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Review

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A Dialogue between the Immune System and Brain, Spoken in the Language of Serotonin

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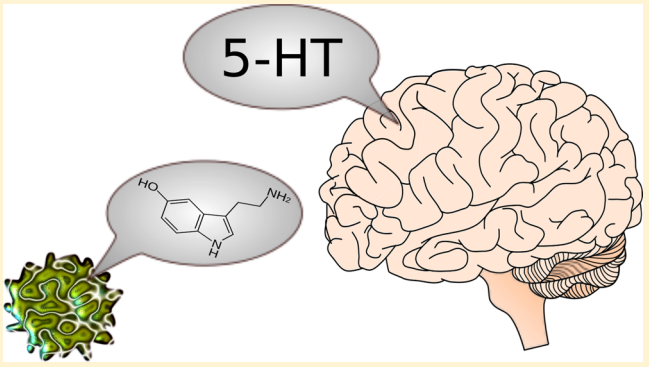
ABSTRACT: Neuropsychiatric disorders have long been linked to both immune system activation and alterations in serotonin (5-HT) signaling. In the CNS, the contributions of 5-HT modulate a broad range of targets, most notably, hypothalamic, limbic and cortical circuits linked to the control of mood and mood disorders. In the periphery, many are aware of the production and actions of 5-HT in the gut but are unaware that the molecule and its receptors are also present in the immune system where evidence suggests they contribute to the both innate and adaptive responses. In addition, there is clear evidence that the immune system communicates to the brain via both humoral and neuronal mechanisms, and that CNS 5-HT neurons are a direct or indirect target for these actions. Following a brief primer on the immune system, we describe our current understanding of the synthesis, release, and actions of 5-HT in modulating immune function, including the expression of 5-HT biosynthetic enzymes, receptors, and transporters that are typically studied with respect to the roles in the CNS. We then orient our presentation to recent findings that pro-inflammatory cytokines can modulate CNS 5-HT signaling, leading to a conceptualization that among the many roles of 5-HT in the body is an integrated physiological and behavioral response to inflammatory events and pathogens. From this perspective, altered 5-HT/immune conversations are likely to contribute to risk for neurobehavioral disorders historically linked to compromised 5-HT function or ameliorated by 5-HT targeted medications, including depression and anxiety disorders, obsessive-compulsive disorder (OCD), and autism. Our review raises the question as to whether genetic variation impacting 5-HT signaling genes may contribute to maladaptive behavior as much through perturbed immune system modulation as through altered brain mechanisms. Conversely, targeting the immune system for therapeutic development may provide an important opportunity to treat mental illness.



KEYWORDS: *Serotonin, serotonin transporter, immune system, P38 MAPK, interleukin-1 beta, depression*

The monoamine neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) regulates many fundamental aspects of physiology and behavior including mood,[1] aggression,[2,3] sleep,[4] appetite,[5] pain sensation,[6] bone mass,[7,8] tissue regeneration,[9] platelet coagulation,[10,11] and gastrointestinal function.[12] In the central nervous system, serotonergic cell bodies are clustered in midbrain and brainstem raphe nuclei from which they project locally, as well as to spinal cord and a large number of forebrain targets.[13,14] Not surprisingly, dysregulation of 5-HT signaling has been implicated in multiple neurobehavioral disorders,[15] including anxiety, depression, obsessive-compulsive disorder (OCD), and addiction. Understanding the mechanisms by which 5-HT neurotransmission is regulated remains an active area of investigation, particularly with respect to how genetic perturbations interact with the environment to modify complex behaviors and drive disease risk.[16,17] Among the genes receiving significant attention in this context, the 5-HT transporter (SERT) gene (*SLC6A4*) arguably tops the list, driven by the transporter's key role in the termination of 5-HT signaling.[18,19] SERT is antagonized by the 5-HT selective reuptake inhibitor (SSRI) class of antidepressant medications that continue to represent the most common pharmacological treatment for affective disorders.[20] SERT also interacts with multiple drugs of abuse, including cocaine[21] and 3,4-methylenedioxymethamphetamine (MDMA; "ecstasy").[22] Alterations in SERT gene expression or transport activity have been associated with multiple neuropsychiatric disorders including depression,[23,24] anxiety,[25] OCD,[26,27] alcoholism,[28] and autism.[29] A more detailed understanding of SERT regulation, particularly in native preparations, is needed to elucidate the contributions of this key regulatory molecule in 5-HT signaling and how its compromised actions contribute to 5-HT linked brain disorders.

Many are unaware that SERT and 5-HT receptors are expressed in the immune system,[30,31] likely due to the fact that the function of these proteins in immune signaling remains ill-

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Table 1. Cells Involved in Mediating Innate and Adaptive Immunity[a]

cell	immune function	serotonergic phenotype
	innate immunity	
macrophage	phagocytosis; APCs; secrete cytokines, GCSF, MCSF, interferons, TNFα and β, TGFβ	SERT; 5-HT receptors 5-HT_{1A}, 5-HT_2, 5-HT_{2A}, 5-HT_3, 5-HT_4, 5-HT_7
mast cell	release heparin, histamine and serotonin; produce leukotrienes, prostaglandins, and PAF	SERT; 5-HT receptors 5-HT_{1A}
dendritic cell	express MHC II (APCs)	SERT; 5-HT receptors 5-HT_{1B}, 5-HT_{1E}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_3, 5-HT_4, 5-HT_7
basophil	release heparin, histamine and serotonin	SERT?
neutrophil	phagocytosis	SERT?; 5-HT receptors 5-HT_7
eosinophil	phagocytosis; release histaminase and other enzymes involved in allergic and parasitic immune responses	SERT?; 5-HT receptors 5-HT_{2A}
natural killer	express MHC I; Apoptosis of "non-self" cells	SERT?; 5-HT receptors 5-HT_{1A}
	adaptive immunity	
T cells	activation of macrophages; cytotoxicity; memory	5-HT receptors 5-HT_{1A}, 5-HT_{1B}, 5-HT_2, 5-HT_3, 5-HT_7
B cells	memory; antibody production	SERT; 5-HT receptors 5-HT_{1A}, 5-HT_2, 5-HT_3, 5-HT_7

[a]Key: antigen-presenting cells (APCs); granulocyte colony stimulating factor (GCSF); monocyte colony stimulating factor (MCSF); tumor necrosis factor (TNF); transforming growth factor (TGF); platelet activating factor (PAF); major histocompatibility complex (MHC).

defined and not yet consistently linked to physiological and behavioral disorders. Nonetheless, biomarkers of immune system dysregulation (e.g., inflammatory cytokine levels) have been found in many subjects with mood disorders and other affective pathologies[32] as well as autism,[33] and 5-HT can be reliably detected in lymphatic tissue,[31] including spleen,[34] thymus,[35] lymph nodes, and lymphatic fluid.[36] These findings dictate that we consider the degree to which genetic, environmental, and pharmacological influences thought to impact brain development, function, and behavior through brain 5-HT signaling actually arise from a perturbation of immune system 5-HT signaling. Additionally, we might ask whether changes in CNS 5-HT signaling can be brought about through primary alterations of immune function. Below we discuss the function of 5-HT in the immune system, and how immune system activation can impact CNS 5-HT signaling, a topic we have been drawn to through our recent findings that CNS SERT can be modulated by the peripheral immune system activation.[37]

A BRIEF PRIMER ON THE STRUCTURE AND FUNCTION OF THE IMMUNE SYSTEM

The complexity of immune system structure and signaling can only be superficially summarized in a brief review. To familiarize the reader with the key components of immune system structure and function that we will discuss in later parts of this review, we present a brief overview of key features. (see ref 38 for a more detailed presentation). Table 1 lists the cells involved in immune responses and summarizes their functions.

