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Posttraumatic Stress Disorder: Does the Gut Microbiome Hold the Key?

Trouble de stress post-traumatique : le microbiome intestinal est-il la clé?

Sophie Leclercq^{1,2}, Paul Forsythe^{1,3}, and John Bienenstock^{1,2}

Abstract

Gut bacteria strongly influence our metabolic, endocrine, immune, and both peripheral and central nervous systems. Microbiota do this directly and indirectly through their components, shed and secreted, ranging from fermented and digested dietary and host products to functionally active neurotransmitters including serotonin, dopamine, and γ -aminobutyric acid. Depression has been associated with enhanced levels of proinflammatory biomarkers and abnormal responses to stress. Posttraumatic stress disorder (PTSD) appears to be marked in addition by low cortisol responses, and these factors seem to predict and predispose individuals to develop PTSD after a traumatic event. Dysregulation of the immune system and of the hypothalamic-pituitary-adrenal axis observed in PTSD may reflect prior trauma exposure, especially early in life. Early life, including the prenatal period, is a critical time in rodents, and may well be for humans, for the functional and structural development of the immune and nervous systems. These, in turn, are likely shaped and programmed by gut and possibly other bacteria. Recent experimental and clinical data converge on the hypothesis that imbalanced gut microbiota in early life may have long-lasting immune and other physiologic effects that make individuals more susceptible to develop PTSD after a traumatic event and contribute to the disorder. This suggests that it may be possible to target abnormalities in these systems by manipulation of certain gut bacterial communities directly through supplementation or indirectly by dietary and other novel approaches.

Abrégé

Le microbiote intestinal est à présent reconnu pour sa capacité à influencer les réactions métaboliques, endocriniennes, immunitaires et nerveuses. Les bactéries présentes dans notre intestin agissent de manière directe ou indirecte via la libération de différentes substances, tels que des composés provenant de la digestion et de la fermentation de nutriments, des composés provenant de l'hôte, voire même des neurotransmetteurs actifs dont la sérotonine, la dopamine et le GABA. La dépression est associée à une augmentation des taux de bio-marqueurs pro-inflammatoires et à une réponse anormale au stress. Les patients atteints du trouble de stress post-traumatique (PTSD) présentent des taux élevés de marqueurs pro-inflammatoires ainsi que de faibles taux de cortisol, et ces facteurs semblent prédisposer les individus à développer un PTSD après un événement traumatique. Ce dérèglement du système immunitaire et de l'axe hypothalamus-hypophyse-surrénale pourrait aussi refléter un trauma antérieur, et plus particulièrement au début de la vie. Le début de vie, incluant la période prénatale, est une période critique chez les rongeurs pour le développement structurel et fonctionnel des systèmes immunitaires et nerveux, et pourrait l'être également chez les humains. Le développement de ces systèmes biologiques semble être sous le contrôle de l'intestin et de ses bactéries. De récentes données expérimentales et cliniques nous permettent d'émettre l'hypothèse selon laquelle une perturbation de la composition du microbiote intestinal en

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début de vie pourrait avoir des conséquences négatives à long terme sur la réponse immunitaire, ce qui rendrait les individus plus sensibles au développement du PTSD après un événement traumatique. Cette revue propose de nouvelles pistes thérapeutiques qui cibleraient le microbiote intestinal, via une supplémentation en certains types bactériens bénéfiques pour la santé, ou via d'autres approches nutritionnelles connues pour modifier favorablement la composition de l'écologie intestinale, dans le traitement du PTSD.

Keywords

posttraumatic stress disorders, gut microbiota, early life, immune system, gut barrier, cortisol

Highlights

- Enhanced levels of proinflammatory cytokines and low cortisol predispose individuals to develop post-traumatic stress disorder after a traumatic event. Childhood trauma contributes to a proinflammatory state and low cortisol in adulthood.
- Stress is a major factor known to alter the gut microbiota and the gut barrier function.
- Early life is a vulnerable period during which the gut microbiome shapes the host immune homeostasis and the nervous system.
- Stress-induced alteration of the gut microbiota early in life may elicit long-lasting immune consequences and increase the risk of developing stress-related disorders later in life. This article offers novel therapeutic approaches via manipulation of the microbiota to improve symptomatology.

In this article, we review recent experimental and clinical evidence that highlights the possible major roles of the immune system, hypothalamic-pituitary-adrenal (HPA) axis, and the overlooked role of the gut microbiota in the development of psychiatric disorders, with special emphasis on posttraumatic stress disorder (PTSD). It has been suggested that the earliest contact with pioneer bacteria probably determine, shape, and even program metabolic, endocrine, immune, and nervous functions.¹⁻⁷ These crucial interactions are likely to be bidirectional with major consequences for short- and long-term health.

While this review focuses on mostly afferent (gut to brain) potential beneficial effects of gut microbiota, it is important to emphasize that brain-gut microbiota pathways may also have significant physiologic and even deleterious effects on health. Lyte et al⁸ emphasized that stress may change the gut microbiome and even promote the activation of virulence genes in *Escherichia coli* and *Pseudomonas*. Another aspect of brain-gut pathways not covered here but that may have relevance to the subject is the controversial topic of the efferent cholinergic anti-inflammatory response.^{9,10} On both topics, the reader is referred elsewhere for further coverage.

The technical advances afforded by molecular biology have uncovered the staggering complexity of the communities of microorganisms that share our bodies. Trillions of bacteria are ever present in our guts, and although we can identify them by their molecular signatures, only a fraction

(<30%) has been cultured to date, rendering cause-and-effect relationships to disordered health lacking in evidence and unconvincing. Nevertheless, the body of evidence that is emerging in work with rodents, and beginning to emerge clinically, is hard to ignore.

It is clear that massive, terrifying, and/or chronic trauma may be associated with subsequent development of PTSD and that such individuals have higher risk of developing another mental health problem including depression, anxiety, alcohol and/or drug abuse, and suicidal thoughts. However, only a certain percentage of people so exposed present subsequent signs of psychopathology. The factors that underlie susceptibility or resilience to stress are still not clear, but biological markers of these traits are beginning to be elucidated.

