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FEATURE REVIEW

Serotonergic vulnerability and depression: assumptions, experimental evidence and implications

LAW Jans[1], WJ Riedel[1], CR Markus[2] and A Blokland[1]

[1]*Faculty of Psychology, Department of Neurocognition, Maastricht University, Maastricht, The Netherlands and*
[2]*Faculty of Psychology, Department of Experimental Psychology, Maastricht University, Maastricht, The Netherlands*

In recent years, the term serotonergic vulnerability (SV) has been used in scientific literature, but so far it has not been explicitly defined. This review article attempts to elucidate the SV concept. SV can be defined as increased sensitivity to natural or experimental alterations of the serotonergic (5-HTergic) system. Several factors that may disrupt the 5-HTergic system and hence contribute to SV are discussed, including genetic factors, female gender, personality characteristics, several types of stress and drug use. It is explained that SV can be demonstrated by means of manipulations of the 5-HTergic system, such as 5-HT challenges or acute tryptophan depletion (ATD). Results of 5-HT challenge studies and ATD studies are discussed in terms of their implications for the concept of SV. A model is proposed in which a combination of various factors that may compromise 5-HT functioning in one person can result in depression or other 5-HT-related pathology. By manipulating 5-HT levels, in particular with ATD, vulnerable subjects may be identified before pathology initiates, providing the opportunity to take preventive action. Although it is not likely that this model applies to all cases of depression, or is able to identify all vulnerable subjects, the strength of the model is that it may enable identification of vulnerable subjects before the 5-HT related pathology occurs.
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Involvement of serotonin in depression

Mood disorders are one of the most prevalent forms of mental illness.[1] According to the DSM-IV, the lifetime risk for major depressive disorder is between 10 and 25% for women and between 5 and 12% for men.[2] Depression has been studied intensively during the last few decades, but the psychological and neurobiological determinants of depression have not yet been precisely defined. Although several factors are known to contribute to the etiology of depression, it is not clear how these factors cause depression, and why some subjects become depressed while others remain unaffected. In terms of biological factors in the etiology of depression, there are several hypotheses. These include neurotransmitter dysfunctions (serotonin, 5-HT; dopamine, DA; norepinephrine, NE); dysregulations in hypothalamic–pituitary–adrenal (HPA) axis activity; and immune system imbalance. The aim of this article is not to discuss all of these hypotheses in detail, but to evaluate the role that serotonin may play within these hypothesized mechanisms.

It is widely accepted that diminished serotonergic (5-HTergic) function is involved in the onset and course of depression.[3,4] The 5-HTergic system is a large and complex system. Although nearly all cell bodies of 5-HTergic neurons are located in the raphe nuclei in the brain stem, the axons of these neurons innervate almost the entire brain. The actions of 5-HT are mediated by 16 types and subtypes of receptors.[5] As the 5-HTergic system is assumed to be a modulatory system, the exact function of 5-HT is not easy to define.[6] However, 5-HT is known to be involved in many physiological and behavioral processes, including mood, appetite, sleep, activity, suicide, sexual behavior and cognition; all of which are affected in depression.[7] Alterations in 5-HTergic function have been observed in many clinical conditions, including affective disorders, anxiety, obsessive-compulsive disorders, eating disorders, aggression, suicide, impulsive disorders, alcohol abuse and premenstrual syndrome.[4,8]

Several altered 5-HT system indices have been reported in depression, including decreased plasma tryptophan levels[9,10] and decreased levels of 5-hydroxyindoleacetic acid (5-HIAA; metabolite of 5-HT) in cerebrospinal fluid (CSF),[11] suggesting decreased 5-HT metabolism in the central nervous system (CNS).

Correspondence: LAW Jans, Faculty of Psychology, Department of Neurocognition, Maastricht University, PO Box 616, Maastricht 6200 MD, The Netherlands.
E-mail: l.jans@psychology.unimaas.nl
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Furthermore, brain imaging studies have reported a reduction in 5-HT_{1A} receptor binding, both pre- and postsynaptically, that failed to normalize after remission.[12,13] Decreased availability of 5-HT reuptake sites in midbrain and brainstem regions has also been found in patients with major depression.[14] Evidence for functional 5-HTergic abnormalities comes from studies showing that depletion of tryptophan – and consequent lowering of brain 5-HT – can cause transient depressive symptoms in individuals that are vulnerable to depression, based on their personal or family history of depression.[15–18] Furthermore, depressed and remitted patients show blunted neuroendocrine responses to drugs that stimulate 5-HT turnover, suggesting decreased 5-HT responsiveness.[19–21] Some 5-HT abnormalities are also seen in remitted depressed patients or in subjects with a family history of depression, suggesting that either some dysfunction in 5-HT systems or an increased sensitivity of the 5-HT system is a trait abnormality in depression. It should be mentioned, however, that not all depressed patients show all these abnormalities in 5-HTergic functioning.[22]

The first monoamine theories of depression proposed that depression was caused by monoamine deficiency. By now, however, the model of a deficit in 5-HTergic neurotransmission being primary in the causation of depressive disorders, and predictive of therapeutic response to drugs enhancing 5-HTergic neurotransmission has become obsolete: not all depressed patients present with 5-HT abnormalities, not all patients benefit from drugs enhancing 5-HTergic neurotransmission, and several drugs that are devoid of major effects on 5-HTergic neurotransmission are known to be effective antidepressants. The categorical view – low 5-HT is specific for depression – has gradually been replaced by a dimensional approach: low 5-HT explains in part the vulnerability to mood liability across diagnoses, rather than depression *per se*. Hence, the thinking has progressively evolved from one of an absolute deficit to a high-risk model: low 5-HTergic neurotransmission is now thought to operate as a biological risk factor, resulting from innate and/or environmental factors, neither sufficient nor necessary, yet when combined may play an important role in the triggering and maintenance of mood disorders.[23] In other words, 5-HT can be considered as a vulnerability factor for developing depression.

5-HTergic vulnerability

In essence, serotonergic vulnerability (SV) means that there is a vulnerability or sensitivity to alterations or dysregulations in the 5-HTergic system (based upon references[21,23–30]). Thus, the idea is that the 5-HTergic functioning of an individual determines the vulnerability of that individual to develop disorders that are related to 5-HT. This assumption implies that there are differences in 5-HT functioning between different individuals, and that the development of depression is associated with the presence of *a priori* abnormalities in the functioning of this system. Abnormalities in the 5-HTergic system could occur at one or more of several levels, including the availability of L-tryptophan (TRP), 5-HT synthesis, release, reuptake or metabolism, or at the level of pre- or postsynaptic receptors.

SV can be demonstrated by challenging the 5-HT system; vulnerable and non-vulnerable subjects will react differently to these manipulations. The 5-HT system can be challenged using tryptophan depletion or 5-HT challenges. In general terms, 5-HT challenge studies can show that the neuroendocrine response to a 5-HT-related stimulus is blunted or enhanced in 5-HT vulnerable subjects as compared to non-vulnerable subjects. In acute tryptophan depletion (ATD), tryptophan – the precursor of 5-HT – is depleted, resulting in lower central 5-HT levels. ATD studies generally report the effects of lower 5-HT on behavior.[16,29]

The focus of this review is on the concept of SV. We advocate that SV is one answer to the question why some people become depressed, for example, when exposed to stress, while others do not. In this article, possible causes of SV are discussed. Furthermore, it is discussed how SV can be studied and what results have been found in studies manipulating the 5-HTergic system. Is there enough evidence to justify the use of the SV concept? Apart from the scientific importance, more knowledge about SV may be relevant from a clinical point of view. When SV can be demonstrated in individuals, vulnerable subjects may be identified before they become ill. This may enable early intervention, perhaps minimizing the risk or preventing the occurrence of depression or other disorders,[17] hence decreasing personal suffering, social and occupational dysfunction, and health care expenditures. In addition, knowledge about the causes of depression may in time help to find the optimal treatment.