Defense against pathogens is mediated by innate and adaptive immune mechanisms that act in the periphery and the CNS.[39] Within minutes of injury or pathogen breach of the skin or mucosa, an acute, systemic inflammatory response occurs, mediated by cells of the innate immune system. This response is marked by changes in vascular permeability, migration of immune cells and the elevation of pro- and anti-inflammatory molecules at the site of infection or injury. This "first wave" of innate immune system function involves macrophages, mast cells, and dendritic cells (DCs), that act in the area of infection or injury, leading to the identification and destruction of pathogens. Innate immunity is primarily responsible for recognizing and eradicating "nonself" molecules presented by the pathogen, and is therefore confined to recognizing extracellular pathogens (bacteria vs viruses). This response is nonspecific with respect to particular invaders, and host tissue can be mistakenly destroyed along with pathogens. Innate immunity provides immediate host defense against pathogens via pattern recognition by toll-like receptors (TLRs). Pathogen-associated molecular patterns (PAMPs) on pathogens (e.g., peptidoglycans, bacterial lipopolysaccharides) bind TLRs on antigen-presenting cells (APCs), namely, DCs[34] and macrophages. APCs then phagocytize pathogens and display pathogen-derived peptides via the major histocompatibility complex (MHC) on their cell surface for recognition by leukocytes of the "adaptive" immune system (see below). APCs also secrete pro-inflammatory cytokines (e.g., IL1β, IL-6, TNFα), prostaglandins, and histamine, which further activate physiological responses, alerting the body to infection/invasion.[38] In addition to cellular protective mechanisms, innate immunity also includes the complement system, which consists of more than 20 glycoproteins, activated in a cascade by foreign substances, antigen−antibody complexes (classical pathway), and Gram-negative bacteria (alternative pathway). This system leads to lysis of cells, increased vascular permeability (allowing antibodies, innate immune cells, and fluid to enter tissue more readily), and chemotaxis. The complement system also helps to activate APCs, namely, follicular DCs and B cells in the specific immune responses. Innate immunity therefore also functions to communicate pathogen presence to cells involved in adaptive immune responses.

The response of a second immune system division, termed the adaptive, or specific, immune system, occurs within hours to days of an infection and involves antigen-specific recognition and destruction of pathogens by T and B lymphocytes. The two components of the adaptive immune system involve cell-mediated and humoral immunity. Cell-mediated immunity is carried out by T cells located in the thymus, lymph nodes, and circulation. APCs that migrate to lymph nodes prime and educate T cells as to the nature of the pathogen. T cells then proliferate and differentiate into either CD4+ T helper inflammatory cells (Th1) that activate macrophages, CD4+ Th2 cells that aid antibody responses, or CD8+ cytotoxic cells that target cells infected with intracellular microbes. It is beyond the scope of the present review to note all of the other various surface antigens (e.g., CD20+, CD3+CD4+,

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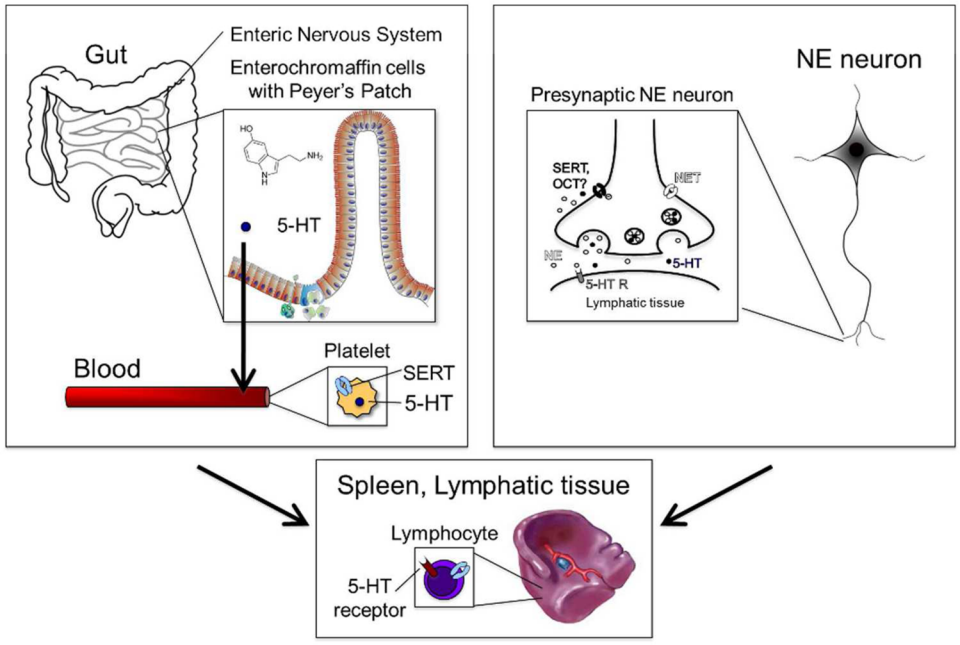

Figure 1. Enterochromaffin cells of the gut produce serotonin (5-HT) and release it into the blood. 5-HT is then taken up by the serotonin transporter (SERT) into platelets. 5-HT can then interact with immune cells in the blood or in the tissue. In lymph tissue, B lymphocytes take up and release 5-HT. 5-HT is also released in lymph tissue by noradrenergic neurons.

CD3+CD8+, etc.) that T and B cells exhibit as they differentiate and carry out specific immune functions. The reader is referred to excellent texts that provide more in depth descriptions of immune cell types.[31,38] The second component of adaptive immunity involves the contributions of B cells, located in lymph tissue, spleen, and in the circulation. Upon stimulation, B cells become plasma cells (with or without the help of Th2),[40] that produce and secrete antibodies (immunoglobulins). Memory T and B cells recognize specific antigens and respond quickly if a specific antigen is encountered again. Thus, the adaptive immune is distinguished from the innate immune system by its ability to identify, remember, and eliminate pathogens that have been designated as nonself. Together, the innate and adaptive immune responses work together, but over different time scales, to provide cellular, molecular, and chemical defenses against potentially harmful substances, microbes, and viruses.

