The Impact of Chronic Psychological Stress on the Gut-Brain Axis: Implications for Immune Function and Gastrointestinal Health

https://atlasbiomed.com/blog/how-stress-impacts-the-gut-via-the-gut-brain-axis/

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Abstract

This literature review investigates the impact of Chronic psychological stress on the gut-brain axis (GBA) which further have important implications on immune function and overall gastrointestinal (GI) health. CPS disrupts the GBA by altering key signaling pathways such as the hypothalamic-pituitary-adrenal (HPA) axis, sympathetic-adrenal-medullary (SAM) axis, and enteric nervous system (ENS). Studies show how HPA dysregulation leads to Glucocorticoid dysregulation with CPS causing either a state of hyper or hypo-activation of the axis. SAM is led to release catecholamine for prolonged periods, therefore negatively affecting motility and contributing to a state of dysbiosis. ENS further adds to these adverse effects and is influenced by the dysregulation of the other two components. This all causes dysregulated neurotransmitter and hormone release leading to systemic inflammation, gut barrier dysfunction and dysbiosis. This state of inflammation is linked to the pathophysiology as well as exasperation of GI disorders, such as Inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). Therefore, this review will also mention a few of the existing potential therapies in order to minimize the adverse effects of CPS as well as highlight the importance of understanding this dysfunction and the need for further research.

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Introduction

Everybody has felt that drop in their stomach, their heartbeat racing and that daunting inability to breathe on their way to give a presentation or the morning before an exam, but why is this our body's usual response if it can hinder performance and makes these situations seem worse than they actually are? This physiological reaction is known as the “fight or flight” response, an evolutionary adaptation to deal with events that are considered potentially threatening, such as public speaking. During these situations the body activates the sympathetic nervous system, which triggers the release of stress hormones such as adrenaline and cortisol. These are what cause the aforementioned physiological reaction we now identify as anxiety. (Hall J.M et al, 2012)

This stress response is also closely linked to other systems in our body, such as the cardiovascular system, the enteric system and the immune system and while these are essential for survival, their involvement during the fight or flight response is not always necessary for coping with modern stressors. In the modern day, stress is an unavoidable phenomenon and has even been considered a global epidemic (Johnson A, 2023), due to the constant demands of contemporary life. However, there is a significant difference between the effects of acute and chronic stress. Chronic stress is a state of persistent psychological stress due to long lasting stressors such as job pressures, relationship conflicts, or financial difficulty in contrast to the nature of acute stress, which is usually short lived. The prolonged activation of the stress response and the constant exposure to elevated levels of stress hormones can lead to a range of problems detrimental to one's health. (Molina-Torres, G et al, 2019; Yang L et al, 2015)

In the midst ofAmid all the bodily systems affected by the stress response, we especially focus on one very crucial system, the gut-brain axis. The gut-brain axis allows for bidirectional communication between the central nervous system (CNS) and the Enteric Nervous System (ENS) and it plays an important role in maintenance as well as digestion, immune response and even mood (Liang S et al, 2018). Since the gut-brain axis is in charge of vital bodily functions and the prevalence of chronic psychological stress, this research aims to address the question: “What are the effects of chronic psychological stress on the gut-brain axis and its impact on immune system functioning and gastrointestinal health?”. This will be possible through answering the following sub questions: (1) How does chronic psychological stress affect the signaling pathways of the gut-brain axis? (2) How does the composition of the gut microbiome change due to chronic stress? (3) What are the consequences of these changes on gut-associated lymphoid tissue (GALT) and overall immune response? (4) How does stress-induced dysregulation of the gut-brain axis contribute to gastrointestinal disorders? (5) What are some effective interventions for mediating the impact of chronic stress on the gut-brain axis?

The purpose of this research is to summarize existing knowledge from scientific studies, present the findings and gain insights into how chronic stress impacts the gut-brain axis, as well as explore potential interventions to minimize unwanted

effects. This is of great importance to understand the role of gut-brain axis and its effects on health and disease, particularly under chronic stress conditions.