Factors that may cause SV

Any factor that has a long-lasting influence on the 5-HTergic system may make the system vulnerable. Several factors are known to be capable of causing abnormalities in 5-HTergic function. Some of these factors are innate, some occur early in life, others can occur at any age.

Innate factors

Family history

Family studies show a familial aggregation of depression.[31–33] The presence of one depressed first-degree relative increases the relative risk for major depression by twofold.[32] Although family history (FH) is a risk factor for depression with high clinical relevance, it is not directly related to either genetic or environmental factors and can, therefore, not elucidate the mechanisms by which these factors may cause SV and/or depression.

It is interesting, however, that in many cases, subjects with a FH of depression also report other disorders in their family members, including affective psychosis, obsessive-compulsive phenomena, panic attacks, eating disorders and alcoholism/substance abuse.[34–36] Perhaps there is a general vulnerability factor involved in these disorders. 5-HTergic functioning may be a good candidate, because 5-HT is involved in all these disorders.[4]

Genetic factors

The inheritability of liability to major depression in most twin studies ranges from 31 to 42%.[32] As depression is related to several altered 5-HTergic indices in the brain and CSF, numerous genes involved in the regulation of 5-HT synthesis, release, uptake and metabolism, or receptor activation, are candidate genes in association studies of depression. Genetics can affect 5-HTergic activity, given that heritability has been reported to account for approximately 35% of the variance in CSF 5-HIAA levels.[37,38] Thus, alterations in the 5-HTergic system that are associated with psychiatric disorders may be inheritable, and could represent heritable vulnerability factors for developing psychopathology.

5-HT transporter. In the search for genetic factors related to depression, the serotonin transporter (5-HTT) has received particular attention, because it is the primary mode of action for selective serotonin reuptake inhibitors (SSRIs). The 5-HTT is involved in the reuptake of 5-HT from the synapse, returning it to the presynaptic neuron where it can be degraded or retained for release later. In this way, the 5-HTT determines the magnitude and duration of the 5-HT synaptic signal and thus plays an important role in the regulation of 5-HTergic neurotransmission. 5-HTT abnormalities are widely reported in depression.[26,39] Both functional imaging[14] and postmortem brain studies[39] have reported fewer 5-HTT sites in the brain of depressed patients, indicating less 5-HTT binding. As the binding to the 5-HTT and the 5-HT uptake capacity remain low after recovery, low 5-HTT activity has been proposed as a trait marker for affective disorders.[40]

The most important 5-HTT polymorphism in this perspective is the deletion/insertion polymorphism in the 5-HT transporter gene-linked promoter region (5-HTTLPR).[41,42] Two 5-HTTLPR alleles have been identified: a 484-base pair denoted as short (s), and a 528-base pair denoted as long (l). The l-variant is more active than the s-variant, resulting in higher 5-HTT expression and function.[41,42] As a result, the s/s and s/l genotypes are associated with 40% fewer 5-HTT-binding sites than the l/l genotype.[39] Consequently, the finding of fewer 5-HTT sites in depression can perhaps be explained by an association between the s-allele and major depression.[39]

Regarding an association between 5-HTTLPR genotype and depression, results have been mixed. Some studies reported no association,[39,43,44] others found an association between the s-allele and depression,[26,45–48] while others conclude that the l-allele is associated with depression.[49] However, most evidence seems to suggest an association between the s-allele of 5-HTTLPR and depression. The lower 5-HT uptake activity caused by the s-allele may result in increased levels of 5-HT in the vicinity of the 5-HTergic cell bodies and dendrites in the raphe complex. This increased extracellular 5-HT may bind to somatodendritic 5-HT_{1A} receptors, exerting negative feedback that leads to an overall decrease of 5-HT neurotransmission in subjects with the s-allele.[40,50] Evidence for this mechanism comes from studies with 5-HTT knockout mice (−/− and +/−) that exhibit no (−/−) or lower (+/−) 5-HTT binding. These mice show reduced 5-HT tissue concentrations and increased extracellular concentrations of 5-HT in the striatum and probably other structures (for review see Murphy *et al*.[51]). The l-allele of the 5-HTTLPR polymorphism is associated with better SSRI antidepressive effects than the s-allele[49,52,53] and with faster response to several SSRI's.[54,55] Combined treatment with a 5-HT_{1A} blocker to prevent negative feedback at the somatodendritic level compensated for the worse antidepressant effect of SSRIs in s/s and s/l genotypes,[56] suggesting higher 5-HT_{1A} negative feedback in s-allele genotypes.

5-HT_{1A} receptor. Human 5-HT_{1A} receptors are expressed presynaptically on 5-HT cell bodies in the raphe (somatodendritic autoreceptors) and postsynaptically in other brain regions. Presynaptic 5-HT_{1A} autoreceptors play a key role in the regulation of 5-HT transmission, they reduce the firing rate of the 5-HT neuron by exerting negative feedback. Although several studies failed to find a clear association between polymorphisms in the 5-HT_{1A} receptor gene and depression (for review see Neumeister *et al*.[57]), Lemonde *et al*.[58] linked a common polymorphism in the human 5-HT_{1A} promoter region[59] to depression and suicide. The homozygous G(−1019) genotype of this polymorphism may cause impaired repression of presynaptic 5-HT_{1A} autoreceptors, resulting in increased expression of these receptors. This may ultimately cause reduced 5-HT neurotransmission, perhaps predisposing individuals to depression and suicidal behavior.[58] Keeping in mind that the abnormalities in 5-HT_{1A} receptor binding are not specific for depression,[60] this finding may reflect a general vulnerability factor for psychopathology, including affective and anxiety-related disorders.

Tryptophan hydroxylase 2. Tryptophan hydroxylase (TPH) is the rate-limiting enzyme for the synthesis of serotonin.[61] TPH catalyzes the oxygenation of tryptophan to 5-hydroxytryptophan, which is then decarboxylated to form 5-HT.[62] Walther *et al*.[63] discovered a new brain-specific TPH isoform, called TPH2. Biochemical studies in mice[64] and humans[65] suggest that a TPH2 polymorphism results in decreased 5-HT synthesis. Zhang *et al*.[65] reported that in a group of elderly patients with a history of

major depression, 10% carried the mutated gene variant encoding the poor producer of serotonin, compared with just 1.4% of the non-depressed controls. Furthermore, the controls carrying the mutant allele, although not diagnosed with depression, did report problems, such as generalized anxiety, mild depression, or family histories of alcohol abuse or mental illness. Zill *et al*.[66] also found evidence to suggest that polymorphisms in the TPH2 gene represent risk factors in the development of major depression.

The mutation may help to predict treatment effectiveness, since depressed patients with the mutant TPH2 allele do not respond well to SSRIs.[65,67] TPH2 represents an interesting candidate gene for affective disorders, but more research is needed in this area before more reliable conclusions can be drawn.