THE PRESENCE AND ROLE OF 5-HT AND ITS TARGETS IN THE PERIPHERAL IMMUNE SYSTEM

Only about 5% of the body's 5-HT is produced in the brain,[41] and therefore, 5-HT in lymphatic tissue is supplied primarily from peripheral sources (Figure 1). The majority of peripheral 5-HT is found in the gut, where approximately 90% is synthesized by enterochromaffin (EC) cells of the gastrointestinal (GI) tract and 5% by myenteric neurons.[42] 5-HT released from EC cells into the bloodstream is transported into blood platelets via SERT. Because 5-HT does not cross the BBB, almost all of the 5-HT in the blood is from this GI pathway.[43] Platelets carry 5-HT to various tissues and represent the major source of 5-HT for immune cells and lymphatic vessels which express MAOA,[44] but not MAOB, for degradation of 5-HT. The blood therefore represents the primary source of 5-HT for lymph tissue, although monocytes,[45] mast cells,[46] and T cells also appear able to synthesize small amounts of 5-HT.[47] In addition to the circulation and immune cells, sympathetic nerves represents another source of 5-HT in lymphatic tissue, which is heavily innervated by sympathetic neurons that appear capable of accumulating 5-HT[48,49] and co-releasing norepinephrine (NE) and 5-HT.[50−53] Whether accumulation of 5-HT by adrenergic nerve terminals is mediated by SERT remains to be determined. Interestingly though, SERT[54] and 5-HT[55,56] have been shown to reside with NE in adrenal chromaffin cells, but again the role of these sites of expression has only been superficially explored. Lymphoid tissue levels of 5-HT can reach up to 1 μM, and therefore, clearance of 5-HT in lymphatic tissue by noradrenergic terminals could be mediated by other low-affinity 5-HT transporters, such as the norepinephrine transporter (NET), one or more organic cation transporters (OCT), or the peripheral monoamine transporter (PMAT). Together these findings provide strong evidence for the presence of 5-HT in immune tissues, though certain aspects related to cellular subtypes, releasable pools, and stimuli that evoke release deserve considerably more attention.

Innate (Nonspecific) Immune System. In Table 1, we provide a summary of the reported 5-HT related characteristics, including expression of SERT and/or 5-HT receptors, of immune cells. Caution should be noted, however, with respect to such tabulations, as for some 5-HT receptors, studies suggest that some are expressed by either rodent or human cells, but not both. With respect to 5-HT transport, DCs,[34] macrophages,[57,58] monocytes,[45] and mast cells[59] have been reported to take up 5-HT. Mast cells also store 5-HT;[60] however, the abundance of 5-HT in mast cells is species-specific. Although rodent mast cells contain 5-HT, human mast cells, which express TPH-1,[46] appear to sequester only small amounts of 5-HT with larger amounts of histamine. The presence of 5-HT receptors and the ability of these cells to take up 5-HT suggests that 5-HT influences the activity of the innate immune cells.[31] Following injury or release of inflammatory substances, such as C3a, C5a, and IgE complexes, and PAF,[61] 5-HT is rapidly

released from mast cells, basophils, and platelets[62,63] and functions in several ways to coordinate the movement and secretion of several cellular cascades involved in carrying out immune responses,[30] including inflammation, chemotaxis, and phagocytosis. 5-HT is chemotactic for eosinophils,[64] dendritic cells,[65] and mast cells,[66] attracting cells to sites of inflammation. In mouse and human mast cells in particular, the chemotaxis-promoting effect of 5-HT was lost in 5-HT_{1A} receptor knockout mice, suggesting this effect is mediated by a 5-HT_{1A} receptor pathway.[66] The chemotactic properties of human eosinophils and mouse DCs have also been reported to be mediated through 5-HT_{2A}[64] and 5-HT_1/5-HT_2[65] receptor pathways, respectively. Although 5-HT has not been reported to be chemotactic for macrophages or NK cells, 5-HT regulates several functions of macrophages and NK cells that relate to cell death. For example in macrophages, 5-HT stimulates phagocytosis[67] of cells that contained zymosan, a component of yeast cell walls that is used to induced sterile inflammation.[68] 5-HT also plays a role in enhancing the cytotoxicity of NK cells[67,69] cells in blood and lymph that are not phagocytic but promote cell lysis when stimulated. Together, these findings indicate that multiple cells of the native immune system possess the machinery to produce, store, release, and respond to 5-HT, with the overall picture being one to mobilize cells to target to, and destroy, pathogens.

DCs, macrophages, and endo/epithelial cells produce cytokines, which are small molecules that are released in response to immune system activation to orchestrate chemotactic and inflammatory processes. Cytokines are key markers of immune system activation, and there is evidence that multiple cytokines are elevated in patients with mental illness, including major depressive disorder (MDD).[70] The bulk of investigations regarding 5-HT and the modulation of cytokine production/release suggest that 5-HT mediates pro-inflammatory responses. For example, 5-HT has been reported to enhance human NK cell production of interferon-gamma (IFN-γ)[71] and alter the types of cytokines released by DCs,[72] enhancing IL-1β and IL-8 and decreasing IL-12 and TNFα.[72] This "cytokine switch" has been shown to alter whether the immune system is stimulated or suppressed.[73] 5-HT also has been shown to enhance the levels of IL-1α and IL-1β in smooth muscle cells,[74] as well as TNFα and IL-6 mRNA expression in hippocampal astrocytes.[75] Pro-inflammatory cytokines, including IFN-β1b and IFN-γ (with a synergistic effect of TNF-α[76]), have been shown to reduce the availability of tryptophan, which is required for 5-HT synthesis, through activation of indoleamine-2,3-dioxygenase (IDO),[77−79] a tryptophan-degrading enzyme, and elevate kynurenine metabolites, that can be neurotoxic.[80] Changes in tryptophan, through manipulation of IDO, influence brain 5-HT. Upregulation of IDO by IL-6 has been shown to reduce 5-HT/tryptophan ratios, increase kynurenine/tryptophan ratios in hippocampus, and produce a depressive-like effect on behavior that is lost in IDO knockout mice.[81]