Chapter 1: Theoretical Framework and Context

1.1 Introduction

This chapter provides the theoretical framework and context for understanding the gut-brain axis and its components, as well as what is defined as “chronic psychological stress”. This is important to have the basic understanding of each component and to easier understand the detailed examination of stress-induced changes in subsequent chapters.

1.2 The Gut-Brain Axis

The brain communicates with virtually every part of the body through various mechanisms, such as through the nervous and endocrine system (Sivadas, A., & Broadie, K, 2020), so therefore an established connection with the Gastrointestinal (GI) System is unsurprising. The GI system consists of a network of organs that work together to digest food, absorb nutrients and eliminate any leftover waste. It is also considered one of the biggest immune and endocrine organs and contains a nervous system known as the enteric nervous system (ENS) or “the second brain” (Liang S et al, 2018).

The ENS, consisting of more than 100 million neurons, is responsible for peristalsis, secretion of enzymes aiding digestion as well as coordination of blood flow. Its importance also stems from its ability to bidirectionally communicate with the CNS through the vagus nerve and therefore makes up a large part of the gut-brain axis (Furness, J. B, 2012; Grundy, D., & Schemann, M, 2006). The ENS sends sensory information to the brain, such as fullness or pain, and the brain responds by appropriately modulating digestive functions through motor commands.

Even though the ENS is one of the most crucial parts of the gut-brain axis, it is not the only one. The gut-brain axis also encompasses gut microbiota, the immune system, and the two important stress response systems, the HPA (Hypothalamic-Pituitary-Adrenal) and SAM (sympathoadrenal medullary) system (Carabotti, M, 2015). A simple overview of the role and interaction of these parts can be seen in Figure 1. There are many studies and existing theories on each of these elements, which will be analyzed in the upcoming Chapters.

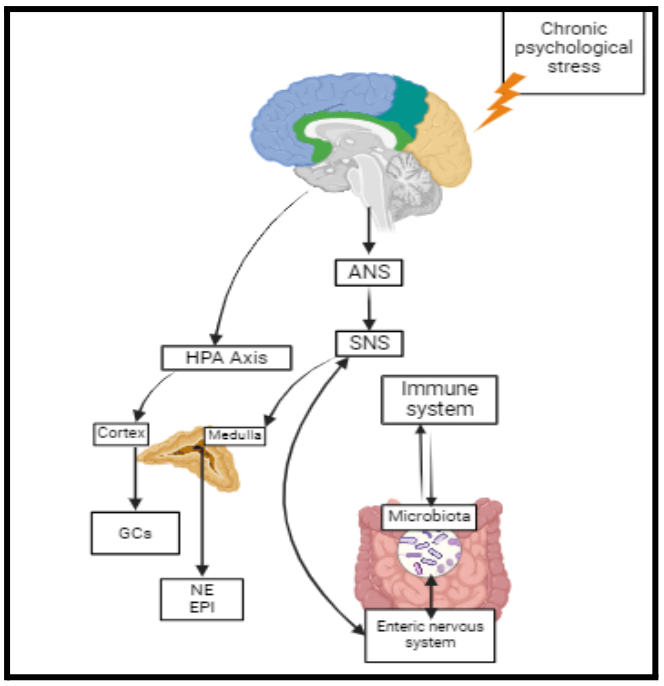

Figure 1: Pathways of the brain-gut axis. Representation of the bidirectional axis and its main connections that are affected by chronic psychological stress. Local systems and their most well-known effectors are visualized (SAM system (sympathoadrenal medullary system): involves the sympathetic nervous system (SNS) and release of epinephrine (EPI) and norepinephrine (NE), HPA axis, hypothalamic-pituitary-adrenal, axis, Glucocorticoids (GCs), as well as the involvement of microbiota (Labanski A et al, 2019; Mawdsley J, 2005)

1.3 Chronic Psychological Stress

To grasp the concept of stress and its impact, it is important to first understand the concept of Homeostasis and Allostasis. Homeostasis is a commonly used concept in biology and refers to the ability of our body to maintain the stability of various physiological systems no matter the external conditions. This means keeping certain factors within a narrow range, such as blood glucose levels, body temperature and pH. This is not the case when faced with a stressor as the initial state of homeostasis no longer aligns with the demands posed by the now altered environment. This is where the concept of allostasis comes into play which compliments homeostasis by also striving for stability but through change.