Personality

As was mentioned before, the heritability of liability to depression in most twin studies ranges from 31 to 42%.[32] However, it is unclear whether depression itself is inherited or some personality traits that cause vulnerability for developing mood disorders. Personality traits appear to have a considerable heredity component[68] and it has been suggested that a certain type of personality or temperament represents a risk factor for developing major depression.[69,70]

Depression has been associated with higher scores on neuroticism (N) and the temperament dimension harm avoidance (HA), the tendency to respond to signals of adverse stimuli with behavioral inhibition.[70–78] Also, low self-directedness (the ability to regulate one's behavior and commit to chosen goals; SD) and cooperativeness (the ability to identify with and accept other people; C) have been found in depressed patients,[77,79] which may increase after treatment.[80] SD and C have been associated with 5-HTergic activity.[81] The fact that antidepressant treatment also leads to increased SD scores[82] and decreased aggression and negative affect[83] in healthy volunteers, might suggest that 5-HT is the causal factor.[81] A reduction in HA has been reported in depressed patients after 5-HTergic antidepressant treatment,[84,85] indicating that some personality change, mainly related to HA and N, is related to clinical improvement.[81]

Higher scores on HA may be related to lower central 5-HTergic turnover or low central basal 5-HT levels,[78,86,87] lower 5-HT_{2A} receptor binding in the cortex[88] and lower platelet 5-HT_{2A} receptor number.[78] Low brain 5-HT turnover, indicated by low CSF 5-HIAA, is characteristic for a subgroup of depressed subjects, who have a history of serious suicide attempts.[89] Low CSF 5-HIAA is associated with increased impulsivity and impaired control of aggressive behaviors[90,91] and with impulsive aggression and suicidal behavior (for review see Asberg[92]). Studies with non-human primates have shown that CNS 5-HT functioning is traitlike, with low CSF 5-HIAA being stable over time and across settings.[93–95] In non-human primates, low CSF 5-HIAA is associated with impaired impulse control, less competent social behavior and impulsive and unrestrained aggression.[95]

In conclusion, personality factors may be involved in SV, but it is far from clear how personality factors may cause SV and whether the personality factors cause 5-HTergic abnormalities or vice versa.

Gender

Epidemiological studies have shown that major depression is more common in females than in males (for review see Piccinelli and Wilkinson[96]). This is found in several countries and ethnic groups.[97] Artefactual factors, including more help-seeking and illness behavior in women, may enhance the female prevalence to some extend, but the gender difference in depression appears to be genuine.[96] The gender difference in the prevalence of depression begins around puberty and persists until midlife. During puberty, women enter their reproductive years,[98] while at the same time the frequency of traumatic events increases and social roles are changing.[99] All these factors may be involved in the gender difference in prevalence of depression.

Besides the prevalence of depression, there are also gender differences in the presentation of the disorder. Men commonly have classical neurovegetative symptoms of depression, such as decreased appetite and insomnia and have higher incidence of comorbid alcohol and substance abuse. Women on the other hand are more likely to present with sleep disturbance, psychomotor retardation, somatization, comorbid psychiatric disorders, mostly anxiety and eating disorders, and atypical symptoms such as increased appetite and weight gain (for review see Kornstein[100]). There are several possible causes of these sex differences in depression, to which 5-HT may be directly or indirectly related. Sex differences in 5-HT system and sex hormones are discussed in this section, whereas sex differences in stress exposure and stress responsivity will be discussed later.

5-HT differences between males and females. Reports of sex differences related to the 5-HTergic system come from animal and human studies. Female rats exhibit increased 5-HT activity in the dorsal raphe nuclei,[101] higher 5-HT levels in the brain stem and limbic forebrain,[102] higher 5-HT synthesis[103] and turnover.[104] The average firing activity of 5-HT neurons has also been found to be higher in males than in cycling females.[105]

Human CSF studies suggest that the rate of brain 5-HT metabolism is higher in females than in males[106,107] while 5-HT_2 receptor-binding capacity in some brain regions[108] and whole brain 5-HT synthesis[109] are lower in women than in men. Women also appear to have lower 5-HTT binding than men.[39] Possibly, sex differences in 5-HT are related to higher SV in females.

There is some data indicating that the decrease in 5-HT transporter availability observed in depression[14] is sex dependent. Staley *et al*.[110] reported an overall decrease of transporter availability in depressed patients. However, this decrease was explained by a substantial decrease in women, with hardly any decrease in men.

Sex hormones. Estrogen may play a neuromodulatory role on several neurotransmitter systems, including the 5-HTergic system.[111] It has been shown, for example, that 5-HT concentrations fluctuate throughout the rodent estrus cycle.[112,113] Estrogen has been reported to increase TPH production in non-human primates.[114] Furthermore, estrogen can alter the expression of genes involved in the 5-HTergic system and can enhance 5-HTergic activity.[115–119] This raises the possibility that estrogen may alter the risk for depression through its effect on 5-HTergic function.[115–119]

Estrogen acts on the central nervous system through two different estrogen receptors, the estrogen receptor α (ER-α), and the estrogen receptor β (ER-β). ER-α may be involved in automatic and reproductive functions, emotional expression and mood regulation, since this receptor subtype is predominantly expressed in hypothalamus and amygdala.[120] ER-β is highly expressed in hippocampus and thalamus, suggesting a role in cognition and memory. Compared to wild-type littermates, female ER-β knockout mice have been found to have significantly lower 5-HT and dopamine content in several brain regions, including reduced 5-HT in the hippocampus and a trend towards decreased 5-HT content in the dorsal raphe nucleus.[121] On a behavioral level, these knockout mice showed increased anxiety.

The effects of estrogen appear to have protective effects in some women, while conferring an added risk for mood disorders in others.[119] Estrogen levels vary along the estrus cycle, perhaps transiently increasing SV in some women. This may be related, for example, to symptoms of premenstrual syndrome.[122]

Environmental factors

Stress

Depression is likely to result from a combination of innate and environmental factors that each modify the risk of an individual to become depressed. Stress is one of the most potent environmental factors.[123] Stress is a difficult concept to define. It consists of a threatening of homeostasis to which the organism reacts with adaptive responses. There is a psychological aspect to stress, in which predictability, control and coping are involved; and a biological aspect, consisting of the activation of specific brain and neuroendocrine circuits.[124] There is an obvious connection between depression and stress. Depression is often preceded by stressors[22] and chances to develop a depressive episode are increased five or six fold after stressful life events.[125]

The response to stress varies vastly across individuals;[126] stress responsivity is the result of complex interactions between genetic susceptibility and environmental stress.[127] Various types of situations can be perceived as stressful. In general, typical stressful events include ending personal relationships, job loss, moving to another town and long-lasting situations including financial problems and problems at work. There are some individual differences in exactly what situations are stressful. A situation that is perceived as stressful activates the stress system and results in several physiological changes. In medical students, for example, academic examinations increased cortisol levels only in students who perceived stress.[128] The biological changes caused by stress are probably an adaptive response, serving to sustain well-being.[123,129] Stressors challenge the capacity of an individual to cope. A person may adapt to the stressor by responding in a way that reduces the impact of the stressor.[130] If this coping fails, various events can result in a long-lasting state of distress, which is reflected in abnormal HPA axis activity and altered limbic function, increasing the risk of developing depression.[131] When the stress-state is long-lasting and cortisol levels remain high, negative consequences may occur.

Stress and depression. Depressed patients show higher levels of circulating stress hormones[22,132,133] and abnormalities in various hormonal challenge tests,[22] indicating HPA axis hyperactivity that appears to be depression-related rather than stress-related. This HPA overactivity is a consistent finding, although it is not found in all patients.[22]

During stress, activity of the brain 5-HT system and HPA axis rises. Serotonin can modulate the HPA axis, 5-HT overdrive increases corticotrophin-releasing factor (CRF) and corticosteroid release.[22] Increased cortisol levels initially cause higher CNS 5-HT turnover by increasing tryptophan availability and stimulation of TPH activity[134,135] and increased responsivity of the $5HT_{1A}$ receptor system.[136,137] However, when cortisol/corticosterone levels remain high, 5-HT turnover and $5\text{-}HT_{1A}$ sensitivity and mRNA expression will be reduced.[22]

Given that high cortisol levels initially cause higher CNS 5-HT turnover, hypothetically, during continuous or frequent exposure to stress, availability of brain TRP and serotonin may diminish and vulnerability to pathology may increase.[123,138] Stress-induced depletion of brain serotonin has been shown in animal research (for review see Markus[123]). As serotonin enhances the negative feedback control of cortisol on the release of CRF and adrenocorticotrophic hormone (ACTH), reduced tryptophan availability and 5-HT depletion may reduce this feedback control, causing increased cortisol concentrations in the blood,[123,139] perhaps by reducing activation of $5\text{-}HT_{1A}$ receptors in the hippocampus.[139]

There is evidence to indicate that impaired glucocorticoid receptors (GR)-mediated feedback may be involved in the pathophysiology of depression.[133,140] The changes in expression and function of GR and mineralocorticoid receptors (MR) in the hippocampus may be mediated by the increased 5-HT release that is evoked by exposure to stress.[141–143] Furthermore, chronic stress and glucocorticoids induce neural atrophy in limbic structures, mainly the hippocampus, and reduce cell proliferation and neurogenesis in the hippocampus.[144–150] These changes have been related to the development of depression, including the cognitive symptoms of this disorder.[151] Antidepressants can increase GR function and expression[140] and normalization of the HPA axis could be important for clinical improvement and prediction of relapse.[133]

Altered HPA axis feedback inhibition may be a trait marker for vulnerability to depression, since reduced HPA axis suppression by dexamethasone has also been shown in first-degree relatives of depressed patients.[152] In healthy individuals with family history of depression, the combined dex/CRF test showed cortisol responses that were intermediate between depressed individuals and healthy controls,[152] indicating that this family history is accompanied by subtle changes in HPA axis functioning, resulting in increased risk of developing depression.