Adaptive (Specific) Immune System. As noted in Table 1, some lymphocytes express SERT[61,82] as well as 5-HT receptors. Whereas B lymphocytes express SERT,[61,83,84] T lymphocytes do not.[34] Possibly, 5-HT uptake in T cells occurs through the dopamine transporter (DAT),[34] as this molecule has been reported to be expressed by blood lymphocytes[85−87] though the studies cited did not differentiate B and T cells, and DAT is a low-affinity 5-HT uptake mechanism.[88,89] Interestingly, T cells have been reported to produce 5-HT.[47] 5-HT regulates murine T cell function,[90] and although there are reports that 5-HT both promotes and inhibits T cell proliferation, depending on the receptor signaling pathway activated, the strongest evidence points to a stimulatory effect of 5-HT on T cell proliferation. Either through an autocrine or paracrine mechanism, 5-HT can stimulate T cells via 5-HT_7 receptors, ERK phosphorylation, and NFkB activation. Alternately, 5-HT has been shown to reverse forskolin inhibition of T cell proliferation by decreasing cAMP levels via 5-HT_{1A} receptor activation.[91,92] Further complicating this picture, T cell activation leads to expression of 5-HT_{2A} and 5-HT_{1B} receptors.[47] Similar to cells of the innate immune system, T helper (Th) cells also secrete cytokines, and one role of 5-HT in the immune response appears to be in modulating the balance of cells promoting the release of pro-inflammatory (Th1) versus anti-inflammatory (Th2) cytokines.[93] The actions of 5-HT in the adaptive immune system are not limited to T-cells, as 5-HT, together with platelet-activating factor (PAF), activates B cells[94] that both express SERT[61,95] and can cross the BBB,[96] albeit in very low numbers without damage to this barrier. It is known that activated T cells also cross the BBB,[97,98] and therefore, the finding that B cells may cross the BBB suggests that humoral, as well cellular, immune mechanisms may be important in the CNS, which has long been considered to exclude most peripheral immune cells.

In studies of platelets, Walther et al. described a SERT-mediated signaling pathway where transported, intracellular 5-HT is used to "serotonylate" small intracellular GTPases (RhoA and Rab4) via the action of intracellular transglutaminase.[99] Because these GTPases are also expressed in lymphocytes and other immune cells, transport of 5-HT and a serotonylation pathway may act intracellularly to affect the secretory functions of lymphocytes.[95,100] Schneider and co-workers have also described an intracellular action of 5-HT in reducing IL-4 production mediated by both SERT and the organic cation transporter 3 (OCT3).[101] How such mechanisms complement the actions of 5-HT on immune cells via cell-surface receptors is unclear, though the same can be said for platelets. What is clear is that 5-HT exerts significant effects on numerous types of immune cells and processes involved in adaptive immunity. Future studies with methods that allow for the isolation of components of the immune system, for example, using flow cytometry to dissect T- and B-cell subsets, methods that target surface versus intracellular actions of 5-HT, and conditional transgenic methods that could manipulate 5-HT targets in these cells, are needed to take us further toward a better understanding of these mechanisms. Nonetheless, enough evidence is present to consider that we are beyond the point of hypothesis related to the possibility of a role for 5-HT in both innate and adaptive immunity. More so, we now need to dig deeper with better cellular and time resolution on where and when 5-HT contributes to pathogen responses.

Although we have discussed the innate and adaptive immune systems in isolation, a role for 5-HT in communication between these two systems has been described.[30] As examples, 5-HT has been reported to contribute to interactions between monocytes and NK cells,[102] 5-HT may promote macrophage-stimulation of T cell activity, and depletion of 5-HT via treatment with the irreversible tryptophan hydroxylase (TPH) inhibitor para-chlorophenylalanine (PCPA) appears to impair the ability of macrophages to activate T cells.[90,103,104] DCs in blood function as APCs, which communicate between innate and adaptive immune systems, and 5-HT has been shown to modulate the

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differentiation of DCs from human monocytes,[105] the blood-borne phagocytic precursors of macrophages.

Serotonin-Immune Cell Link to Neurobehavioral Disorders. The role of 5-HT in regulating cells involved in neuroinflammation is interesting given evidence for altered immune function in several psychiatric disorders where compromised 5-HT signaling has also been illustrated. For example, one group found that activated microglia and astrocytes are located near senile plaques in inflamed tissue of Alzheimer's patients[106] and that 5-HT_{2C} receptors are pathologically expressed in NK cells in Alzheimer's patients.[107] Furthermore, alterations in immune function have been reported in subjects with autism;[33] autism is also associated with elevated blood levels of serotonin.[108] A human, gain-of-function SERT coding variant, Gly56Ala, was identified among four other similar hyperactive variants in a study of rare gene variation in subjects with autism.[109] The Gly56 to Ala56 change leads to hyperphosphorylation of SERT, and mice carrying the Ala56 variant display hyperserotonemia, 5-HT receptor hypersensitivity, social impairment, and repetitive behavior,[110] characteristics displayed by subjects with autism. Since autism is often associated with gastrointestinal disorder (GID)[111] and immune disturbances,[112] these findings have elevated our interest in 5-HT actions in the immune system. In preliminary studies, our group has found changes in gene expression linked to activation of p38 MAPK in lymphoblastoid cells from autism-associated SERT gene variant carriers,[113] with pathway analysis pointing to activation of p38 MAPK signaling, as well as molecular circuits responding to oxidative stress. As we note below, p38 MAPK pathways also regulate SERT, suggesting the opportunity for a positive feedback regulation of 5-HT accumulation in immune cells should the balance between uptake, metabolism, and release of 5-HT be altered. Lymphoblastoid cells are derived from B lymphocytes following immortalization with Epstein−Barr virus (EBV). Since these cells are typically the source of patient DNA samples, they are a convenient resource to connect genotype with phenotype, when the molecule of choice is expressed in B cells (e.g., SERT). However, it must be recognized that as they are transformed, these cells may exhibit characteristics distinct from native B cells. For example, we find that SERT protein levels and 5-HT uptake activity are much higher in native B cells than in EBV-transformed cells (Han and Blakely, unpublished findings).

Since the discussion above notes a connection between inflammatory cytokine signaling, SERT, and neurobehavioral disorders, it is interesting to note that functional coding variation exists in the transporter across inbred strains of mice. Many inbred mouse strains express Glu-39 and Arg-152 coding sequences (ER isoform) whereas a few others, and notably C57Bl/6 express Gly-39 and Lys-152 (GK isoform).[114] Since these two haplotypes are present in one each of the progenitors of BXD recombinant inbred lines, Carneiro and colleagues were able to demonstrate that not only does the ER SERT variant exhibit elevated 5-HT uptake capacity compared to the GK isoform, but also that the ER variant is associated with reduced T cell proliferation and T cell receptor expression.[114] Although it is not possible to know if this association is driven by a direct effect of SERT activity in the immune system proper, this seems a parsimonious hypothesis. Possibly, elevated 5-HT clearance in the immune system diminishes extracellular 5-HT availability that in turn limits T cell proliferation. As both the mouse ER/GK variation and the human Ala56Gly SERT variant associate with behavioral changes, studies are now needed that distinguish their immune system impact from other sites of action of the transporter and 5-HT.

