Neuroscience and Psychiatry

Serotonin States and Social Anxiety

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Social anxiety disorder is characterized by fear and avoidance of situations in which an individual believes he or she may be subject to scrutiny and at risk for embarrassment or humiliation. It is the most common of the anxiety disorders, affecting more than 5% of the general population, with an early age at onset that is frequently associated with high rates of depressive comorbidity.[1] Social anxiety disorder is frequently treated pharmacologically with selective serotonin reuptake inhibitors (SSRIs) or serotonin-norepinephrine reuptake inhibitors, several of which are approved by the US Food and Drug Administration for this indication. Nonetheless, only 30% to 40% of patients have full and satisfactory responses to these agents.[2] Attempts have been made to enable genetic prediction of response to SSRIs in patients with social anxiety disorder,[3] but such efforts are still in the early stages and have, to our knowledge, yet to be replicated.

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Nevertheless, some patients with social anxiety disorder—particularly certain children and adolescents[4]—respond exceptionally well to SSRIs. This fact, in concert with the observation that social anxiety disorder is a frequent precursor to major depressive episodes and is the most common comorbid disorder in patients with anxious depression, has motivated interest in identifying a serotonergic basis for social anxiety disorder. In a study reported in this issue, Frick et al[5] use positron emission tomography to measure what they consider to be indices of serotonin synthesis and serotonin transporter density. They find that both measures are increased in patients with social anxiety disorder. Interpreting these findings and putting them in context with other related models of anxiety and depression requires an understanding of the functioning of the brain's serotonin system.

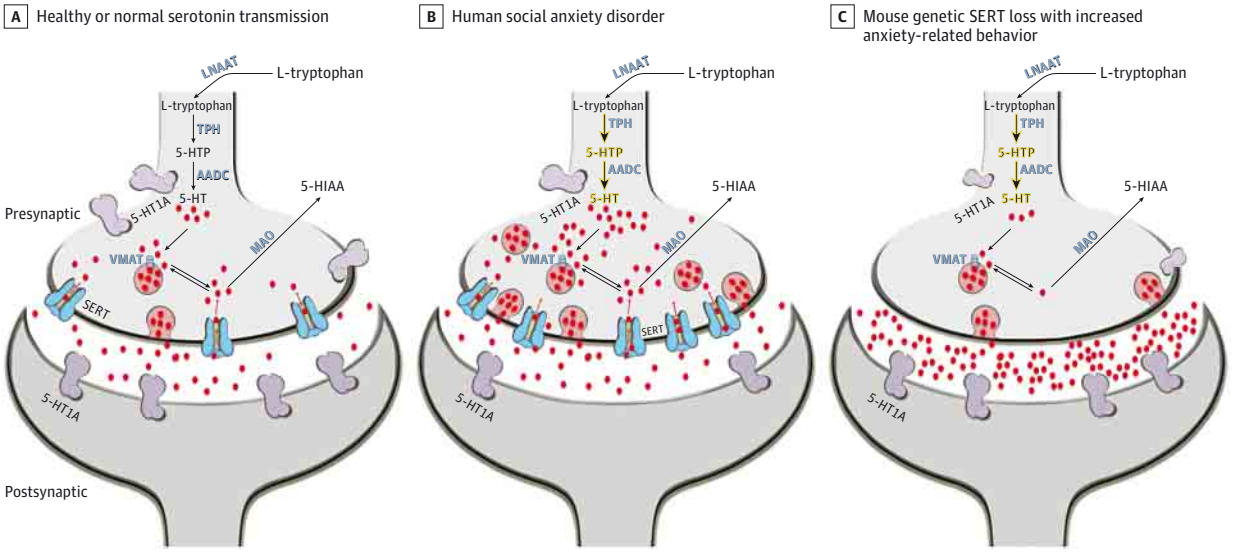
Serotonin neurons number fewer than 300 000 in humans (0.0003% of brain neurons) and are clustered in 9 brainstem groups called the *raphe nuclei*. The 3 most rostral clusters project forward to anterior brain structures, including the amygdala, striatum, hippocampus, thalamus, insula, and anterior cingulate cortex studied by Frick et al.[5] These authors administered a radioactive serotonin precursor with a short half-life ([^{11}C]5-HTP) to patients and healthy volunteers (**Figure**, A and B). Serotonin synthesis, which was estimated by measuring influx rates of [^{11}C] in various brain regions, was higher in individuals with social anxiety disorder (Figure, B). Two processes control serotonin neurotransmission. The strength of serotonin signals is determined by release, which is influenced by neuronal firing rates, vesicle filling (which is proportional to synthesis), and other factors (Figure, A). Reuptake, which largely occurs by serotonin transporters (SERT), further controls extracellular serotonin levels. In the study by Frick et al,[5] SERT density was estimated from [^{11}C]DASB binding potential and was higher in patients with social anxiety disorder than in healthy controls (Figure, B).