In conclusion, stress causes CRF and corticosteroid overdrive, in time resulting in decreased 5-HT turnover and 5-HT_{1A} responsivity. In this way inability to cope with a chronic stressor[153] or exposure to a single acute life event can cause changes in HPA axis activity and corticosteroid action,[154] imposing a risk for depression and other diseases.[131]

Prenatal stress. Exposure of a pregnant woman to physical and/or psychological stress (prenatal stress; PS) can have structural, behavioral and pharmacological effects on the offspring.[126,127,135] A large body of evidence relates stress-induced disturbances in the maternal HPA axis activity to impaired development. In humans, paternal death, malnutrition or drug use during pregnancy, and familial or marital problems can predispose individuals to the development of affective and anxiety disorders in adulthood (for review see Kofman,[126] Maccari *et al.*,[135] Morley-Fletcher *et al.*[155]). It has been reported, for example, that the prevalence of depression was increased in children prenatally exposed to the earthquake in Tangshan, China, in 1976.[156]

In rat studies, PS has been reported to result in developmental abnormalities that may have long-term detrimental consequences for brain functioning and may lead to increased susceptibility to psychopathology, including depression, in adulthood.[157,158] Prenatal stressors such as repeated saline injections, daily restraint stress, or crowding (for review see Kofman,[126] Maccari *et al.*[135]) have been associated with several deficits in the offspring, such as reduced birth weight, increased infant morbidity, locomotor and cognitive retardation, circadian abnormalities and sleep disturbances, alterations in sexual or social behavior, and learning deficits.[127,159] Rat PS offspring show enhanced response to stress[126,127,135,155,160] and higher behavioral emotionality in several stressful situations.[155,159,161–164] Adult PS offspring show many characteristics similar to those seen in depressed humans[165] including HPA dysregulations and anhedonic behaviors.[166,167] Abnormalities in brain neurotransmitter systems have also been reported, most importantly low central 5-HT activity in adult offspring after high maternal and fetal 5-HT.[160]

The mechanisms accounting for the impact of PS on postnatal life are not yet fully understood. Investigations on the putative mechanisms involved have focused mainly on the HPA axis. The generally held hypothesis is that PS leads to activation of the maternal HPA axis and elevated plasma levels of glucocorticoids in the pregnant animal, which influences fetal brain development. This results in downregulated glucocorticoid receptors in the fetal hippocampus, altered receptor sensitivity and neurotoxic effects on hippocampal cells, finally causing decreased negative feedback in the PS offspring.[126,127,135]

As glucocorticoids may play a role in brain development, PS may result in alterations of other biochemical systems in the brain, including 5-HT.[127] The 5-HTergic system develops early in the mammalian brain and is widespread, therefore it can influence the maturation of many other cells in the brain.[168] In addition to influencing the development of target regions, 5-HT also has a negative feedback effect on the developing fetal 5-HT neurons.[169] As 5-HT neurons develop, 5-HT levels increase until a point is reached at which the growth is curtailed through a negative feedback mechanism.[169] When 5-HT levels are high early in development, when the blood–brain barrier is not fully formed yet, 5-HT can enter the brain of a developing fetus and thus cause loss of serotonin terminals through negative feedback. In this way, increased 5-HTergic activity during development causes loss of 5-HT terminals in the adult.[169] Several drug treatment studies have shown this negative feedback of 5-HT on development of 5-HTergic neurons.[170–180] Alterations in 5-HTT function[181] and hypothalamic 5-HT levels[182] may be in part responsible for altered HPA axis activity in adult PS offspring.[183] PS may lead to an altered set-point of the HPA axis and 5-HT system, increasing susceptibility to later (depressive) disorders.[127]

Early life experiences. Aversive or stressful early life experiences, such as childhood neglect, childhood sexual or physical abuse, exposure to war, or parental loss, are important environmental factors contributing to the development of psychiatric disorders, including depression, anxiety, impulsive behavior and substance abuse in adulthood.[184–187] Alterations in mother-infant interactions also have major influences on development and subsequent functioning of the HPA axis.[188,189]

Human children, as well as rodent pups, are characterized by a stress hypo-responsive period,

developing gradually during the first year of life.[154,190] Healthy newborn infants react to stressors with an adrenocortical response,[191] but the HPA axis of a 12–18 months old child does not respond to mild stressors,[192,193] although the child does show a behavioral response to such a stressor. More severe stress, however, will cause an acute rise in corticosterone levels during the stress-hyporesponsive period.[194]

The effects of early life experiences depend on their nature. Rodents exposed to the mild stress of short periods of neonatal handling show decreased HPA responsivity to stress in adulthood,[195] although a sex difference in this effect has been reported, with an increase in the ability to cope with stressful stimuli in males and a decrease in females after neonatal handling.[196] The more severe stress of maternal deprivation, on the other hand, causes behavioral abnormalities resembling symptoms of depression and anxiety in adulthood,[127,197,198] these negative effects may be more marked in males than in females.[198]

In rodents, early-life stress induces 5-HTergic dysfunctioning and persistent changes in HPA responsiveness and CRF expression.[131,154,186] Adverse early life experiences appear to interact with chronic stress in adulthood to alter physiology and behavior.[199] The release of corticosterone in response to mild stress is heightened and prolonged in adult male rats that were maternally deprived as pups, indicating impaired glucocorticoid-mediated feedback sensitivity[200] that may results from downregulation of hippocampal GR.[201]

Another long-term effect of stressful experiences during development, is that they can permanently affect neurogenesis.[202] Inhibition of adult neurogenesis in the dentate gyrus might play a role in depressive illness,[203] since several antidepressant treatments increase neurogenesis with a delay that parallels clinical improvement.[145,204] Early adverse experience inhibits structural plasticity via hypersensitivity to glucocorticoids and diminishes the ability of the hippocampus to respond to stress in adulthood.[205]

Immune system and cytokines

Psychological, physical and systemic stressors, the latter including infection, chronic inflammation and tissue injury, have been reported to influence cytokine production.[206–210] Cytokines are the signaling molecules of the immune system, and may also act to signal the CNS about the presence of an immunological challenge. They can be divided into two general categories, that is anti-inflammatory cytokines and proinflammatory cytokines, the latter including interleukin (IL)-1, IL-6, interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α).[211]

Dysregulated activity of the immune system in depression, with increased proinflammatory activity, has repeatedly been demonstrated.[212–216] In addition, inflammatory diseases are typically associated with depressed mood.[217,218] Administration of proinflammatory cytokines (e.g. in cancer or hepatitis C therapy) has been found to induce depressive symptoms. Several studies in healthy volunteers have suggested that increased concentrations of IL-6, but also of TNF-α and IL-1 receptor antagonist (IL-1Ra) may cause depressed mood.[218–220] The increase of several proinflammatory cytokines appear to correlate with the severity of depressive symptom.[214,215,217,218,221] The 'cytokine theory of depression' implies that proinflammatory cytokines, acting as neuromodulators, represent the key factor in the (central) mediation of the behavioral, neuroendocrine and neurochemical features of depressive disorders.[211,222]