This means it deviates from the original “set points” to adapt to the new circumstances and stressors known as the “allostatic state”, which ensures that the physiological conditions remain optimal even during varying conditions. In the context of this review, allostasis is the process used by the body to respond to psychological stress and restore homeostasis (Russell, G., & Lightman, S, 2019; McEwen, B. S., & Wingfield, J. C, 2003). During chronic exposure to such stressors, there is a constant activation of these allostatic mechanisms, including the SAM and HPA axes, which leads to what is termed "Allostatic load” a cumulative wear and tear on the body over time. This is what ultimately leads to physiological dysregulation and health problems.

In this research, the stressor we are focusing on is psychological stress. Psychological stress can be categorized into two types: Acute and Chronic. Acute stress is a short term form of stress that results from immediate stressors or during

challenging situations. It triggers the fight or flight response leading to the “known” physical symptoms such as increased heart and breathing rate, mediated by the release of stress hormones like adrenaline and cortisol. This response can be beneficial when faced with such situations, as it can prepare the body to respond accordingly by increasing energy through increase of glucose availability and blood flow to muscles as well as enhance cognitive functions, such as focus and alertness (Rohleder N, 2019). When exposed to such psychological stressors the physiology of the stress response consists of the 2 aforementioned components, the slow response consisting of the activation of the HPA axis by releasing cortisol and a fast response mediated by the SAM axis through the release of epinephrine and norepinephrine

While exposure to acute stress is primarily considered beneficial, chronic stress is more so considered detrimental as it contributes to an increase in allostatic load. In the case of chronic stress, where the stressors are persistent, it leads to the prolonged activation of the HPA and SAM axis and a constant elevated exposure to the relevant stress hormones. This can have detrimental effects for both physiological and psychological aspects. In the context of this research, chronic stress significantly impacts the gut-brain axis through the dysregulation of these two systems. The continuous release of stress hormones affects gut motility, permeability, and the composition of the gut microbiota, leading to various negative consequences. The specific effects of chronic stress on these aspects of the gut-brain axis will be explored in the subsequent chapters.

Chapter 2: Effect of chronic stress on Gut microbiome composition and function

2.1 Introduction

This chapter will elaborate on the impact of Chronic psychological stress on the gut microbiome, focusing on changes in composition and function. This will be achieved through examining current research findings from various animal and human studies.

2.2 Gut microbiome

2.2.1 Baseline Gut microbiome composition

A term that is widely used is “microbiome” which includes all the microorganisms, such as bacteria, archaea, fungi and viruses that are inhabiting the gastrointestinal (GI) tract, collectively also known as the gut-microbiota. This gives rise to the term gut–microbiota–brain axis’ which refers specifically to the network of connections that allows bidirectional communication between gut bacteria and the brain. The relationship between humans and their microbiome is mutually beneficial. While we provide the microbes with a nutrient-rich and stable environment, they help us with digestion, maintenance of the gut barrier, metabolism, immune function, mental health and even protection from pathogens (Morais, L. H, 2020; Hou K et al, 2022).

The gut microbiome differs between species but also within a species depending on various factors such as age, genetics and environment. Nevertheless, there are similarities that create an overview of what a “healthy and balanced” human microbiome should consist of. Among the mentioned microorganisms, the majority consists of bacterial species (Hou K et al, 2022; Rinninella E et al, 2019) which are classified into different taxonomic levels such as phyla. It is mostly composed of 6 phyla including Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia, among which Firmicutes and Bacteroidetes make up 90% of the gut bacteria.

The microbiota and the brain communicate with each other via various routes, including the immune system, the vagus nerve, and the ENS. Existing research has shown how the gut microbiome influences stress responses and stress-related behaviors. Results show that infection can trigger anxiety, germ free animal experiments alter stress responses, probiotic treatments reduce stress and even in the absence of normal microbiota early in life responses to stress are hindered, which is reversible through recolonization (Morais, L. H, 2020). While these studies highlight how the gut microbiome affects stress and anxiety, in this review we aim to

understand how psychological stress can, in turn, affect the already established gut microbiome.