Serotonin synthesis is highly regulated at many levels. There is evidence that brain tryptophan concentrations, as well as oxygen and pteridine cofactor amounts, modulate tryptophan hydroxylase activity and, thus, the production of serotonin. Moreover, tryptophan hydroxylase activity may increase in response to an activity-dependent rise in intracellular Ca^{2+} and subsequent enzyme phosphorylation. Serotonin$_{1A}$ autoreceptors, which are sensitive to extracellular serotonin levels, are also believed to regulate serotonin synthesis. Beyond synthesis, SERT is dynamically controlled (eg, protein synthesis, plasma-membrane localization, and affinity states). Activation of protein kinase C, in response to increased intracellular Ca^{2+}, causes phosphorylation of SERT and sequestration from the plasma membrane. However, regulatory modes have been studied mainly in cell systems and many have yet to be verified in intact brains. Thus, it is difficult to predict the net effect of sometimes opposing regulatory mechanisms on serotonergic transmission in vivo.

There is a long and storied history of serotonin system abnormalities being associated with and, in some models, causative of anxiety and depressive behaviors and traits (eg, neuroticism) in animals and humans, particularly in the context of stress. These observations have, in fact, driven much of the development of pharmacologic approaches to treating these conditions in the past 30 years. In humans, volumes have been written about the association of 5-HTTLPR, a variable-number tandem repeat polymorphism mapped to the 5′ region of the gene that codes for human SERT, and anxiety- and depression-related traits. Whereas this literature has had its fair share of failures to replicate, an argument can be made that among the most widely accepted findings in biological psychiatry is that copies of the short (s) allele—believed to confer reduced expression of SERT—are associated with an array of anxiety-related phenotypes, including but not limited to hypervigilance, accentuated fear-potentiated startle, and behavioral sensitivity to stress. The latter phenotype—often associated with reduced emotional resilience—is becoming increasingly recognized as a feature of certain alterations in serotonin brain systems that may explain some of the core symptoms in anxiety and depressive syndromes and, therein, transcends diagnostic labels (eg, social anxiety disorder vs generalized anxiety disorder vs major depression). In humans, having 1 or 2 copies of the *s* allele of the 5-HTTLPR has been associated with self-reported reduced emotional resilience.[6]

Mice (and rats) with genetically engineered reductions in SERT show a consistently reported phenotype characterized by increased anxiety-related (conflict and avoidance) behavior and stress sensitivity, as well as decreased fear extinction and social behavior.[7] These behaviors in rodents have strong correlations with aspects of human anxiety and depression. Genetic reductions in SERT drive neuroadaptive alterations in the serotonin system, including increased extracellular serotonin concentrations,[8] decreased serotonin$_{1A}$ autoreceptor function,[9] and reduced intracellular (recycled) serotonin accompanied by compensatory increases in serotonin synthesis (Figure, C). Observations in rodent models support the notion of reduced serotonin availability, either through relative deficits in serotonin recycling (eg, via reduced SERT) or serotonin

Figure. Serotonin States Associated With Social Anxiety



A, Serotonin (5-HT) is synthesized from L-tryptophan by the rate-limiting enzyme tryptophan hydroxylase (TPH) to produce 5-hydroxytryptophan (5-HTP) followed by amino acid decarboxylase (AADC). After sequestration by vesicle monoamine transporters (VMAT), serotonin is released in response to neuronal firing. Extracellular serotonin interacts with receptors (eg, 5-HT_{1A} receptors, which function as heteroreceptors and autoreceptors, the latter providing negative feedback in raphe networks). Serotonin transporters (SERT) remove serotonin for vesicular repackaging or catabolism by monoamine oxidase (MAO) into 5-hydroxyindoleacetic acid (5-HIAA). B, Using positron emission tomography, Frick et al[5] find that [^{11C}]5-HTP influx is increased, which is interpreted as an indicator of increased serotonin synthesis. Moreover, [^{11C}]DASB binding potential is increased, suggesting greater numbers of plasma membrane SERT in social anxiety disorder. C, By contrast, mice with constitutively reduced SERT show increased anxiety-related behavior and are characterized by increased serotonin synthesis, decreased intracellular (vesicular) serotonin levels, higher extracellular serotonin levels, and desensitized 5-HT_{1A} autoreceptor function. LNAAT indicates large neutral amino acid transporter.