Proinflammatory cytokines increase 5-HT turnover, which may lead to depletion of 5-HT in combination with several other mechanisms that lower 5-HT.[211,215] First, proinflammatory cytokines may induce tryptophan depletion by reducing food intake.[219,223] A second possibility is that they set off depressive symptoms by modulating the activity of the HPA axis.[208,211] Proinflammatory cytokines may disturb the negative feedback inhibition of corticosteroids on the HPA axis, causing HPA axis hyperactivity (for review see Schiepers *et al*.[211]). Thirdly, cytokines may induce depressive symptoms by downregulating the synthesis of serotonin. Several cytokines, including IL-1, IL-2, IL-6 and INFs reduce TRP availability by activating the TRP-metabolizing enzyme indoleamine-2,3-dioxygenase (IDO), the rate-limiting enzyme in the TRP-kynurenine pathway that converts L-TRP to *N*-formylkynurenine.[224] In this way, there is less TRP available for serotonin synthesis, which may lead to depletion of serum TRP and reduction of 5-HT systhesis.[225,226] Along with the cytokine-induced stimulation of IDO, the production of certain metabolites of the IDO-mediated kynurenine pathway increases, including 3-hydroxy-kynurenine (3OH-KYN) and quinolinic acid (QUIN). These are neurotoxic substances and may cause hippocampal volume loss, loss of glucocorticoid receptors and increased HPA activity.[224,227] Furthermore, proinflammatory cytokines also stimulate the utilization of TRP and other peripheral amino acids for synthesis of positive acute-phase proteins (APPs).[226,228] Moreover, it has been suggested that proinflammatory cytokines result in reduced extracellular 5-HT levels[208,229–231] based on the finding that several proinflammatory cytokines have been shown to upregulate 5-HTT expression.[232–234] Ultimately, neurotrophic factors such as brain derived neurotrophic factor (BDNF) may be affected. The activation of proinflammatory cytokines and/or inhibition of anti-inflammatory may be involved in depression by means of processes related to neuroplasticity.[235,236] It is possible that cytokines contribute to the pathogenesis of depression through their actions on 5-HT and BDNF, which co-regulate each other.[236,237]

Stressors, including cytokine challenges, can proactively influence the response to later challenges. Neuronal systems may sensitize, so that later exposure results in exaggerated neurochemical changes.[206] Cyto-

kine administration increases the response to stressors or further cytokine exposure.[206,238–241] It is possible that exposure to stress, including the activation of the inflammatory immune system, may result in greater vulnerability to stressor-related pathology (for review see Connor and Leonard,[125] Hayley *et al*.[236]).

Preexisting vulnerability may also affect the outcome of acute immune challenges.[242] For example, some reports showed that pretreatment depression scores and past psychiatric history were predictive for the occurrence of depression-like changes over the course of chronic cytokine therapy.[208,243,244]

Drug use

Hypothetically, any drug that influences the 5-HTergic system may cause that system to become vulnerable. Some popular drugs that may influence the 5-HTergic system are discussed.

±3,4-Methylenedioxymethamphetamine (MDMA). MDMA is a popular recreational drug taken for its acute effects, which include euphoria, increased sociability and energy.[245] Acutely, MDMA causes the release of stored 5-HT from nerve terminals, prevents reuptake of 5-HT from the synaptic cleft, and inhibits TPH, thereby inhibiting the synthesis of replacement 5-HT.[246] These acute effects are followed by a temporary attenuation of central 5-HT.[247]

There is evidence to indicate that MDMA has neurotoxic effects on the 5-HTergic system in animals[248] and humans,[249,250] mainly characterized by a decreased number of serotonin transporters.[249,251] Animal studies have indicated that MDMA administration results in long-term attenuation of brain 5-HT and 5-HIAA as well as attenuated TPH activity and reductions in the density of 5-HT uptake sites (for review see Curran *et al*.[252]) and there is evidence to indicate that recovery may remain incomplete for several years.[253,254] In human studies the decreased 5-HTT binding is generally reported to be at least partly reversible.[249,251,255,256] However, it should be mentioned that the recovery of 5-HTT densities does not necessarily guarantee normal functioning.[257] Studies on the functional consequences of MDMA induced 5-HT neurotoxicity show converging evidence of memory impairments.[258] There are several reports indicating that current and former heavy MDMA use results in negative effects on mood and depression, but results are mixed.[255,257,259,260–264]

Alcohol. Alcohol dependence and depression co-occur commonly in clinical populations.[265,266] Presumably, a deficit in 5-HTergic transmission is involved in the etiology and maintenance of alcoholism and 5-HT may mediate ethanol intake (for review see LeMarquand *et al*.[267,268]). Distinguishing the effects of long-term alcohol intake from putative premorbid 5-HT dysfunction is a problem.[268] Alcohol can damage the nervous system, either directly or indirectly through damage to other organs.[269] Chronic alcohol intake may have neurotoxic effects on the 5-HT system[270] that may result in loss of central 5-HTergic function and negative mood states.[271]

Overall evidence suggests that acute ethanol facilitates 5-HTergic neurotransmission (for review see LeMarquand *et al*.[268]), although there is some evidence that alcohol may have a biphasic effect on the 5-HTergic system, with an initial facilitation of 5-HTergic activity followed by a decrease several hours later.[272,273] Animal studies suggest that acute and chronic ethanol result in transient increases in 5-HT levels and functioning, but withdrawal of chronic ethanol causes a decrease in 5-HT and 5-HIAA levels and 5-HTergic functioning (for review see LeMarquand *et al*.[267]). Low CSF 5-HIAA and low plasma TRP availability have been reported in alcoholics, suggesting lowered central 5-HT neurotransmission (for review see LeMarquand *et al*.[268]) and it has been demonstrated that heavy drinkers can experience mood symptoms as a consequence of their heavy drinking.[274]

Heinz *et al*.[271] found a significant reduction in the availability of brainstem 5-HTT in alcoholics after 3–5 weeks of withdrawal compared to healthy controls, that was correlated with ratings of depression and anxiety during the withdrawal. The reduction in raphe 5-HTT also correlated with lifetime alcohol consumption, suggesting that this reduction in transporter density may be caused by the cumulative toxic effects of ethanol consumption.[271]

Cannabis. There is evidence to indicate that heavy or problematic cannabis users have an increased chance of developing depression (for review see Degenhardt *et al*.[275]). One hypothesis to explain this increased risk is that large doses of the primary psychoactive ingredient of cannabis, delta-9-tetrahydrocannabinol (THC) affect 5-HT and other neurotransmitters.[275] THC acts upon the cannabinoid system, a neuromodulatory system in the brain that is involved in regulating some of the same physiological processes as the 5-HTergic system. Acutely, THC has been reported to suppress 5-HT neurotransmission in the hippocampus by inhibiting 5-HT release.[276] Long-term cannabinoid administration can affect the response to a single challenge of a 5-HT_{1A} and 5-HT_{2A} receptor agonist. Twelve days of pretreatment with a cannabinoid receptor agonist resulted in an apparent upregulation of 5-HT_{2A} receptor activity and a downregulation of 5-HT_{1A} receptor activity.[277] The effects of THC on 5-HTergic functioning and receptor activity resemble the changes seen in depressed patients.