Depression has long been linked to aberrations in both the immune and serotonergic systems. Patients diagnosed with major depressive disorder often display elevated levels of immune markers, as well as associations with single nucleotide polymorphisms (SNPs) in pro-inflammatory cytokine genes,[115] such as IL-1β,[116,117] TNFα,[118,119] and cyclooxygenase-2.[120] The observation that pro-inflammatory cytokine levels are elevated in depressed patients[121] led Smith to propose a "macrophage theory of depression".[122] Evidence that cytokine elevations may be causally related to mental illness derives from the depressive symptoms that occur in patients treated with cytokines (IFN-α) as part of cancer therapy.[123,124] SSRIs, such as Prozac and Zoloft, elevate 5-HT levels in the brain and periphery, and effects of SSRIs on adaptive immune cells have been described. In turn, SSRIs enhance the cytolytic function of NK cells,[125] whereas fluoxetine and paroxetine treatment can reverse the blunted NK cell activity reported in depressed patients.[126] Long-term SSRI treatment has also been shown to enhance NK and B cell proliferation.[127] Zimelidine increases MHC class II expression on macrophages in vitro.[128] Finally, paroxetine and sertraline inhibit CNS microglial activation via inhibition of IFN-γ-induced increases in intracellular Ca^{2+}.[129] Finally, SSRI treatment has been shown to normalize elevated cytokine levels in depressed patients.[130] Another link between immune function and SSRI action arises from findings that reduced lymphocyte SERT expression in patients with MDD is normalized by administration of SSRIs.[131] A caveat to a mechanistic interpretation of these studies is the possibility that the actions of SSRIs in immune system function may be mediated by proteins other than SERT, particularly when the evidence derives from in vitro studies of cell suspensions where blockade of the transporter is unlikely to elevate extracellular 5-HT to an extent that would impact cell-surface 5-HT receptors in the way we expect in intact systems. We recently developed a knock-in mouse strain (SERT I172M) that results in significantly reduced sensitivity to multiple SSRIs.[132] The SERT I172 M model may be useful in pursuing this question.

Several lines of evidence suggest that treatment with antidepressant medications can have anti-inflammatory properties.[133] Sacre et al.[134] reported that fluoxetine and citalopram reduced inflammation by inhibiting TLR function. Elevated cytokine levels in depressed patients have also been shown to normalize following SSRI treatment;[135,136] however, normalization of cytokine levels appears not to occur in patients that did not respond to SSRIs,[137] raising the possibility that immune system status could be a useful biomarker in structuring personalized depression therapies. Since T cells appear not to express SERT[47] (though note the caveat mentioned regarding T cell subtypes above), they do express 5-HT receptors[47] and thus the effects of SSRIs in vivo may also be indirect. In the CNS, SSRIs have been reported to potently inhibit microglial TNFα and NO production after stimulation by LPS.[138] Contrary to these findings, others have reported that SSRIs and other antidepressants elevate levels of cytokines in the brain, including TNFα and IFNγ, but this effect is lost when animals are cotreated with a nonsteroidal anti-inflammatory (NSAID) drug.[139] Warner-Schmidt et al.[139] recently reported that NSAIDs attenuate the antidepressant effects of SSRIs in both mice and humans. The discrepancies in the ability of SSRIs to increase or reduce cytokine levels could be a result of

varying concentrations of these agents across studies and the duration of exposure to these drugs, as well as brain region specificity, and thus will require further study. Interestingly, macrophage migration inhibitory factor (MMIF) has been reported to be required for the antidepressant effects of voluntary exercise,[140] as these effects of exercise are lost in MIF knockout mice, extending the immune system connection to treatments for depression beyond those of antidepressant medications.

Polymorphisms in interleukin genes linked to inflammation and depression have been identified, raising the possibility that a genetic predisposition to mood disorders (or resiliency) could arise not from the brain but from an immune system action. Individuals homozygous for the −511T allele of the IL-1β gene have fewer symptoms associated with depression and respond better to fluoxetine[141] and paroxetine[142] treatment than those[143] carrying the −511C isoform, but not in patients carrying a variable number of tandom repeats (VNTR) polymorphism of IL-1Ra, an IL-1R antagonist.[142] Another study showed a positive association between a polymorphism in the IL-11 gene (rs1126757) and response to escitalopram.[144] Finally, two SNPs (rs2929115 and rs2929116) in the *IDO2* gene, which has been linked to both 5-HT homeostasis (via tryptophan metabolism) and immune function, have been associated with SSRI efficacy.[145] Since these genes are expressed in the brain, as well as the periphery, work remains to understand where these polymorphisms may impact behavior and/or antidepressant action.

Loss of SERT in knockout mice has been reported to trigger changes in levels of pulmonary immune molecule transcripts,[146] to exacerbate intestinal inflammatory/immune responses to 2,4,6-trinitrobenzene sulfonic acid (TNBS) exposure,[147] and to reduce severity of immune responses in an experimental autoimmune encephalomyelitis paradigm.[148] TPH1 knockout mice have been shown to have reduced levels of 5-HT and production of cytokines in the gut, as well as reduced severity of colitis.[149] In humans[80,140] and rhesus monkeys,[150,151] a commonly studied SERT promoter polymorphism, the 5-HTTLPR, has been reported to impact SERT mRNA levels, protein expression, and uptake function.[83,143] Carvalho and co-workers have reported that patients diagnosed with fibromyalgia expressed different T-cell surface antigens, depending on whether they were carriers of the long "*l*" or short "*s*" variants of the 5-HTTLPR. In addition, NK cells were also reduced in *s* carriers.[152] Additionally, Lima and colleagues described effects of 5-HTTLPR status on lymphocyte SERT expression and found that SERT mRNA expression is reduced in lymphocytes of depressed patients carrying the *l* allele.[153] The sensitivity of SERT gene expression to IL-4 stimulation in EBV-transformed lymphoblasts has also been reported to depend on 5-HTTLPR status.[154] Finally, multiple studies indicate that polymorphism status reveals itself with respect to mental illness in the context of a significant life history of stress.[16] In this regard, Matsunaga and colleagues reported that amygdala activity occasioned by seeing a "favorite person" is correlated with levels of NK cells.[155] Thus, further work is needed to determine whether environment/5-HT gene interactions might drive changes in immune function that can ultimately alter behavior.