Specific Brain Biomarkers Associated with Susceptibility to Stress

Using the “social defeat” paradigm, a model of PTSD and depression, genetically identical mice can be split into 2 groups according to their biological and behavioural responses to chronic stress.¹¹ “Susceptible” mice showed symptoms typically associated with PTSD such as social avoidance, depression-like behaviour (e.g., anhedonia, weight loss), and increase in addictive drug reward. The “resilient” mice were resistant to these behavioural changes. Increased brain-derived neurotrophic factor (BDNF) levels (a key regulator of the mesolimbic dopamine pathway) and signalling in the nucleus accumbens induced the susceptibility to social defeat stress. Moreover, a postmortem brain analysis in depressed patients revealed that the protein expression of BDNF was increased in the nucleus accumbens compared with nondepressed subjects.¹¹

Another recent study highlighted the potential role of the γ -aminobutyric acid (GABA)_{B(1)} receptor in mediating social avoidance and depressive-like behaviour following social defeat.¹² Mice lacking GABA_{B(1a)} receptors were susceptible whereas mice lacking GABA_{B(1b)} receptor were resilient to chronic stress-induced behavioural abnormalities.

These studies therefore suggest that the effects of chronic stress are associated with changes in the expression of genes involved in certain neurotrophic activity and neurotransmission.

Dysregulation of the Immune System and HPA Axis Are Involved in the Psychopathology of Stress-Related Disorders

Numerous clinical studies have found positive correlations between the levels of proinflammatory cytokines, particularly interleukin (IL)-6, tumor necrosis factor- α , and the acute phase reactant C-reactive protein (CRP) and depression.¹³⁻¹⁵ Although such correlations do not imply a cause-effect relationship, several recent experimental and clinical studies have highlighted the predictive value of inflammation in the development of psychological symptoms, suggesting that the proinflammatory immune response precedes the onset of depression.

Using the social defeat paradigm, Hodes et al¹⁶ showed that, compared with resilient mice, susceptible mice that later developed a depressive-like phenotype had increased leucocyte counts before stress exposure. Furthermore, these immune cells released higher amounts of IL-6 in response to acute stress or when stimulated *ex vivo* with lipopolysaccharide (LPS), suggesting that dysregulated immune physiology and higher IL-6 levels are risk factors for stress susceptibility. These results are in line with those found in a prospective clinical study performed in more than 1800 war zone-deployed Marines and Navy combatants.¹⁷ Plasma CRP levels before deployment were significantly associated with postdeployment PTSD symptom emergence, suggesting that levels may be prospectively associated with resilience versus risk for PTSD. Indeed, data obtained in Bosnian war PTSD sufferers identified higher IL-6 levels released by LPS-stimulated blood cells compared with control subjects.¹⁸

Social and psychological stresses activate the HPA axis, which results in increased corticotropin-releasing hormone (CRH) levels in the cerebrospinal fluid. PTSD is hypothesized to reflect a sustained stress response. However, although patients have high levels of CRH, they have unexpectedly low levels of cortisol compared with healthy subjects, suggesting dysregulation of the negative feedback system of the HPA axis.¹⁹ Disruption of the HPA axis appears to be sustained as low cortisol levels persist decades after the initiating traumatic event.¹⁹ Ehring and McFarlane have shown that an initially low cortisol concentration immediately after the trauma predicts subsequent PTSD diagnosis,^{20,21} and prior trauma is a well-described risk factor that predisposes for later PTSD development. Emerging research findings also indicate that low cortisol levels shortly after the traumatic event may reflect a previous earlier trauma and increase the risk of developing PTSD later in life.²² A recent meta-analysis has confirmed that childhood trauma contributes to a proinflammatory state in adulthood.²³

Although the mechanisms of action are unclear, cognitive-behavioural therapy (CBT) has been shown to be effective in reducing depression for treatment-resistant patients.²⁴ Kéri et al²⁵ showed that CBT reduced the initially

elevated expression of TLR4 and NF κ B in peripheral blood mononuclear cells of depressed patients. Greater reduction of proinflammatory markers during CBT was associated with a more pronounced clinical improvement.

Although it is established that inflammation is associated with depression²⁶⁻²⁸ and, more recently, with stress-related disorders, the origin of inflammation in patients with psychiatric disorders remains poorly determined. One factor could be disordered gut barrier function since its impairment may allow translocation to the internal milieu²⁹ of bacteria and their components such as LPS and peptidoglycans, which induce the synthesis of proinflammatory cytokines. Furthermore, a focus on the gut is even more appropriate since specific families of gut bacteria and their products are largely responsible for the generation and maintenance of counterinflammatory cellular systems.^{30,31} Stress is also a major factor known to increase the permeability of the gut barrier,^{32,33} and depression as well as alcohol dependence are psychiatric conditions associated with a "leaky gut."^{34,35} Thus, a major candidate source of systemic inflammation in stress and (or) depression could well be the result of disordered gut barrier function.

Not surprisingly, in view of the correlation of inflammatory changes, impaired gut barrier function, and depression, there have been attempts to determine if anti-inflammatory treatment might be therapeutic. A double-blind, randomized, placebo-controlled clinical trial showed that addition of a COX-2 inhibitor to standard antidepressant therapy induced a greater improvement of depressive symptoms than antidepressant alone.³⁶ However, the trial consisted of a small number of patients and has yet to be replicated in a large cohort. Regarding the effects of antidepressants on systemic inflammation, the results are highly controversial: studies have demonstrated that antidepressants can both decrease or increase inflammatory cytokines.³⁷ Another important and neglected area for investigation is the possibility that many of the drugs that seem to be psychoeffective might be having their effects through action on the gut microbiome.³⁸

Since recovery from depression is associated, in some but not all studies, with reduced and normalized biomarkers of inflammation,³⁹ greater attention to restoration of impaired barrier gut function in psychiatric diseases in general is warranted.