Cocaine. Cocaine binds to all three monoamine transporters, and acutely inhibits the reuptake of monoaminergic neurotransmitters into presynaptic neurons.[278,279] This causes an initial increase in extracellular neurotransmitter concentrations, which leads to compensatory decreases in cell firing and neurotransmitter synthesis (for review see Levy *et al*.[280]). During cocaine withdrawal, symptoms of depression are common.[281]

Repeated exposure to cocaine may alter functioning of monoamine neurotransmitter systems. Cocaine appears to result in subsensitive postsynaptic 5-HT_{1A} receptors and supersensitive 5-HT_2 receptors.[280,282,283]

Biopsychological interactions

It is likely that interactions between innate (genetics, gender, personality, prenatal stress) and environmental factors (postnatal stress, drug use) determine the vulnerability to develop depression. Figure 1 presents an overview of the factors that may contribute to SV.

Evidence appears to suggest that the 5-HTTLPR s-allele increases sensitivity to the depressogenic effects of environmental insults ranging from stress to MDMA use and neurochemical challenges such as ATD. The s-allele may predispose for a more reactive arousal system. The s-allele appears to predispose towards increased anxiety and exerts a negative influence on the capacity to cope with stress (for review see Hariri and Holmes[284]). It has been shown that carriers of the s-allele have an elevated risk of depression in the context of environmental adversity.[73,285,286] Furthermore, in rhesus macaques, which have the same 5-HTTLPR length variations as humans, the s-allele is associated with decreased 5-HTergic function, indicated by lower CSF 5-HIAA concentrations, only in monkeys reared in stressful conditions.[287] In addition, s-allele carriers showed increased activation of the amygdala in response to fearful or threatening stimuli.[288–290] In terms of interactions between genes and MDMA, Roiser *et al*.[291] reported that 5-HTTLPR genotype mediates emotional processing in ecstasy users, as only ecstasy users carrying the s-allele showed abnormal emotional processing. Interestingly, the abnormalities in emotional processing were similar to those reported in healthy volunteers after ATD.[292]

The l-allele of the 5-HTTLPR may account for a certain flexibility in the 5-HTergic system, or the possibility to react and adapt to changes that are imposed on the system. First, alcoholics carrying the s-allele showed no difference in 5-HTT availability, whereas l/l genotype alcoholics show a reduction in raphe 5-HTT availability.[293,294] Second, the l/l genotype is associated with an initially low response to alcohol.[295] Third, l-allele carriers have better antidepressant response to SSRI treatment than s-allele carriers.[53]

Personality factors may also determine how an individual interacts with the environment. An association exists between 5-HTTLPR genotype and some personality factors, given that carriers of the s-allele are more likely to demonstrate anxiety-related personality traits, including neuroticism and harm avoidance, than l/l genotypes.[42,296–298] It is unclear whether personality can directly cause SV. For example, it is also possible that certain personality traits simply co-occur with SV or depression, or that they represent the state of SV. Neuroticism scores, however, appear to predict future depression and 55% of the genetic risk of depression appears to be shared with neuroticism.[70] Personality characteristics are known to interact with other factors, because they influence the interpretation of events, the ability to cope with stress and the experienced level of cognitive control over a stressful situation. To make matters even more complex, individuals with a genetic risk for depression are not only more susceptible to the depressive episode-triggering effects of an adverse event, but may also be more likely to expose themselves to such an adverse event.[299]

Several types of stressors, for example, psychological stress and the systemic stress caused by pro-

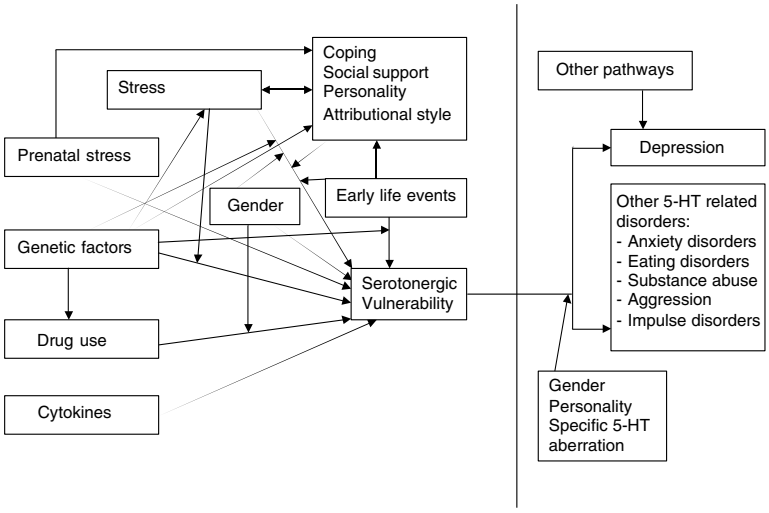

Figure 1 The etiology of SV and depression. The factors that may cause SV and possible interactions between these factors are depicted on the left. On the right are the possible pathological outcomes of SV. As the figure shows, the nature of the outcome may depend on personality variables and the gender of the individual, as well as on the location of the 5-HT aberration.

inflammatory cytokine activation, may result in HPA axis overactivity, decreased 5-HT turnover and reduced 5-HTergic functioning. Prenatal stress and adverse early life events may lead to an altered set-point of the HPA axis and 5-HT system, influencing the response to later stressors and hence increase vulnerability to psychopathology. Stress reactivity in adulthood is influenced by genetic background and early life experiences. Genetic background can modulate the response to adversity in early life as well as in adulthood.[131] Adversity early in life may also influence personality.

Sex hormones or sex differences in 5-HTergic activity and the 5-HTergic system may be directly responsible for higher SV in women. Sex differences in exposure and response to stress may also affect SV. Women are more likely than men to develop depression following a stressful life event early in life[300] and their HPA axis is more susceptible to PS-induced programming.[301–303] Women have a lower mean rate of 5-HT synthesis compared to men, while men and women appear to have similar stores of brain 5-HT.[109] Thus, in times of increased 5-HT utilization, a lower rate of synthesis in women may cause 5-HT levels to decline more in women than in men, possibly increasing vulnerability to depression.[109] Some types of stressful events may be more common in women than in men. The rates of childhood sexual abuse, for example, are higher in girls than in boys, and as a result, as much as 35% of the gender difference in adult depression could be explained by this higher incidence of childhood abuse of girls compared to boys.[304] Furthermore, there are some indications that women may experience more chronic stress in everyday life than men.[99,305]

Some studies found an association between the s-allele and stress only in women.[306] The effects of adverse life experiences are mediated by personality characteristics, attributional style and coping.[307,308] Several authors have suggested the possibility that women appraise some threatening events as more stressful than men.[309,310] It has also been reported that for women, life events appear to be less controllable and more negative.[305] There is evidence to indicate that there is a sex difference in response to stress. In rats, exposure to an acute stressful event can facilitate learning in males, but impairs performance in female rats.[311,312] Furthermore, exposure to an elevated plus-maze, a rodent anxiety test, leads to decreased 5-HTergic activity in the dorsal raphe nuclei in female rats, but to decreased 5-HTergic activity in the medial raphe nuclei in males.[101] There is also data to indicate that male rats may adapt better to stress than females.[313] Furthermore, the female HPA axis may be more reactive to stress, possibly due to sex hormones.[300] In terms of coping styles, women have been reported to use more emotion-focused coping than men, while men more often use rational and problem-focused coping.[305] Women appear to be more likely than men to respond to stress with rumination – focusing inward on feelings of distress and personal concerns rather than taking action to relieve their distress.[99]

Gender may also interact with drug use. Subjective MDMA response,[314] the effects of MDMA on 5-HTT densities[249] and reductions in CSF 5-HIAA concentrations caused by MDMA[315] have been reported to be stronger in women than in men.

Possible pathological outcomes of SV

In terms of the pathology in which the SV finally becomes manifest, there may be several factors involved. First, it is possible that the location of the vulnerability-causing factor within the 5-HT system plays a role. There is some evidence to indicate that abnormalities in the 5-HT_{1B} receptor are related to substance abuse, impulsive aggression and motor impulsivity[316,317] while disturbances in 5-HT_{1A} neurotransmission have been associated with less reactivity and higher anxiety[318,319] and 5-HTT abnormalities with depression.[14] Gender may also be involved in forms of manifestation of SV, with substance abuse, aggression and impulsivity being more common in men while anxiety and depression are more common in women. Furthermore, personality traits appear to be involved, since mood lowering after ATD tends to occur in subjects with mean baseline depression scores at the upper end of the normal range.[15,320] Furthermore, TRP depletion produces a marked rise in the ratings of aggression during provocation in subjects with high trait aggression, but has little effect in subjects with low-trait aggression (for review see Young and Leyton[321]).