2.2.2 Effects of stress on Gut microbiome

There are several studies that show how chronic psychological stress affects the gut microbiome composition, typically resulting in a reduced number of beneficial gut flora and an increase of harmful microbes. These studies will be analyzed further and categorized into:

Animal studies

In one study (Cryan, J. F & Dinan, T. G, 2012; Almand, A.T et al,2022) Researchers performed the Social Disruption (SDR) stressor in mice. After SDR exposure, changes in microbiota composition were analyzed. The results showed that SDR significantly reduced microbial diversity and altered the community structure of the gut microbiota, characterized by an increase in the harmful *Clostridium spp*. and a decrease in the beneficial *Bacteroides spp.* Additionally, SDR-induced increases in circulating cytokines were correlated with changes in the abundance of specific microbiota members. effects were prevented when given antibiotics (Goudarzi, M et al, 2014; Zafar, H., & Saier, M. H, 2021).

In another study the effects of prolonged restraint stress (RST) were investigated on male mice. Microbiota analysis revealed significant changes in microbial populations in stressed mice compared to non-stressed control mice such as a decrease in *Porphyromonadaceae*, which are part of a healthy gut microbiome. These alterations stayed beyond the stress period, suggesting that prolonged stressors can lead to lasting changes in gut microbiota composition (Bailey MT et al, 2010).

The study investigated the impact of maternal separation-induced psychological stress on the microbiome of infant monkeys, aiming to understand its potential implications for pathogen susceptibility. Infant rhesus macaques were assessed before and after separation from their mothers, measuring changes in fecal bacterial species, particularly *Lactic acid bacteria (Lactobacilli)*, a species considered highly beneficial. Cortisol levels were also measured. Significant findings revealed a marked decrease in *Lactobacilli* levels, correlated with stress-related behaviors rather than cortisol secretion. Overall, these results are consistent with several animal studies providing evidence that stress decreased the number of beneficial gut flora. This can be because of the bacteria reacting to the stress-induced alterations in gut physiology creating an environment less conducive to beneficial bacteria (Bailey, M. T., & Coe, C. L, 1999; Dempsey, E. & Corr, S. C, 2022).

Human studies

In this study the subjects were male astronauts experiencing stressors during the study. Fecal samples collected show an increase in *B. fragilis subsp.*

thetaiotaomicron levels, particularly in response to situations provoking anger induced psychological stress. These microbiomes are considered part of the normal microbiome, but an increase can lead to gastrointestinal issues. These results are also supported by similar findings in other studies involving individuals experiencing more chronic stressors such as personality conflicts and academic stress (Holdeman, L. V et al, 1976).

Based on another study that was investigating the impact of academic stress on the lactic acid bacteria activity in undergraduate students which composes the gut microbiota, fecal samples were collected and questionnaires on stress perception, gastrointestinal symptoms, and nutritional intake were also taken. Results showed significantly lower levels of the highly beneficial fecal lactic acid bacteria during the high-stress exam period compared to the low-stress baseline condition. This decline correlated with higher perceived stress levels reported by the students during the exam week. Lastly, a review study which analyzes a lot of existing human studies shows a decrease in the abundance of Proteobacteria and Verrucomicrobia, one of the most abundant phyla in the human gut microbiota, and increased abundance of Euryarchaeota (Ma, L., Yan et al, 2023).

Chapter 3: Impact of Chronic Psychological Stress on Gut-Brain Axis Signaling Pathways

3.1 Introduction

In this chapter we are focusing on how chronic psychological stress (CPS) effects the key signaling pathways of the gut brain axis, specifically the hypothalamic pituitary adrenal axis (HPA), the sympatho-adrenal medullary (SAM) axis and the enteric nervous system (ENS). The exact impact of CPS on these pathways will be analyzed through examining current research findings and models (Chu, B et al, 2024; Morais, L. H et al 2020).