The Gut Microbiota: A Novel Actor Mediating Behavioural Changes

Numerous experiments in the past few years, especially those using germ-free (GF) mice have confirmed the gut microbiome as a major new player in the development, structure, and function of both the enteric and central nervous systems^{40,41} as well as the maintenance and integrity of the blood-brain barrier.⁴² Recently, experimental and clinical data have suggested the important role of intestinal bacteria in mediating changes in brain function and behaviour such as depression, anxiety, and cognition.¹ For instance, GF mice

exhibit reduced anxiety-like behaviour^{40,43} and impaired working memory⁴⁴ compared with normal conventionally raised mice. Bercik et al,⁴⁵ in a landmark study, showed that the behavioural traits of a more anxiety-like phenotype could be effectively adoptively transferred to mice that exhibited a less anxious phenotype by colonization with their donor gut bacteria. Behaviour was therefore correlated with a specific gut microbial community. Other studies have shown that the administration to mice of specific strains of beneficial bacteria such as *Lactobacillus rhamnosus* or *Bifidobacterium longum* can ameliorate anxiety- and depressive-like behaviours.^{46,47} Changes in behaviour induced by modification of the gut microbiota were accompanied by changes in brain neurochemistry including changes in BDNF and N-methyl-D-aspartate receptor expression.^{43,45} However, how gut bacteria can communicate with the brain to affect the behaviour is highly complex and involves several metabolic, neural, and immune pathways that are summarized hereafter.

Intestinal microbiota have a huge metabolic activity. Colonic bacteria ferment and digest host-derived and dietary components such as carbohydrates, proteins, and lipids and convert them into various metabolites that can be either beneficial or harmful for health.⁴⁸ For instance, fermentation of carbohydrates leads to production of short-chain fatty acids, mainly acetate, propionate, and butyrate, which can have beneficial impact for health. They provide energy for colonocytes, improve ion absorption, have anti-inflammatory properties, and regulate serotonin production and enterochromaffin cell numbers.⁴⁹⁻⁵¹ Furthermore butyrate, an effective histone deacetylase inhibitor, had greater antidepressant-like effects than fluoxetine in a mouse model.⁵² Conversely, digestion of proteins results in the production of potentially toxic metabolites such as phenolic and sulphur-containing compounds.⁵³ Intestinal epithelial cells exposed to phenol show, in a dose-dependent manner, an increase in paracellular permeability due to delocalization of the intercellular tight junctions.^{54,55}

Also, molecules with neuroactive properties, such as GABA, serotonin, and dopamine, can also be produced by commensal bacteria.⁵⁶ These secreted neurotransmitters, which are not transported across the blood-brain barrier, may stimulate intestinal epithelial cells to release molecules that in turn modulate neural signalling to influence brain functions and behaviour.¹ In addition to the products secreted by the bacteria, it has been shown that membrane components of bacteria such as capsular polysaccharide A from *Bacteroides fragilis* as well as membrane vesicles budded from the cell surface of *B. fragilis* or *L. rhamnosus JB-1* can have profound anti-inflammatory and neuronal effects that recapitulate effects of parent bacteria.^{57,58}

The tryptophan/kynurenine pathway could also be involved in gut-brain interactions. The essential amino acid tryptophan is the precursor of serotonin, and serum levels depend on the presence or absence of gut bacteria.⁵⁹

Tryptophan is also converted into kynurenine, a very potent immunoregulatory component, by indoleamine dioxygenase and subsequently into other metabolites, such as kynurenic acid and quinolinic acid, each of which has potent neurotoxic or neuroprotective properties. To complicate matters even more, the production of systemic serotonin is dependent on the presence of gut bacteria,⁵¹ and consumption of a probiotic (*Bifidobacterium infantis*) altered the tryptophan/kynurenine pathway and was associated with antidepressant effects in a rodent study.⁶⁰

Another important mediator in the gut-brain communication is the vagus nerve, as shown in vagotomy mouse model studies,^{46,47} in which its section interrupts the behavioural effects of certain orally administered probiotics. The vagus is the major nerve of the parasympathetic system, which conveys information from internal organs to the brain. How intestinal bacteria activate the vagal afferents has been poorly studied, but good evidence exists that this activation is not direct but occurs through intrinsic primary afferent neurons (IPANs) located within the gut wall.⁶¹ IPANs are thought to be the first responders to luminal stimuli such as bacteria, their metabolites, or neuroactive substances released by intestinal epithelial cells, and then to relay the signal to the vagus. Indeed, commensal bacteria produce metabolites that signal to the colonic enterochromaffin cells, which in turn produce serotonin basolaterally that then stimulates the enteric nervous system.⁵¹ The important role of the vagus is emphasized by the fact that electrical vagal stimulation has been authorized by the US Food and Drug Administration for the treatment of drug-resistant depression and intractable epilepsy.⁶²⁻⁶⁴

Finally, the HPA axis has also been demonstrated to be part of the gut-brain axis. Sudo et al⁶⁵ have shown that exposure to stress induced an exaggerated activation of the HPA axis in GF mice, which was fully reversed by reconstitution with the probiotic *B. infantis* early in life.

An important clinical study investigated the gut microbiome in a well-characterized group of alcohol-dependent subjects admitted to hospital for withdrawal treatment.⁶⁶ A major subgroup presented with increased intestinal permeability and strong alterations of specific gut microbiota composition, whereas the remainder showed gut permeability and microbiota similar to those of healthy controls. The dysbiosis was characterized by decreased levels in the anti-inflammatory bacteria *Faecalibacterium prausnitzii* and *Bifidobacterium*, higher plasma levels of the leukocyte chemotactic factor IL-8, and increased scores of depression, anxiety, and alcohol craving. This study also suggested that specific metabolites produced by the bacteria, such as phenol and indole compounds, could affect the intestinal barrier integrity.