PATHWAYS MEDIATING IMMUNE–BRAIN COMMUNICATION

The impact of immune system activation on behavior and the intersections noted above between antidepressant action and immune signaling raise the critical question as to the path(s) by which peripheral immune system activation could initiate or stabilize changes in behavior. Activation of the immune system, whether ectopic or via peripheral injection of lipopolysaccharide (LPS), a constituent of the cell walls of gram-negative bacteria, leads to elevation of levels of pro-inflammatory cytokines, such as IL-1β,[156] both systemically and centrally.[157−159] In the periphery (Figure 2), LPS binds to

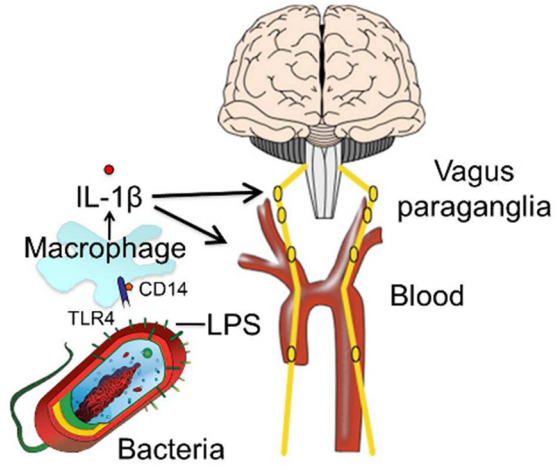

Figure 2. Peripheral lipopolysaccharide (LPS) binds to toll-like receptor-4 (TLR4) on macrophages and stimulates release of interleukin 1β (IL-1β). IL-1β in the blood can enter the brain through circumventricular organs (CVOs) or active transport mechanisms. IL-1β also can stimulate the vagus paraganglia.

pathogen-associated molecular pattern (PAMP)-recognizing receptors, toll-like receptor-4 (TLR4) and CD14 expressed on macrophages, dendritic cells, and neutrophils. LPS stimulates monocytes and macrophages to synthesize IL-1β, which then enters the blood. IL-1β generated in the periphery does not readily cross the BBB because the peptide is fairly large (~15 kDa) and hydrophilic. Cytokines such as IL-1β, however, can cross into the brain at the circumventricular organs (CVOs),[160] such as the organum vasculosum lamina terminalis, where the BBB is weak or absent. Products of both the innate (i.e., monocytes,[161] cytokines[162]) and adaptive (T cells/B cells) immune systems that do enter the brain activate central immune cells, though it is important to note that many peripherally activated components of the immune system are unable to cross the tightly packed cells of the BBB, including many antibodies. Furthermore, under physiological conditions, only T cells that have been activated have been shown to cross the BBB easily and to accumulate in CNS tissue.[98] Several immune components, including cytokines,[163] influence the permeability of the BBB. Astrocytes assist in BBB function through influencing the permeability of brain microvascular endothelial cells that comprise the BBB,[164] and activated microglia damage the BBB via NFκB, JAK-STAT, and JNK stress kinase signaling pathways.[165] The BBB, in turn, also has been shown to release cytokines in response to peripheral administration of LPS[166] or IL-1β.[167] A compromised BBB would allow peripheral cytokines to access enter the brain more easily and thereby influence central neural pathways, likely compounding other problems. Another route by which cytokines could gain access to the brain is through active transport mechanisms. Indeed, active transport mechanisms

have been reported which carry cytokines across the BBB,[168] including IL-1α,[169] IL-1β,[162,170−172] TNFα,[173] and IL-1RA.[174] Multiple other mechanisms for transport of cytokines across the BBB have been described,[162] although the specific details of which proteins mediate these transport activities remain an area of investigation.

Other lines of evidence point to, in addition to a BBB-mediated route, a neural mechanism of immune−brain communication. One study reported that peripheral LPS increased CSF IL-1β within 15 min.[175] In another study, Fos protein expression was measured 1 h following immune activation with IL-1β, suggesting that the *cfos* gene must have been activated within minutes,[176] since blood-borne mechanisms would have a more delayed action. Together, these findings that initial responses to peripheral LPS and IL-1β are rapid suggest a role for activation of neural inputs to the CNS following peripheral immune activation. The vagus nerve is one neural mechanism that mediates neural communication between the brain and peripheral immune system. The vagus is important in controlling autonomic responses and innervates lymphatic tissue, including spleen,[177] thymus,[178] and lymph glands,[179] as well as sites where pathogens enter the body (lungs, peritoneum, and organs important for innate immunity such as the liver). The vagus nerve contains both efferent and afferent fibers both from and to the CNS, respectively, linking brain and lymphatic tissue.[180] The efferent, cholinergic fibers of the vagus have been shown to suppress microglia activation. In addition, IL-1β increases activity of vagal efferent fibers.[181] Peripheral immune activation (via i.p. injection of LPS[182] or IL-1β[183]) also activates afferent vagal fibers[182] and induces perivagal immune cells to rapidly express IL-1β immunoreactivity.[184] Activation of afferent vagal fibers has been shown to produce neural changes characteristic of sickness.[185] Severing the vagus reduces or eliminates the ability of LPS to produce sickness behavior[186,187] but not to produce fever. This suggests different effects of LPS on vagal circuitry controlling behavioral and hypothalamic processes. Together, these lines of evidence point to a key role of the vagus nerve in relaying information between the brain and immune system via afferent and efferent pathways. It is important to note, however, that the vagus is not the only nerve that can signal occurrence of immune activation. The skin and oral cavity also respond to infection/injury but are not innervated by the vagus nerve, and therefore, other nerves in those regions may function to communicate immune activation to the CNS.[188] Taken together, these lines of evidence suggest that peripheral cytokines such as IL-1β employ several routes of communication (through the BBB or peripheral nerves) to the brain to potentially influence behavior and suggest that blood-bourne and neural inputs collaborate in communicating to the brain in the presence of peripheral inflammation.

IMMUNE SYSTEM ACTIVATION AND CNS 5-HT NEUROTRANSMISSION

There are several mechanisms by which inflammatory cytokines acting within the CNS can modulate the functions of key neural circuits, including 5-HT, that drive various physiological and behavioral responses. Although there is evidence that IL-1β can inhibit the firing of 5-HT neurons in the dorsal raphe,[189] there is overarching consensus that acute activation of the immune system and IL-1β increase extracellular 5-HT levels in the brain. Microdialysis studies show that administration of IL-1β either i.p.[190] or centrally[191] elevates extracellular brain 5-HT, and systemic LPS increases 5-HT in hippocampus.[192] Increased 5-HT synthesis in nerve terminals could explain elevations in central 5-HT following immune activation; however, systemic administration of IL-1β elevates levels of both the 5-HT precursor, tryptophan, and its metabolite, 5-HIAA,[193−195] an effect that appears to depend on activation of the sympathetic nervous system.[196] This finding is interesting in light of clinical evidence that serum tryptophan levels are reduced in patients with treatment-resistant depression.[197] Levels of 5-HT are also influenced by cytokines other than IL-1β, as peripheral IL-6 increases 5-HT levels in rat striatum.[198]

