased anxiety.4 Moreover, we found an increased serotonin synthesis rate in the dorsal ACC in patients with SAD relative to controls and a negative relationship between the number of serotonin reuptake sites in this region and severity of social anxiety symptoms, consistent with a proposed role for the dorsal ACC in fear expression.66 Collectively, these findings suggest that extracellular serotonin in the amygdala and dorsal ACC is positively related to severity of social anxiety symptoms. Also, a previous study7 reported an association between SAD and reduced levels of inhibitory serotonin 1A heteroreceptors in the amygdala and the ACC, further indicating a link between serotonin and heightened fear circuit reactivity in SAD.5,47

Upregulated presynaptic activity observed in the present study and downregulated postsynaptic serotonin 1A receptors reported previously in SAD7 are consistent with findings of increased serotonin transporter availability30,31 and reduced sero-

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Abbreviations: ACC, anterior cingulate cortex; MNI, Montreal Neurological Institute; SAD, social anxiety disorder.

a Corrected for familywise error.

b Voxel size is 8 mm3.

* Indicates peak voxel coordinates in MNI space.
Figure 2. Correlations Among Severity of Social Anxiety Symptoms, Serotonin Synthesis Rate, and Serotonin Transporter Availability

A. Significant cluster defining the anatomic extent of the positive relationship between serotonin synthesis rate in the amygdala (Montreal Neurological Institute [MNI] coordinates: 24, 4, -16), indexed by the serotonin synthesis rate of [11C]5-HTP Kᵢ, plotted against the Liebowitz Social Anxiety Scale (LSAS) in patients with social anxiety disorder (SAD). The [11C]5-HTP Kᵢ values plotted against the LSAS score for illustrative purposes. The LSAS scores range from 0 to 144, with higher scores indicating greater severity of symptoms. Data points indicate individual values; diagonal line, fit line.

B. Significant cluster defining the anatomic location of the negative relationship between serotonin transporter availability in the dorsal anterior cingulate cortex (MNI coordinates: -10, 32, 26), indexed by the binding potential of [11C]-labeled 3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile ([11C]DASB BPND), and severity of social anxiety symptoms as measured by the LSAS in patients with SAD. The [11C]DASB BPND plotted against the LSAS score for illustrative purposes. AU indicates arbitrary units; data points, individual values; and diagonal line, fit line.

Serotonin Synthesis and Reuptake in Social Anxiety Disorder

In contrast to SAD, major depressive disorder has been associated with decreased serotonin transporter availability in the amygdala.28 This finding suggests similar serotonergic alterations across these psychiatric disorders. Oler and colleagues27 noted positive correlations among serotonin transporter availability, reactivity, and anxious temperament in the amygdala in rhesus monkeys. In contrast to SAD, major depressive disorder has been associated with a reduced rate of serotonin synthesis28 and serotonin transporter availability;7 although patients with SAD often have comorbid depressive disorder. Furthermore, posttraumatic stress disorder has been associated with decreased serotonin transporter availability in the amygdala.28 This finding suggests a complex relationship between serotonin and mood, anxiety, and stress-related disorders. Although we cannot test causal effects in the present study, we speculate that, because raphe nuclei serotonin 1A autoreceptors exert inhibitory feedback on serotonin synthesis and firing,8 downregulation of inhibitory raphe serotonin 1A autoreceptors previously reported in social anxiety7 and panic disorder27,68 leads to increased serotonin synthesis, and augmented reuptake may be a compensatory mechanism. The serotonin system is under strong internal feedback regulation, and alterations in synthesis, reuptake, or autoreceptor expression would lead to compensatory changes in the other components.27 Furthermore, although serotonin synthesis and reuptake may exert differential effects on extracellular serotonin concentration, they are important contributors to serotonergic firing through replenishment of the releasable pool of serotonin in vesicles.73 Increased synthesis rate and reuptake may consequently suggest a heightened serotonergic firing rate capacity in SAD. Moreover, we cannot exclude that our results are region specific. However, when relaxing the statistical threshold, the trend always pointed to both measures of serotonin function being enhanced in SAD, suggesting that SAD is characterized by a general overactive presynaptic serotonergic system.

Among the study limitations, we could not correlate [11C]5-HTP and [11C]DASB PET measures in a meaningful way because these tracers were collected in different SAD cohorts. This lack of correlation limits the inferences that can be made of the interaction between serotonin synthesis and reuptake. Moreover, some issues regarding the capacity of [11C]5-HTP to measure the serotonin synthesis rate have been raised. For example, since the decarboxylation of 5-HTP to serotonin involves the enzyme amino acid decarboxylase, which is found not only in serotonergic but also in dopaminergic and noradrenergic neurons, the [11C]5-HTP tracer trapping may reflect amino acid decarboxylase activity. However, current evidence indicates that [11C]5-HTP tracer trapping reflects synaptic serotonin synthesis capacity in serotonergic cells,27 and a review concluded that [11C]5-HTP is the most suitable PET tracer for measurement of serotonin synthesis currently available.24
Conclusions

We demonstrate invariably increased serotonin synthesis and transporter availability in patients with SAD relative to healthy controls, which supports an overactive presynaptic serotonin system. Correlations between social anxiety symptoms and serotonergic measures in fear-expressing brain regions further suggest the region-specific anxiogenic effects of serotonin. Our findings are widely consistent with previous imaging reports of anxiety conditions and could provide important insights into the pathogenesis of these impairing disorders.

Figure 3. Increased Serotonin Transporter Availability in Social Anxiety Disorder (SAD)

A, Mean serotonin transporter availability indexed by the binding potential of carbon 11-labeled 3-amino-4-(2-dimethylaminomethyl phenylsulfanyl)-benzonitrile ([11C]DASB BPND) in patients with SAD. B, The [11C]DASB BPND in healthy control participants. C, Clusters of significantly increased [11C]DASB BPND in SAD in the brainstem corresponding to the raphe nucleus region, caudate nucleus, putamen, thalamus, insula cortex, and amygdala. The amygdala is shown at P < .05, uncorrected, for illustrative purposes. Parametric BPND images are overlaid on a standard magnetic resonance image. All rows depict sections at Montreal Neurological Institute coordinates -2, 0, 0. The color bar indicates [11C]DASB BPND for the 2 top rows (given in arbitrary units).

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