Others have reported alteration of gut microbiota in patients with major depressive disorders including increased levels of *Alistipes* and *Oscillibacter*,^{67,68} reduced levels of *Faecalibacterium*,⁶⁷ as well as increased fecal levels in a bacterial product, isovaleric acid.⁶⁹ Taken together, these

recent clinical data suggest that the anxious and depressive symptomatology, commonly found in PTSD patients, may well be associated with alterations of the gut microbiota composition and functionality. The data further open the possibility that through dietary and (or) beneficial bacteria supplementation, it may eventually be possible to affect disordered behaviours.

Although stress is known to induce gut barrier alterations,³² the effect of social stress on gut microbiota remains largely unknown. One study showed that mice exposed to a social stressor exhibited decreased bacterial diversity and richness associated with increased levels of circulating proinflammatory cytokines.⁷⁰ It remains to be determined if in the social defeat paradigm stress resilience and susceptibility are associated with different patterns of gut microbiota. Correspondingly, whether there is an association between gut microbiota composition and severity of symptoms in PTSD sufferers deserves to be explored.

In conclusion, the balance of communities of commensal bacteria seems to play an important role in the regulation of the gut barrier function as well as the immune and nervous systems, which in turn can affect brain function and behaviour.

Lasting Consequences of Early-Life Stress

The acquisition of microbes occurs at birth, although even this dogma has been recently questioned since the intrauterine environment may not be sterile.⁷¹⁻⁷³ Indeed, orally administered *Enterococcus fecium* to pregnant mice were detected in the amniotic fluid and meconium of pups immediately after birth by caesarean delivery.⁷³ Aagaard et al⁷⁴ showed that the human placenta harbors a nonpathogenic and metabolically rich microbiome. How and if the gut microbes routinely access the foetus remains unknown, although bacterial translocation from the maternal gut to bloodstream and thence to the amniotic fluid, which is constantly swallowed by the foetus, is one possible explanation.⁷⁵

Many rodent physiologic systems are programmed by the time of weaning (i.e., the same time that their increased gut permeability is restored to normal adult function). The adult microbiome gut profile is also thought to be achieved at weaning, and attempts to change this with antibiotics only result in a tendency to revert to the characteristic profile normally seen in adults.^{76,77} Although it has not been established that an equivalent to weaning in rodents occurs at all in humans, it is generally accepted that the human gut achieves an adult and characteristic individual profile after about 1 to 3 years of life.⁷⁸⁻⁸⁰ Bäckhed et al⁸¹ showed that cessation of breast-feeding is required for maturation into adult-like microbiome.

Experimentally, stress in early life is associated with alterations of the bacterial colonization of the gut in infants^{82,83} and induces long-term effects in adulthood both immunologically and from the point of view of abnormal behaviour.^{2,84} Maternal separation of pups during the

neonatal period is used in rodents to mimic early-life stress and is associated with enhanced activity of the HPA axis⁸⁵ as well as increased colonic permeability in pups due to elevated CRH. Barreau et al⁸⁶ showed that rats submitted to maternal separation exhibited long-term alteration of gut physiology in adulthood: increased colonic permeability, bacterial translocation, and intestinal inflammation. Also, Sudo et al⁶⁵ were the first to show that colonizing GF mice early in life with a single bacterial species (*B. infantis*) was effective in normalizing the HPA axis response to stress in adulthood.

Bacterial colonization of GF mice early in life promoted normalized behaviour, whereas colonization in adult GF mice failed to do this.^{40,87} Examination of the contribution of microbiota in early life to the development of social behaviour in GF mice revealed increased social avoidance and reduced preference for social novelty in adulthood.⁸⁸ Post-weaning bacterial colonization normalized the social avoidance but failed to reverse the reduced novel social interactions, suggesting that in rodents, only certain behaviours are programmed early in life through microbial colonization.

The extent, if any, to which early life represents a critical window in humans is not known and is viewed with scepticism, but the timing of an insult must be taken into account when analyzing its potential for long-term effects. As mentioned in the previous section, the positive relationship between inflammatory markers and depressive symptoms has been found in numerous studies, which were mostly performed in high-income and industrialized countries. Compared with North American populations, the plasma levels of CRP and IL-6 are exceptionally low in young adults living in low-income countries, despite higher burdens of infectious diseases.⁸⁹ This cannot be explained by lower levels of obesity/overweight or by genetic differences. Rather, converging lines of evidence point to the potential importance of early-life environmental factors in shaping the inflammatory phenotype. The microbiota hypothesis indeed suggests that the higher microbial exposure in infancy may explain the lower level of proinflammatory markers and the subsequent absence of correlation between inflammation and depression in adulthood among low-income nations.^{90,91} On the other hand, diminished exposure to microbes in the perinatal period (as in Western societies) may affect the gut microbiota, enhance the inflammatory response, and increase the risk of developing psychiatric conditions later in life when stress occurs.⁹¹ Also, epidemiologic studies reveal that stressful events in childhood can undoubtedly influence the subsequent onset of psychiatric disorders such as depression and anxiety.⁹²⁻⁹⁴ The underlying mechanisms are still unclear, but studies reported that early-life stress leads to a proinflammatory phenotype as well as alteration of hippocampal neurogenesis that could have long-lasting negative effects.^{95,96}

Further studies addressing more physiologically relevant alterations in the early-life gut microbiota such as

psychological stress^{70,82,97} or the use of antibiotics are required. Regarding stress, only one human study⁹⁸ reported that maternal stress during pregnancy was associated with a disturbed pattern of infant gut microbiota demonstrating decreased levels of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* and increased levels of potential pathogens *Escherichia* and *Enterobacter*. This aberrant colonization pattern was related to more gastrointestinal symptoms and allergy in infants. The assessment of the psychological symptoms of these infants later in life would be interesting to make a clinical link between early-life stress and long-term behaviour. Furthermore, associations have now been recorded between the use of antibiotics in early life and the occurrence of diseases in adulthood—mainly allergic asthma^{99,100} and obesity.^{101,102} There is strong evidence that immune and metabolic alterations associated with early-life antibiotic treatment in mice could be driven by alterations of the gut microbiota.^{103,104} Overall, these recent data also suggest that early life represents a critical window during which alteration of the gut microbiota may have long-lasting consequences in term of immune, metabolic, and behavioural responses. More data in clinically relevant animal models and human psychiatric conditions are urgently needed.