Cytokines have been shown to control proteins that regulate 5-HT levels. Cytokine regulation of SERT transcription and activity has been previously demonstrated by several groups (for review, see ref 199). The pro-inflammatory cytokines TNFα[200] and IL-1β[201] have been reported to elevate SERT activity via increased production of SERT mRNA levels in human JAR cells, a choriocarcinoma cell line derived from the placenta that constitutively expresses SERT. Similarly, interferon (IFN)-α and IFN-γ, also pro-inflammatory, have been found to increase SERT activity in BeWo cells, another human choriocarcinoma cell line.[202] An anti-inflammatory cytokine, IL-4, was shown to reduce SERT activity in B lymphoblastoid cell lines, which express SERT.[203] In general, chronic stimulation by inflammatory cytokines appears to stimulate SERT transcription, whereas anti-inflammatory cytokines reduce SERT activity. Altered SERT expression and activity has long been linked to neuropsychiatric disorders, such as depression. Clinically, carriers of the *s* allele of the SERT-linked polymorphic region (5-HTTLPR), which several groups report as producing reduced SERT expression, showed elevated risk for depression and circulating levels of IL-6, another anti-inflammatory cytokine.[204] Other clinical studies have found a correlation between cytokine levels and depression, reporting higher levels of IL-6 in men with depression,[205] and two other meta-analyses indicate that levels of IL-6 and TNFα were elevated in depressed subjects.[206,207] It may seem odd to have a pro-inflammatory cytokine elevate raphe firing and extracellular levels of 5-HT yet associate with depression or elevated SERT expression (and activity, as noted below). One way to reconcile these findings, and our working model, is that changes in 5-HT transport capacity reflect a homeostatic mechanism, scaled with elevated serotonergic signaling. The more 5-HT is secreted, the greater the need for uptake activity via SERT to ensure that the elevated amplitude of 5-HT signals can scale in magnitude, without also broadening the temporal or spatial impact of these effects. Seen in this light, disease risk can arise is when these homeostatic mechanisms are activated independent of the normal stimulus (such that 5-HT modulation occurs in a context devoid of the actions of cytokines elsewhere in the brain) or when inflammatory stimuli that should drive a coordinated serotonergic response, produce one involving an incomplete constellation of contributors (e.g., uptake modulation without a change in raphe firing). In this regard, our group recently reported that a SERT coding variant that lacks a capacity for regulation by cytokine-linked signaling pathways[109,208] produces behaviors reminiscent of autism when expressed in transgenic mice.[110]

Since SERTs are critical modulators of 5-HT signaling and are the primary target for many antidepressant medications, our lab and others have investigated how this transporter can be regulated by multiple signaling pathways and interacting proteins.[209] In relation to this review, our lab discovered that

pathways activated in the CNS by the pro-inflammatory cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNFα) regulate SERT via rapid effects mediated by p38 mitogen activated protein kinase (MAPK)-linked pathways.[210] The ability of cytokines to regulate SERT activity derives from studies initially exploring how SERT is regulated by G-protein coupled receptors. SERT proteins do not reside constitutively at the plasma membrane, but rather, like many receptors and transporters, are dynamically trafficked as a consequence of protein−protein associations and post-translational modifications, including those mediated by PKC[211,212] glycosylation,[213] PKG/p38 MAPK,[214] and cGMP.[215] SERT is a phosphoprotein,[216] and alterations in transporter phosphorylation also appear to modulate both trafficking and SERT catalytic activity.[213,214,210] Several receptor-signaling pathways are now known to regulate SERT. Thus, activation of the A3 adenosine receptor (A3AR),[214] α2-adrenoreceptor,[217] 5-HT$_{1B}$ autoreceptor,[218] and interleukin-1 receptor (IL-1R)[37] have been reported to rapidly modulate the intrinsic activity or membrane density of SERT. Our group found that A3AR activation elevates SERT plasma membrane density in cultured cells and synaptosomes via PKG1α signaling and catalytic activation of SERT in parallel via PKG-dependent regulation of p38 MAPK.[209] Activation of both PKG1α and p38 MAPK isoforms (not yet well-defined, though antagonist SB203580 targets α and β subtypes) leads to SERT phosphorylation. Ramamoorthy and co-workers have identified Thr276 as the essential phosphorylation site on SERT that supports PKG1-dependent SERT regulation.[219] Although PKG1α activation can modulate SERT trafficking, we have argued[209] that the T276A site may be phosphorylated following PKG1-dependent p38 MAPK activation and, therefore, is of greatest relevance to catalytic activation. Recent studies by Rudnick and colleagues have found that, in transfected cells, PKG does not directly phosphorylate SERT, nor does p38 MAPK appear to be the critical intermediate, necessitating further studies.[220] Finally, we have recently demonstrated using cultured raphe cell line (RN46A cells) that the IL-1R/p38 MAPK pathway mobilizes cell surface SERT in lipid-raft microdomains through detatchment of the transporter from cytoskeletal anchors that normally stabilize the transporter in less effective conformations.[221] Clearly, a sophisticated network of controls is in place for altered immune function to modulate at least one critical component of 5-HT signaling regulation.

Finding that IL-1β and TNFα stimulated SERT activity in RN46A cells, as well as in mouse brain synaptosomes,[222] we asked whether IL-1β effects could be blocked by the IL-1R antagonist IL-1Ra, as well as a p38 MAPK inhibitor, SB203580,[210] and in both cases the answer was yes. We next wanted to determine whether peripheral induction of IL-1R secretion by stimulation of the native immune system could modulate CNS SERT activity. We recently reported that peripheral administration of LPS rapidly stimulates SERT activity (as much as a 100% increase) in mouse midbrain and hippocampal synaptosomes, as well increases the clearance rate of 5-HT in the CNS.[37] These effects occur within 1 h of peripheral LPS injection, suggesting that they arise from a post-translational modification of SERT, one that our report indicates results in a shift of SERT to a high affinity state for 5-HT.[37] Use of an IL-R KO or systemic administration of SB203580 abolished this effect, suggesting that the activation of IL-1R affects SERT via an IL-1R and p38 MAPK-dependent pathway (Figure 3). In chronoamperometric studies, peripheral

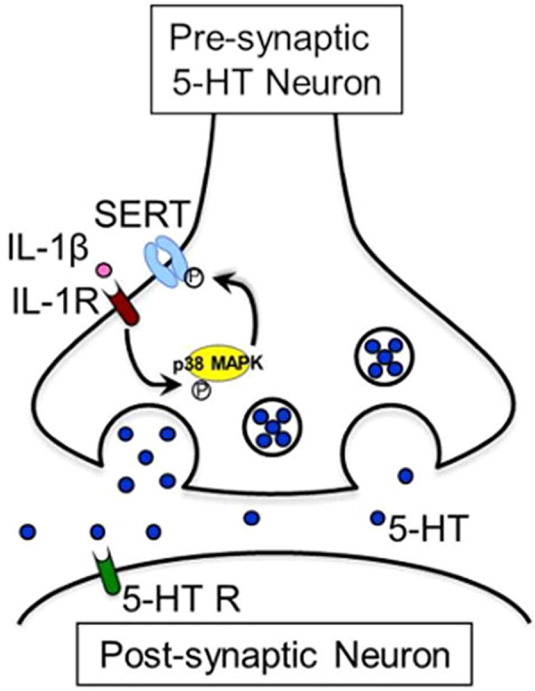

Figure 3. Interleukin-1β (IL-1β) stimulates SERT activity through an interleukin-1 receptor (IL-1R)- and p38 MAPK-dependent signaling pathway.