Experimental manipulations of 5-HT in relation to SV factors

Challenge studies

5-HTergic challenge tests are used to measure 5-HTergic responsivity *in vivo*. These tests involve the administration of a 5-HTergic probe and the subsequent assessment of one or more anterior pituitary hormones, such as cortisol, ACTH, growth hormone and prolactin.[19,20] The secretion of prolactin is regulated by 5-HTergic mechanisms in the brain.[322,323] Therefore, increased plasma prolactin can be interpreted as an index of enhanced brain serotonin function[324,325] and hypersensitivity of the 5-HTergic system.[326,327] Neuroendocrine responses to 5-HT agonists are an indication of postsynaptic receptor sensitivity, with relatively high hormonal responses being indicative of receptor hypersensitivity while relatively low hormonal responses suggest receptor hyposensitivity.[328]

Many substances with different sites of action have been used in 5-HTergic challenge studies, including L-TRP,[329] fenfluramine,[330–334] buspirone,[335] *meta*-chlorophenylpiperazine (*m*-CPP)[336] and several 5-HTergic reuptake inhibitors.[337–339]

Administration of D-fenfluramine results in a dose-dependent rise in prolactin levels,[340] which has been

used as an index of central 5-HT responsivity.[341–343] The prolactin response to fenfluramine in depression has been studied extensively, with mixed results (for review see Kavoussi *et al*.[340]). Studies that compare prolactin response to fenfluramine in depressed patients before and after antidepressant treatment have yielded mixed results.[344–346]

Citalopram is a highly selective 5-HT reuptake inhibitor that produces dose-related increases in prolactin and cortisol in normal subjects.[347,348] Intravenous challenge with citalopram has been reported to result in a blunted prolactin response in depressed patients compared to healthy controls,[3,334] while cortisol secretion was reported not to differ between groups.[19] Bhagwagar *et al*.[21] showed that the prolactin response to citalopram was blunted similarly in both acutely depressed and recovered drug-free euthymic subjects. The cortisol responses, however, were blunted in the acutely depressed patients, but not in the recovered subjects. These data support the notion that some aspects of impaired 5-HT neurotransmission, such as the blunted 5-HT-mediated prolactin release, may be trait markers of vulnerability to depression, since they persist into clinical remission. However, it could be a consequence of having been depressed or treatment with antidepressants, rather than a marker of vulnerability.[21]

Studies measuring hormonal responses to L-TRP infusion generally demonstrate attenuated prolactin and growth hormone responses in patients with major depression, indicating an impairment in postsynaptic 5-HT_{1A} receptor function (for review see Porter *et al*.[349]).

Nutrients can influence 5-HT synthesis, with carbohydrate ingestion generally increasing 5-HT synthesis, while protein ingestion has been reported to decrease 5-HT synthesis or not to change it.[350–352] The amount of TRP available to the brain can be increased with alpha-lactalbumin (LAC), a whey protein with high TRP content,[353,354] leading to enhanced 5-HT synthesis. While TRP availability to the brain influences 5-HT synthesis,[350,351] extracellular 5-HT levels reflect the neuronal activity of release and reuptake of the neurotransmitter.[355] LAC ingestion not only affects 5-HT synthesis, but also its release. Long-term LAC diet was reported to elevate extracellular 5-HT and may induce beneficial effects on mood.[356]

In humans, LAC has been reported to increase the plasma TRP/LNAA ratio by 43–48% in subjects with high and low stress vulnerability.[353,357] This increase in plasma TRP-LNAA ratio is considered to be an indirect index of elevated brain TRP and 5-HT concentrations.[353] However, LAC had a positive effect on mood[358] and cognitive performance[353] only in the group of high stress-vulnerable subjects. These results may suggest that high stress-vulnerability is related to poor 5-HTergic functioning, in a way that the stress-vulnerable subjects appear to need more 5-HT than they have, in order to keep up performance under stress conditions. The LAC intake may compensate for this deficiency by enhancing brain 5-HT function.

In short, a blunted neuroendocrine response to 5-HTergic agonists has been found in depressed patients.[3,330,334,359] The consensus is that 5-HT neurotransmission is impaired in unmedicated depressed patients, but it is unclear whether this abnormality persists following clinical recovery.[21] Results are mixed and it is difficult to distinguish possible trait markers of vulnerability to depression from abnormalities that occur as a result of having been depressed.

Blunted prolactin responses to fenfluramine have been reported in euthymic men and women with a personal history of depression, indicating persistent disturbances of 5-HTergic activity in these subjects.[20] Heavy drinkers also show bunted prolactin responses to fenfluramine challenge compared to healthy controls, suggesting impaired central 5-HTergic neurotransmission in heavy drinkers.[360] During withdrawal from chronic cocaine use, hormone responses to some specific 5-HT challenges are altered (for review see Baumann and Rothman,[283] Levy *et al*.[280]). Furthermore, healthy 5-HTTLPR l/l individuals show significantly greater prolactin responses than s/s individuals after challenge with the tricyclic antidepressant clomipramine[361] or the SSRI citalopram.[362] Thus, it appears that heavy drinking, withdrawal from cocaine use and 5-HTTLPR genotype are associated with altered hormonal responses to 5-HT challenges, which may indicate SV.

Depletion/ATD

Serotonin is synthesized from its amino acid precursor tryptophan (TRP), which is taken up from the blood. Most of the TRP in plasma is protein-bound, with only 5% being left free and available for transport into the CNS. This free TRP is transported into the brain across the blood–brain barrier by an active transport system for which TRP competes with five other large neutral amino acids (LNAAs; valine, leucine, isoleucine, phenylalanine, tyrosine).[363] Once in the brain, TRP is converted into 5-HT. The availability of free plasma TRP is a limiting factor in the synthesis of serotonin. Therefore, ATD can be used to study the effects of lower 5-HT. In humans, an amino-acid based mixture is used for ATD, whereas in rats a protein-mixture is used.[364] ATD lowers plasma TRP[365] and alters 5-HT synthesis, metabolism, and release.[366] Brain 5-HT levels are temporarily lowered[367,368] and there is a decrease in brain 5-HT synthesis,[109] CSF 5-HT[369] and 5-HIAA concentrations.[366,370,371] Nishizawa *et al*.[109] studied the effects of ATD on 5-HT synthesis and found that the rates of 5-HT synthesis were reduced by ATD by a factor of about 9.5 in men and a factor of about 40 in women. Apparently, the effect of ATD on 5-HT synthesis is larger in women than in men.

In rats, ATD can cause changes in cognitive functions and depression- and anxiety-like behavior.[372] In humans, ATD has cognitive effects and

can cause mild, transient mood effects. Although ATD causes approximately the same decrease in TRP levels in all subjects, not all subjects experience symptoms like mood lowering following ATD. Mood lowering in response to ATD demonstrates individual vulnerability of the 5-HTergic system.[24,27–30,373]

In currently depressed untreated patients, ATD does not cause any mood-lowering (for review see Maes and Meltzer,[23] Bell *et al.*,[320] Young and Leyton[321]). However, ATD can cause mood changes in subjects with a personal history of mood disorders.[321] Remitted depressed patients can experience lowered mood after ATD, especially when they had more than one depressive episode[24] and/or were treated with SSRIs or MAOIs.[321,374] Duration of treatment and/or remission may also play a role (for review see Bell *et al.*,[320] Van der Does[374]). Patients with a history of self-injury or suicidal activity are also more likely to report mood-lowering effects after ATD (for review see Booij *et al.*,[24] Young *et al.*,[321] Van der Does[374]).