New Targets in the Treatment of PTSD

Finally, we think that targeting the gut microbiota, the potential key modulator of the immune and nervous systems, could lead to a greater improvement in the emotional symptoms of patients suffering from depression or anxiety. The composition and the function of the bacterial community inhabiting the gut can be improved through dietary interventions or the use of beneficial bacteria such as probiotics. In this respect, clinical trials have largely been inadequate in design or in numbers of subjects involved. However, those that have been conducted have shown that the administration of different species of *Lactobacillus* and *Bifidobacterium* were associated with an improvement in mood,¹⁰⁵ a decrease in anxiety,¹⁰⁶ and a decrease in psychological distress,¹⁰⁷ particularly in subjects with low cortisol levels.¹⁰⁸ Also, administration of fermented milk products containing probiotics was shown to affect the activity of brain regions that control central processing of emotions in women.¹⁰⁹ However, all of these studies have been performed in healthy volunteers. There is an urgent need for well-designed, double-blind, placebo-controlled clinical trials aimed at determining the effect of bacterial supplements and controlled changes in diet on psychological symptoms and cognitive functions in patients with well-documented mental health problems. This particularly applies to PTSD patients characterized by long-lasting low cortisol levels.

Conclusion

Accumulating data suggest that enhanced peripheral levels of proinflammatory cytokines and low cortisol predispose

individuals to develop PTSD after a traumatic event.^{16,17,20,21} Childhood trauma contributes to a proinflammatory state and low cortisol in adulthood.²³

There is now evidence, at least in rodents, of the existence of a vulnerable window in early life during which the gut microbiome shapes the host immune homeostasis, particularly gut immunity^{110,111} as well as the nervous system.² The concept of “early life” is not clearly defined in the literature. Recent studies have demonstrated that the intrauterine environment is not sterile,⁷³ as originally thought. Therefore, the prenatal period may also be part of this critical window, as the development of immune and nervous systems may be influenced by microbial exposure or metabolites originating from the maternal microbiota. Therefore, results obtained from GF studies have to be very cautiously transposed to human health and disease.

The microbiota hypothesis⁹¹ postulates that colonization with a “healthy” diverse microbiota community during this specific vulnerable period may decrease susceptibility to diseases later in life, whereas dysbiosis induced by antibiotic treatment or stress in childhood may increase the risk of developing immune, metabolic, and behavioural disorders.

The effectiveness of current antidepressant drug therapies in treating PTSD is limited, and it is interesting that CBT alone may reduce a proinflammatory profile, especially an inflammatory profile linked to leaky gut.²⁵ To our knowledge, the role of the gut microbiota in the development of PTSD has never been investigated. Studies should aim at determining whether childhood trauma is associated with alterations of the gut microbiota and whether dysbiosis early in life could promote the development of PTSD later in life when subjects are reexposed to traumatic events. This would consider the gut microbiota as a new biological factor mediating vulnerability versus resilience to stress and may suggest new targets in the treatment of PTSD.

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References

1. Forsythe P, Kunze WA. Voices from within: gut microbes and the CNS. *Cell Mol Life Sci*. 2012;70(1):55-69.
2. Borre YE, O’Keeffe GW, Clarke G, et al. Microbiota and neurodevelopmental windows: implications for brain disorders. *Trends Mol Med*. 2014;20(9):509-518.
3. Collins SM, Surette M, Bercik P. The interplay between the intestinal microbiota and the brain. *Nat Rev Microbiol*. 2012;10(11):735-742.