synthesis as critical mechanisms by which the buffering of emotional stress is affected in anxious and depressed states. Moreover, adaptive changes in the development of the serotonin system and its targets in brain circuitry underlying fear- and anxiety-related behavior occur in response to early life alterations in serotonin transmission associated with constitutive or environmental factors.[9]

Current understanding of the neural systems basis for social anxiety disorder emphasizes abnormal, traitlike dysfunction in distributed circuits involving the amygdala, insula, hippocampus, and orbital frontal regions.[10] Often referred to as components of the "fear system," these (and other) regions to which they are functionally connected are known to be modulated by serotonin states, providing a theoretical basis for expecting that altered serotonin function is associated with anxiety-related dysfunction. Prior studies specifically looking at aspects of serotonin function in social anxiety disorder have been mixed, mostly marked by small sample sizes and few to no replications. Frick et al[5] refer to their positron emission tomographic findings as indicative of "an overactive presynaptic serotonin system, with increased serotonin synthesis and transporter availability." Studies on animal models of increased serotonin synthesis associated with reduced or absent SERT expression (Figure, C) most often find that an anxious phenotype results. In fact, changes in anxiety-related behavior in mice and rats appear to accompany underexpression or overexpression of SERT. Thus, it is not at first clear why anxiety would result when increased serotonin synthesis is accompanied by increased SERT availability (Figure, B). Frick et al[5] speculate that potentiated serotonergic neuronal activity (ie, firing rates) and concomitantly increased extracellular serotonin levels might constitute the key underpinnings.

How, then, to reconcile the findings of increased SERT availability reported by Frick et al[5] with findings of mice and rats with genetically engineered deficiencies in SERT as similarly contributing to heightened anxiety-related states? An answer is to focus on the outcome for, arguably, the most important aspect of the serotonin system: extracellular serotonin signaling levels. Frick and coauthors[5] hypothesize that extraneuronal serotonin levels are increased in individuals with social anxiety disorder as a result of potentiated serotonin synthesis and, furthermore, that increased SERT binding is a neuroadaptive response to higher extracellular serotonin levels. Mice having a primary defect in SERT expression similarly exhibit increased extracellular serotonin levels; however, in this case, increased extracellular serotonin levels were derived directly from a lack of reuptake.[8] Unfortunately, there are as yet no direct methods of assessing extracellular serotonin signaling in humans that might be useful for further investigating the hypothesis by Frick et al.

Moreover, how do we understand the apparent paradox that potentiated serotonin signaling might underlie increased anxiety-related endophenotypes and possible predisposition for developing anxiety disorders with the fact that some patients respond to SSRIs, which presumably further increase extracellular serotonin levels? One possibility, of course, is that SSRIs might function through alternate adaptive presynaptic and postsynaptic mechanisms. Another is that all individuals with categorically similar symptoms may not have the same underlying pathologies. In expensive, difficult-

to-perform studies in humans, there is often a tendency to consider small, between-group differences as reflective of abnormalities that occur at the level of the individual, ignoring the potential heterogeneity that exists within the group (as well as the fact that the range of values typically overlaps extensively between patients and control participants). In the case of social anxiety disorder, there is considerable evidence that an early-onset and more pervasive form of the syndrome (formerly referred to as *generalized social anxiety disorder* in *DSM-IV*) is genetically and biologically distinct from more discrete forms of social anxiety (eg, performance anxiety only about public speaking).[1] Paying attention to this likely biological heterogeneity in the patients we study will pay dividends going forward. We have been working in this regard to investigate SERT activity in peripheral blood cells as a potential predictor of SSRI efficacy.[11]

Finally, there are caveats to the study by Frick et al[5] that benefit from additional clarification. First, as acknowledged by the authors, [^{11}C]5-HTP is converted to serotonin in other monoamine neurons when the brain is exposed to high levels of this precursor. Since dopamine and norepinephrine neurons innervate brain regions associated with fear and anxiety circuitry (eg, amygdala, orbital frontal cortex, and hippocampus), it is unknown whether increased [^{11}C]5-HTP influx measured by positron emission tomography is completely reflective of native endogenous increases in serotonin synthesis in individuals with social anxiety disorder. Second, positron emission tomography for [^{11}C]5-HTP and [^{11}C]DASB was not performed in the same individuals. Thus, both of these aspects of the serotonin system may not always be elevated simultaneously in patients with social anxiety disorder. Nonetheless, the findings by Frick et al point to a potentially novel disrupted regulatory sequence in serotonin neurochemistry resulting from overactive serotonin signaling and having aspects in common with other preclinical and human models of increased anxiety traits and behavior. Larger replication studies, possibly using additional imaging ligands and modalities, will be valuable for further exploring these theories.

ARTICLE INFORMATION

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