Of the healthy subjects, some report mood lowering after ATD, while others do not. Female gender[375,376] and a positive family history for affective illness[16,377] are associated with mood effects on ATD. Higher baseline depression score also increase chances of ATD-induced mood lowering (for review see Bell *et al.*,[320] Van der Does[374]). MDMA use may also influence the effects of ATD, as Taffe *et al.*[378] demonstrated that monkeys exposed to a short-course, high-dose repeated regimen of MDMA showed no observable effects of the MDMA under unchallenged conditions 1 year later, but did show alterations in electrophysiological and behavioral sensitivity to ATD.

The extent of TRP depletion is not consistent across studies, which may explain some mixed results and negative findings. There is evidence to indicate that a threshold exists that needs to be exceeded before behavioral effects occur.[379,380] Van der Does[379] reviewed several ATD studies and suggests that the threshold for possible mood effects to occur lies somewhere around a 60% reduction of free plasma TRP. In a study comparing high and low dose ATD in remitted depressed patients, Booij *et al.*[381] reported impaired processing of positive information in all and return of depressive symptoms in some of the participants in the high depletion group (80–90% depletion of plasma TRP levels). Participants in the low depletion group (40–50% depletion) did not show effects on mood or impaired processing of positive information.

Proposed model and conclusions

There appear to be various factors that can disrupt 5-HT functioning in any person. The factors that may cause SV have one common final result: a change in 5-HTergic activity, mostly reduced 5-HT indices or reduced 5-HTergic activity. The proposed model is one in which each factor results in some disturbance in the 5-HTergic system, making the system more vulnerable. Depression is hypothesized to result from the combination of several factors in one person. As long as the number of vulnerability factors is limited, the disturbances in the 5-HTergic system can be compensated for and there are no overt signs to indicate the vulnerability. However, when several vulnerability factors occur in one individual, a threshold is reached where the system can no longer compensate and depression or other 5-HT related disorders occur. This may be similar to the threshold hypothesis stated by Van der Does,[379] which claims that a certain level of TRP depletion is required in order for mood changes to occur after ATD. Some 5-HT challenge and ATD studies appear to confirm the model and the threshold hypothesis. Both models state that the 5-HT system requires a significant amount of challenging for symptoms of depression or other 5-HT related disorders to occur.

This model may be able to provide an explanation for the issue why one person becomes depressed after, for example, a stressful life event, while other individuals may appear unaffected. In individuals with an existing SV-probably caused by genetics and/or early life events – such a stressor may cause depression, but in subjects without this pre-existing vulnerability the stressor will not cause depression. However, this stressor may influence 5-HTergic functioning in this person, resulting in increased vulnerability to develop depression in reaction to further adversity. In other words, a stressor may cause depression in one subject, and SV in another. In this way, the stressor can have very different effects in different individuals, depending on their 5-HTergic functioning when encountering the stressor.

The etiology of both depression and SV are complex, and both can be caused by various factors. In depressed patients, it is often difficult to pinpoint the possible causes of the depression. Although the same is true for SV, the main advantage of the concept of SV is that SV can be demonstrated in individuals before behavioral and emotional symptoms occur. The amount of adversity or compromising of the 5-HT system that is needed to make the system vulnerable probably varies per individual, but with 5-HT manipulations vulnerable subjects can be identified at a point in time where disorders may be prevented. 5-HT manipulation studies can indicate whether a person is close to the threshold where the system can no longer compensate for the compromising factors. This is important because SV will not necessarily lead to pathology, symptoms will only occur if adversity continues to affect the 5-HTergic system. Identifying vulnerable subjects before 5-HT related pathology occurs provides the opportunity to take preventive action. In these vulnerable subjects, pathology may be prevented by improving coping skills or by increasing TRP intake in times of stress.[358]

A mood response to ATD appears to be the best way to identify subjects with SV, because this mood response indicates that the 5-HTergic system lacks the ability to compensate for the transient decrease in

TRP and 5-HT. ATD has already been described as a way to identify a subgroup of patients with a specific 5-HTergic 'vulnerability'[30] and as a suitable model of vulnerability to depression.[382] ATD causes mood lowering in some populations, but results are generally mixed, and ATD does not cause mood lowering in currently depressed patients. These findings suggest that decreased 5-HTergic activity is not the limiting factor in the severity of depression in untreated major depression.[23] Also, it is not the primary or sole cause of affective disorders.[320] This is consistent with the hypothesis of diminished 5-HTergic activity as a vulnerability factor in depression.[23]

It may also be important to identify SV patients, because if they do become depressed, 5-HT being a causal factor may have consequences for treatment outcome. The s-allele of the 5-HTTLPR, the described 5-HT_{1A} polymorphism and TPH2 are all associated with lower responsiveness to SSRI treatment. Although it may appear logical to use treatment targeting the 5-HT system when 5-HT is a causal factor in the pathology, this may not work because in these patients the 5-HT system is not responsive and flexible enough to react to the treatment and benefit from it.

Limitations of the model

It is important to keep in mind that depression is a complex and heterogeneous disorder, both on phenotypical and biological levels. Depression is not likely to result from a single gene or a single external event, but is caused by the complex interactions between an individual and its environment. The specific symptoms of depression, both the subjective feelings and complaints and the physiological indices, are different in different patients. Obviously, this complicates research, because it may not always be valid to compare one group of depressed patients with another.

It is only logical to assume that this model does not explain all cases of depression. It is not likely that all depressed patients share this etiology of factors causing SV and later developing depression. It is known that other neurotransmitters are involved in depression as well, and these may be causal in depression, either on their own or in combination with 5-HT. Apart from SV, there are other paths leading to depression.

Another limitation is that although SV can be demonstrated before depression occurs, it is not possible to determine whether depressed patients had SV before their depression occurred. In depressed patients, ATD does not cause further mood lowering[320] and if mood changes are reported in remitted patients after ATD, this may indicate an acquired vulnerability of the system as a result of the depression instead of representing a causal mechanism. In other words, the changes in 5-HTergic functioning may be secondary.

Furthermore, it cannot be excluded that there are vulnerable subjects that do not show vulnerable responses to challenges. For example, if a person does not report mood lowering after ATD, this may not necessarily mean that this person has no vulnerability to develop depression. These false negatives cannot be excluded. In addition, the mood response to ATD is an indication of SV at a given moment in time. SV can occur later in life as a result of for example stress or drug use. If the circumstances of an individual change, so may the 5-HTergic functioning of that individual. In short, normal responses to 5-HT challenges do not guarantee that an individual cannot develop a 5-HT-related pathology.

It may be difficult to find evidence against the model in human studies because there are always factors to explain mixed results and negative findings, such as level of 5-HT depletion, heterogeneity of the disorder, or not correcting for possible genetic SV, PS, stressful life events early or later in life or other factor that may influence SV. It should be mentioned though that evidence against the model has been found in an ATD study in Parkinson's disease (PD) patients.[383] This study found no support for SV in PD patients, although these patients generally have reduced 5-HT activity and an increased risk of depression. These results may debilitate the model, although there may be several possible explanations for the negative finding.

Conclusions

In conclusion, there is evidence to indicate that there are various factors that may interfere with 5-HTergic functioning. The proposed model is one in which each factor disrupts the 5-HT system, until a threshold is reached where the system can no longer compensate and pathology occurs. In this way, a combination of the factors described may result in depression or other 5-HT related disorders. The etiology of depression and SV are complex, but the concept of SV offers an opportunity to identify individuals that are vulnerable to developing depression and/or other 5-HT related disorders. This may enable preventive actions in these individuals and may give an indication of what treatment to choose if these people do become depressed. However, it is unlikely that this model applies to all cases of depression, and 5-HT challenges may not be able to identify all vulnerable subjects.

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