4. Mayer EA, Tillisch K, Gupta A. Gut/brain axis and the microbiota. *J Clin Invest*. 2015;125(3):926-938.
5. Rook GAW, Lowry CA, Raison CL. Microbial "old friends", immunoregulation and stress resilience. *Evol Med Public Health*. 2013;2013(1):46-64.
6. Bested AC, Logan AC, Selhub EM. Intestinal microbiota, probiotics and mental health: from Metchnikoff to modern advances: Part I—autointoxication revisited. *Gut Pathog*. 2013;5(1):5.
7. Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci*. 2012;13:701-712.
8. Freestone PPE, Sandrini SM, Haigh RD, et al. Microbial endocrinology: how stress influences susceptibility to infection. *Trends Microbiol*. 2008;16(2):55-64.
9. Olofsson PS, Rosas-Ballina M, Levine YA, et al. Rethinking inflammation: neural circuits in the regulation of immunity. *Immunol Rev*. 2012;248(1):188-204.
10. Martelli D, McKinley MJ, McAllen RM. The cholinergic anti-inflammatory pathway: a critical review. *Auton Neurosci Basic Clin*. 2014;182:65-69.
11. Krishnan V, Han M-H, Graham DL, et al. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell*. 2007;131(2):391-404.
12. O'Leary OF, Felice D, Galimberti S, et al. GABAB(1) receptor subunit isoforms differentially regulate stress resilience. *Proc Natl Acad Sci U S A*. 2014;111(42):15232-15237.
13. Gimeno D, Kivimäki M, Brunner EJ, et al. Associations of C-reactive protein and interleukin-6 with cognitive symptoms of depression: 12-year follow-up of the Whitehall II study. *Psychol Med*. 2009;39(03):413-423.
14. Zalli A, Jovanova O, Hoogendijk WJG, et al. Low-grade inflammation predicts persistence of depressive symptoms. *Psychopharmacology (Berl)*. 2015 Apr 16; Epub ahead of print.
15. Dinan TG. Inflammatory markers in depression. *Curr Opin Psychiatry*. 2009;22(1):32-36.
16. Hodes GE, Pfau ML, Leboeuf M, et al. Individual differences in the peripheral immune system promote resilience versus susceptibility to social stress. *Proc Natl Acad Sci U S A*. 2014;111(45):16136-16141.
17. Eraly SA, Nievergelt CM, Maihofer AX, et al. Assessment of plasma C-reactive protein as a biomarker of posttraumatic stress disorder risk. *JAMA Psychiatry*. 2014;71(4):423.
18. Rohleder N, Joksimovic L, Wolf JM, et al. Hypocortisolism and increased glucocorticoid sensitivity of pro-inflammatory cytokine production in Bosnian war refugees with posttraumatic stress disorder. *Biol Psychiatry*. 2004;55(7):745-751.
19. Yehuda R. Post-traumatic stress disorder. *N Engl J Med*. 2002;346(2):108-114.
20. Ehring T, Ehlers A, Cleare AJ, et al. Do acute psychological and psychobiological responses to trauma predict subsequent symptom severities of PTSD and depression? *Psychiatry Res*. 2008;161(1):67-75.
21. McFarlane AC, Barton CA, Yehuda R, et al. Cortisol response to acute trauma and risk of posttraumatic stress disorder. *Psychoneuroendocrinology*. 2011;36(5):720-727.
22. Pervanidou P. Biology of post-traumatic stress disorder in childhood and adolescence. *J Neuroendocrinol*. 2008;20(5):632-638.
23. Baumeister D, Akhtar R, Ciufolini S, et al. Childhood trauma and adulthood inflammation: a meta-analysis of peripheral C-reactive protein, interleukin-6 and tumour necrosis factor- α . *Mol Psychiatry*. 2015 Jun 2; Epub ahead of print.
24. Wiles N, Thomas L, Abel A, et al. Clinical effectiveness and cost-effectiveness of cognitive behavioural therapy as an adjunct to pharmacotherapy for treatment-resistant depression in primary care: the CoBaT randomised controlled trial. *Health Technol Assess Winch Engl*. 2014;18(31):1-167.
25. Kéri S, Szabó C, Kelemen O. Expression of toll-like receptors in peripheral blood mononuclear cells and response to cognitive-behavioral therapy in major depressive disorder. *Brain Behav Immun*. 2014;40:235-243.
26. Dantzer R, O'Connor JC, Freund GG, et al. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci*. 2008;9:46-56.
27. Maes M. Major depression and activation of the inflammatory response system. *Adv Exp Med Biol*. 1999;461:25-46.
28. Meyer U, Feldon J, Dammann O. Schizophrenia and autism: both shared and disorder-specific pathogenesis via perinatal inflammation? *Pediatr Res*. 2011;69:26R-33R.
29. Duerkop BA, Vaishnav S, Hooper LV. Immune responses to the microbiota at the intestinal mucosal surface. *Immunity*. 2009;31(3):368-376.
30. Rakoff-Nahoum S, Medzhitov R. Role of the innate immune system and host-commensal mutualism. *Curr Top Microbiol Immunol*. 2006;308:1-18.
31. Furusawa Y, Obata Y, Fukuda S, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature*. 2013;504(7480):446-450.
32. Söderholm JD, Perdue MH. Stress and gastrointestinal tract. II. Stress and intestinal barrier function. *Am J Physiol Gastrointest Liver Physiol*. 2001;280(1):G7-13.
33. Saunders PR, Santos J, Hanssen NPM, et al. Physical and psychological stress in rats enhances colonic epithelial permeability via peripheral CRH. *Dig Dis Sci*. 2002;47(1):208-215.
34. Leclercq S, Cani PD, Neyrinck AM, et al. Role of intestinal permeability and inflammation in the biological and behavioral control of alcohol-dependent subjects. *Brain Behav Immun*. 2012;26:911-918.
35. Maes M, Kubera M, Leunis J-C, et al. Increased IgA and IgM responses against gut commensals in chronic depression: further evidence for increased bacterial translocation or leaky gut. *J Affect Disord*. 2012 ;141(1):55-62.
36. Müller N, Schwarz MJ, Dehning S, et al. The cyclooxygenase-2 inhibitor celecoxib has therapeutic effects in major depression: results of a double-blind, randomized, placebo controlled, add-on pilot study to reboxetine. *Mol Psychiatry*. 2006;11(7):680-684.
37. Kenis G, Maes M. Effects of antidepressants on the production of cytokines. *Int J Neuropsychopharmacol*. 2002;5(4):401-412.
38. Davey KJ, O'Mahony SM, Schellekens H, et al. Gender-dependent consequences of chronic olanzapine in the rat: effects