administration of LPS also stimulated 5-HT uptake in hippocampus,[37] further supporting the notion that CNS SERT activity *in vivo* is under the control of the peripheral immune system. Behaviorally, mice treated with LPS spent more time immobile in the tail suspension test (TST), a screen for antidepressant efficacy. The depressive-like effect of LPS on behavior is lost in mice treated with SB203580, as well as in IL-1R KO and SSRI-treated mice. Together, these biochemical and behavioral studies provide strong evidence that LPS stimulates SERT activity through a p38 MAPK/IL-1R pathway, and may explain components of the depressive-like phenotype that follows immune activation in humans.[223,224] However, there are many steps between LPS activation of the peripheral immune system and CNS SERT regulation at which loss of IL-1Rs or pharmacological antagonism of p38 MAPK could attenuate SERT upregulation. For example, these manipulations may simply depress peripheral immune system reactivity that leads to IL-1β stimulation. We have reported that if synaptosomes prepared from LPS-injected mice are treated with SB203580, stimulation of SERT is lost. These data indicate that ongoing CNS p38 MAPK activity in serotonergic terminals is required at least to sustain rapid upregulation of neuronal SERT initiated by peripheral immune system activation.

In addition to SERT, pro-inflammatory cytokines have been shown to influence the function of other proteins, such as p11 (S100A10), that interact with 5-HT receptors.[225,226] The level of p11 expression has been negatively correlated with depressive behavior, with p11 knockout mice displaying increased immobility time in both the forced swim (FST) and tail suspension (TST) tests.[225] Interestingly, SSRIs, tricyclic antidepressants (TCAs), and electroconvulsive therapy (ECT) all appear to increase both cytokine (specifically IL-1β, IL-3, IL-6, IFNγ, TNFα, and IL-12)[139] and p11 levels in some brain regions of mice.[225,227] The SSRI-induced elevation of p11

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was abolished in mice with genetic depletion of cytokines, suggesting that SSRIs could elevate cytokine levels via a p11-mediated pathway. These findings suggest that SSRI-stimulation of cytokine production leads to elevated p11 and has an antidepressant effect on behavior. How elevated p11 elicits an effect on mood, however, remains unknown. In this regard, there is evidence that p11 binds to and upregulates surface expression of both 5-HT_{1B}[225] and 5-HT_4[226] receptors. p11 could therefore play a role in mediating immune-brain communication and the risk for depression. Again, one would presume that regulation of p11 has likely not evolved to establish a mood disorder, but in concert with other immune targets such as those expressed presynaptically by 5-HT neurons, to provide for highly regulated brain signaling and behaviors responsive to inflammatory insults.

IMMUNE MODULATION OF SEROTONIN-RELATED BEHAVIORS

As noted earlier, peripheral LPS[228] and pro-inflammatory cytokines trigger "sickness behavior"[70,229] that is characterized by fatigue, loss of appetite, inactivity, malaise, and lack of sociability. This "sickness syndrome" is strikingly similar to symptoms presented by depressed patients.[70] Importantly, vaccination and LPS administration can induce sickness behavior and depressive behavior in both humans and rodents.[230] Our lab has previously shown that systemic treatments with LPS altered behavior in behavioral assays that are commonly used to screen for antidepressant drugs, namely, the FST and TST.[37] This effect of LPS on behavior was lost in mice treated with SB203580 or with a constitutive knockout of the IL-1R, supporting the hypothesis that the despair-like effect of LPS is p38 MAPK- and IL-1R-dependent. As described above, cytokines can alter levels of 5-HT in the brain,[70] and IL-1β upregulates SERT density and function and enhances SERT activity. As p38 MAPK-dependent activation of SERT could involve other cytokines that signal through p38 MAPK besides IL-1β, we have initiated studies of the constitutive elimination of p38 MAPK in raphe neurons ($p38MAPK^{flox/flox}$:ePet-1::Cre). Our studies[231,232] indicate that animals positive for both ePet-1:Cre and floxed p38 MAPKα are viable with no gross evidence of developmental perturbations as expected from studies using virus-based raphe excision of p38 MAPKα by Chavkin and co-workers.[233] Our preliminary biochemical and behavioral studies[231,232] with these mice are consistent with a requirement for p38 MAPKα expression by raphe neurons in producing both a rise in synaptosomal 5-HT uptake, as well as alterations in 5-HT and SSRI-linked behaviors. To determine if LPS effects arise from IL-1R stimulated MAPK pathways, we have also engineered mice that express a floxed allele of the IL-1R gene ($IL\text{-}1R^{flox/flox}$), and these mice recently achieved germ-line transmission. As with constitutive IL-1R KO mice, these animals are viable, of normal size and fecundity, and appear to have no gross morphological alterations of the CNS or perturbation of 5-HT levels. Following initial characterization to ensure that the floxed construct does not perturb IL-1R expression, we can achieve serotonin neuron-specific elimination of IL-1R signaling potential using raphe-specific Cre expression, further defining a signaling path by which peripheral inflammation can modulate behaviors linked to depression and antidepressant action.

Although we have much work to do before we can say that we have defined the molecular and circuit-level underpinnings of mental illness, the studies reviewed above make a strong case that we cannot remain brain-centric in our thinking as to mechanism, both in terms of physiological processes involved, in general, and the contributions of compromised 5-HT signaling, in particular. It seems likely that the behavioral changes arising as a consequence of immune system activation have evolved to provide survival benefit following pathogen invasion. A reduction in eating, a conservation of energy resources, and social withdrawal are things an organism should do to prevent exacerbation of infections and the potential for spread of potentially fatal agents to other members of the community. In this view, risk for mood disorders in some subjects may arise from a propensity for an enhanced or ectopic activation of what normally is a beneficial immune−brain dialogue, one that often speaks the language of 5-HT. Although this evolution-centered hypothesis is straightforward in description, specific mechanisms remained to be defined, as receptors and signaling pathways for immune molecules are widely expressed, as are determinants of 5-HT signaling. We must also remember that early actions that perturb maternal, embryonic, and neonatal immune and 5-HT signaling likely have enduring consequences on behavior.[234−237] Regardless of complexity, the inclusion of the immune system in our concepts of the determinants of both normal behavior and risk for mental illness has the opportunity to provide new opportunities for improved diagnosis and treatment of disorders long linked to changes in 5-HT signaling.

AUTHOR INFORMATION

Author Contributions

N.B. and R.B. researched and wrote the article.

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