- on body weight, inflammatory, metabolic and microbiota parameters. *Psychopharmacology* (Berl). 2012;221(1):155-169.
39. Hannestad J, DellaGioia N, Bloch M. The effect of antidepressant medication treatment on serum levels of inflammatory cytokines: a meta-analysis. *Neuropsychopharmacology*. 2011;36(12):2452-2459.
 40. Heijtz RD, Wang S, Anuar F, et al. Normal gut microbiota modulates brain development and behavior. *Proc Natl Acad Sci U S A*. 2011;108:3047-3052.
 41. Neufeld KAM, Perez-Burgos A, Mao YK, et al. The gut microbiome restores intrinsic and extrinsic nerve function in germ-free mice accompanied by changes in calbindin. *Neurogastroenterol Motil*. 2015;27(5):627-636.
 42. Braniste V, Al-Asmakh M, Kowal C, et al. The gut microbiota influences blood-brain barrier permeability in mice. *Sci Transl Med*. 2014;6(263):263ra158.
 43. Neufeld KM, Kang N, Bienenstock J, et al. Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterol Motil*. 2011;23(3):255-264.
 44. Gareau MG, Wine E, Rodrigues DM, et al. Bacterial infection causes stress-induced memory dysfunction in mice. *Gut*. 2011;60(3):307-317.
 45. Bercik P, Denou E, Collins J, et al. The intestinal microbiota affect central levels of brain-derived neurotrophic factor and behavior in mice. *Gastroenterology*. 2011;141(2):599-609.e3.
 46. Bercik P, Park AJ, Sinclair D, et al. The anxiolytic effect of *Bifidobacterium longum* NCC3001 involves vagal pathways for gut-brain communication. *Neurogastroenterol Motil*. 2011;23(12):1132-1139.
 47. Bravo JA, Forsythe P, Chew MV, et al. Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc Natl Acad Sci U S A*. 2011;108(38):16050-16055.
 48. Hamer HM, De Preter V, Windey K, et al. Functional analysis of colonic bacterial metabolism: relevant to health? *Am J Physiol Gastrointest Liver Physiol*. 2012;302:G1-G9.
 49. Fukumoto S, Tatewaki M, Yamada T, et al. Short-chain fatty acids stimulate colonic transit via intraluminal 5-HT release in rats. *Am J Physiol Regul Integr Comp Physiol*. 2003;284(5):R1269-R1276.
 50. Hamer HM, Jonkers D, Venema K, et al. Review article: the role of butyrate on colonic function. *Aliment Pharmacol Ther*. 2008;27(2):104-119.
 51. Yano JM, Yu K, Donaldson GP, et al. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell*. 2015;161(2):264-276.
 52. Schroeder FA, Lin CL, Crusio WE, et al. Antidepressant-like effects of the histone deacetylase inhibitor, sodium butyrate, in the mouse. *Biol Psychiatry*. 2007;62(1):55-64.
 53. Windey K, De Preter V, Verbeke K. Relevance of protein fermentation to gut health. *Mol Nutr Food Res*. 2012;56:184-196.
 54. Hughes R, Kurth MJ, McGilligan V, et al. Effect of colonic bacterial metabolites on Caco-2 cell paracellular permeability in vitro. *Nutr Cancer*. 2008;60:259-266.
 55. McCall IC, Betanzos A, Weber DA, et al. Effects of phenol on barrier function of a human intestinal epithelial cell line correlate with altered tight junction protein localization. *Toxicol Appl Pharmacol*. 2009;241:61-70.
 56. Lyte M. Microbial endocrinology in the microbiome-gut-brain axis: how bacterial production and utilization of neurochemicals influence behavior. *PLoS Pathog*. 2013;9(11):e1003726.
 57. Al-Nedawi K, Mian MF, Hossain N, et al. Gut commensal microvesicles reproduce parent bacterial signals to host immune and enteric nervous systems. *FASEB J*. 2015;29(2):684-695.
 58. Shen Y, Torchia MLG, Lawson GW, et al. Outer membrane vesicles of a human commensal mediate immune regulation and disease protection. *Cell Host Microbe*. 2012;12(4):509-520.
 59. Wikoff WR, Anfora AT, Liu J, et al. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc Natl Acad Sci U S A*. 2009;106:3698-3703.
 60. Desbonnet L, Garrett L, Clarke G, et al. The probiotic *Bifidobacteria infantis*: an assessment of potential antidepressant properties in the rat. *J Psychiatr Res*. 2008;43(2):164-174.
 61. Perez-Burgos A, Mao Y-K, Bienenstock J, et al. The gut-brain axis rewired: adding a functional vagal nicotinic "sensory synapse." *FASEB J*. 2014;28(7):3064-3074.
 62. Goodnick PJ, Rush AJ, George MS, et al. Vagus nerve stimulation in depression. *Expert Opin Pharmacother*. 2001;2(7):1061-1063.
 63. Cristancho P, Cristancho MA, Baltuch GH, et al. Effectiveness and safety of vagus nerve stimulation for severe treatment-resistant major depression in clinical practice after FDA approval: outcomes at 1 year. *J Clin Psychiatry*. 2011;72(10):1376-1382.
 64. García-Navarrete E, Torres CV, Gallego I, et al. Long-term results of vagal nerve stimulation for adults with medication-resistant epilepsy who have been on unchanged antiepileptic medication. *Seizure*. 2013;22(1):9-13.
 65. Sudo N, Chida Y, Aiba Y, et al. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *J Physiol*. 2004;558(pt 1):263-275.
 66. Leclercq S, Matamoros S, Cani PD, et al. Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. *Proc Natl Acad Sci U S A*. 2014;111(42):E4485-E4493.
 67. Jiang H, Ling Z, Zhang Y, et al. Altered fecal microbiota composition in patients with major depressive disorder. *Brain Behav Immun*. 2015;48:186-194.
 68. Naseribafrouei A, Hestad K, Avershina E, et al. Correlation between the human fecal microbiota and depression. *Neurogastroenterol Motil*. 2014;26(8):1155-1162.
 69. Szczesniak O, Hestad K, Hanssen JF, et al. Isovaleric acid in stool correlates with human depression. *Nutr Neurosci*. 2015 Feb 24; Epub ahead of print.
 70. Bailey MT, Dowd SE, Galley JD, et al. Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. *Brain Behav Immun*. 2011;25(3):397-407.

71. DiGiulio DB, Romero R, Amogan HP, et al. Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: a molecular and culture-based investigation. *PLoS One*. 2008;3(8):e3056.
72. Jiménez E, Fernández L, Marín ML, et al. Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. *Curr Microbiol*. 2005;51(4):270-274.
73. Jiménez E, Marín ML, Martín R, et al. Is meconium from healthy newborns actually sterile? *Res Microbiol*. 2008;159(3):187-193.
74. Aagaard K, Ma J, Antony KM, et al. The placenta harbors a unique microbiome. *Sci Transl Med*. 2014;6(237):237ra65.
75. Funkhouser LJ, Bordenstein SR. Mom knows best: the Universality of Maternal Microbial Transmission. *PLoS Biol*. 2013;11(8):e1001631.
76. Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proc Natl Acad Sci U S A*. 2011;108(suppl 1):4554-4561.
77. Modi SR, Collins JJ, Relman DA. Antibiotics and the gut microbiota. *J Clin Invest*. 2014;124(10):4212-4218.
78. Matamoros S, Gras-Leguen C, Le Vacon F, et al. Development of intestinal microbiota in infants and its impact on health. *Trends Microbiol*. 2013;21(4):167-173.
79. Palmer C, Bik EM, DiGiulio DB, et al. Development of the human infant intestinal microbiota. *PLoS Biol*. 2007;5(7):e177.
80. Yatsunenkov T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. *Nature*. 2012;486(7402):222-227.
81. Bäckhed F, Roswall J, Peng Y, et al. Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host Microbe*. 2015;17(5):690-703.
82. Bailey MT, Lubach GR, Coe CL. Prenatal stress alters bacterial colonization of the gut in infant monkeys. *J Pediatr Gastroenterol Nutr*. 2004;38(4):414-421.
83. O'Mahony SM, Marchesi JR, Scully P, et al. Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. *Biol Psychiatry*. 2009;65(3):263-267.
84. Sánchez MM, Ladd CO, Plotsky PM. Early adverse experience as a developmental risk factor for later psychopathology: evidence from rodent and primate models. *Dev Psychopathol*. 2001;13(3):419-449.
85. Gareau MG, Jury J, MacQueen G, et al. Probiotic treatment of rat pups normalises corticosterone release and ameliorates colonic dysfunction induced by maternal separation. *Gut*. 2007;56(11):1522-1528.
86. Barreau F, Ferrier L, Fioramonti J, et al. Neonatal maternal deprivation triggers long term alterations in colonic epithelial barrier and mucosal immunity in rats. *Gut*. 2004;53(4):501-506.
87. Neufeld KA, Kang N, Bienenstock J, et al. Effects of intestinal microbiota on anxiety-like behavior. *Commun Integr Biol*. 2011;4(4):492-494.
88. Desbonnet L, Clarke G, et al. Microbiota is essential for social development in the mouse. *Mol Psychiatry*. 2014;19(2):146-148.
89. McDade TW, Borja JB, Adair LS, et al. Depressive symptoms are not associated with inflammation in younger and older adults in the Philippines. *Evol Med Public Health*. 2013;2013(1):18-23.
90. McDade TW. Early environments and the ecology of inflammation. *Proc Natl Acad Sci U S A*. 2012;109(suppl 2):17281-17288.
91. Rook GAW, Raison CL, Lowry CA. Microbiota, immunoregulatory old friends and psychiatric disorders. *Adv Exp Med Biol*. 2014;817:319-356.
92. Bailer J, Witthöft M, Wagner H, et al. Childhood maltreatment is associated with depression but not with hypochondriasis in later life. *J Psychosom Res*. 2014;77(2):104-108.
93. Bernet CZ, Stein MB. Relationship of childhood maltreatment to the onset and course of major depression in adulthood. *Depress Anxiety*. 1999;9(4):169-174.
94. Gibb BE, Chelminski I, Zimmerman M. Childhood emotional, physical, and sexual abuse, and diagnoses of depressive and anxiety disorders in adult psychiatric outpatients. *Depress Anxiety*. 2007;24(4):256-263.
95. Cattaneo A, Macchi F, Plazzotta G, et al. Inflammation and neuronal plasticity: a link between childhood trauma and depression pathogenesis. *Front Cell Neurosci*. 2015;9(40):1-12.
96. Mirescu C, Peters JD, Gould E. Early life experience alters response of adult neurogenesis to stress. *Nat Neurosci*. 2004;7(8):841-846.
97. Bailey MT, Coe CL. Maternal separation disrupts the integrity of the intestinal microflora in infant rhesus monkeys. *Dev Psychobiol*. 1999;35(2):146-155.
98. Zijlmans MAC, Korpela K, Riksen-Walraven JM, et al. Maternal prenatal stress is associated with the infant intestinal microbiota. *Psychoneuroendocrinology*. 2015;53:233-245.
99. Kozyskyj AL, Ernst P, Becker AB. Increased risk of childhood asthma from antibiotic use in early life. *Chest*. 2007;131(6):1753-1759.
100. Stensballe LG, Simonsen J, Jensen SM, et al. Use of antibiotics during pregnancy increases the risk of asthma in early childhood. *J Pediatr*. 2013;162(4):832-838.e3.
101. Bailey LC, Forrest CB, Zhang P, et al. Association of antibiotics in infancy with early childhood obesity. *JAMA Pediatr*. 2014;168(11):1063-1069.
102. Murphy R, Stewart AW, Braithwaite I, et al. Antibiotic treatment during infancy and increased body mass index in boys: an international cross-sectional study. *Int J Obes (Lond)*. 2014;38(8):1115-1119.
103. Cox LM, Yamanishi S, Sohn J, et al. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. *Cell*. 2014;158(4):705-721.

104. Russell SL, Gold MJ, Hartmann M, et al. Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma. *EMBO Rep.* 2012;13(5):440-447.
105. Benton D, Williams C, Brown A. Impact of consuming a milk drink containing a probiotic on mood and cognition. *Eur J Clin Nutr.* 2006;61(3):355-361.
106. Rao AV, Bested AC, Beaulne TM, et al. A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. *Gut Pathog.* 2009;1(1):6.
107. Messaoudi M, Lalonde R, Violle N, et al. Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in rats and human subjects. *Br J Nutr.* 2011;105(05):755-764.
108. Messaoudi M, Violle N, Bisson J-F, et al. Beneficial psychological effects of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in healthy human volunteers. *Gut Microbes.* 2011;2(4):256-261.
109. Tillisch K, Labus J, Kilpatrick L, et al. Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology.* 2013;144(7):1394-1401.
110. Lee YK, Mazmanian SK. Has the microbiota played a critical role in the evolution of the adaptive immune system? *Science.* 2010;330(6012):1768-1773.
111. Olszak T, An D, Zeissig S, et al. Microbial exposure during early life has persistent effects on natural killer T cell function. *Science.* 2012;336(6080):489-493.