3.2 HPA axis

3.2.1 HPA axis background

The brain communicates with the gut through various pathways, one of which is known as the HPA axis, a big part of the gut-brain axis. The HPA axis is a very crucial part of our homeostatic regulatory system and one of the main components of the stress response (Russell, G. & Lightman, S, 2019; Rusch, J. A. et al, 2023). When under stress, the paraventricular nucleus of the hypothalamus releases CRH (Corticotropin releasing hormone) which travels to the Anterior Pituitary and triggers the release of ACTH (Adrenocorticotropic hormone) into the bloodstream. ACTH then targets and stimulates the adrenal cortex to secrete glucocorticoid hormones, such as cortisol. (Kazakou, P et al, 2023). GCs follow a circadian rhythm with the highest production in the morning and through a negative feedback loop they can prevent prolonged exposure of their effects. During stressful situations the body prioritizes immediate survival, therefore Glucocorticoids (GCs) are involved in functions aiming to conserve energy and resources. These include suppression of the immune system, increasing fat and protein metabolism and enhancing gluconeogenesis.

In this section, we are focusing on how the HPA axis interconnects with the gut, specifically how chronic psychological stress affects this established communication. Therefore, how the gut affects the stress response will not be analyzed. As mentioned before GCs are involved in prioritization of survival through energy conservation by altering various physiological functions, many of which involve the gut. These include reduction of gut motility, suppression of immune function in the gut and impact on gut permeability. The GC hormone, cortisol, directly acts through the expressed cortisol receptors on epithelial cells, enteroendocrine cells and immune cells in the gut. Cortisol can also affect the gut microbiome by changing the time food and waste takes to pass through the GI tract and influencing nutrient availability (Rusch, J. A et al, 2023).

3.2.2 Under chronic stress conditions

While the activation of the HPA axis during acute stress is usually efficient with just a temporary spike in cortisol, that is rarely the case. More often stress is prolonged which can lead to the up or downregulation of the aforementioned feedback mechanism.In the case of upregulation, there will be overly elevated cortisol levels in humans and corticosterone levels in rodents as the body attempts to cope with the persistent stressor (Mayer, E. A, 2000). This could be due to a decrease in GC receptors caused by chronic stress (Jacobson, L. & Sapolsky, R, 1991) leading to the activation of compensatory mechanisms to maintain cortisol/corticosterone levels (Yehuda R et al, 1991; Unsal, H. & Balkay, M, 2012).

Studies show that chronic psychological stress leads to disrupted tight junctions, increased permeability and visceral hypersensitivity, increased mast cell and increased proinflammatory cytokine secretions. For example, a study performed on rats investigating the effect of chronic CRH administration results in an elevated secretory state and permeability dysfunction (Teitelbaum, A et al, 2018). Another study used male rats subjected to a water avoidance stress (WAS) test or were control rats that received subcutaneous corticosterone injection, in the presence or absence of corticoid-receptor antagonist. Changes observed in the rats subjected to the WAS test were similar to the control rats. Results show decrease in epithelial tight junction protein levels in the colon and increased epithelial permeability (Zheng, G et al, 2013). This altered permeability can trigger local and systemic inflammation in both humans and animals (Leigh, S et al, 2022; M, L., C, K, 2019).

In other cases, chronic stress leads to a phenomenon known as “habituation”.
Habituation refers to the gradual reduction of GCs after repeated exposure to the same stressor, to conserve energy and as a result of a stronger negative feedback inhibition. This state is characterized by decreased basal cortisol levels and increased number of GC receptors. For example, adult animals that have received chronic neonatal stress show a lower baseline corticosterone level (Hess et al, 1969). This is counterintuitive, exacerbating a chronic stress state as the organism continues to experience stress but now lacks effective stress coping mechanisms (Herman, J. P, 2016). In a study involving Lewis rats with a diminished HPA axis response and Fischer rats with an overactive HPA response, they show that Lewis rats are more susceptible to autoimmune and inflammatory diseases such as IBS and colitis (Bonaz, B. L., & Bernstein, C. N, 2013).

3.3 SMA axis

3.3.1 SPA axis background

SAM axis is another important mediator of the stress response which in contrast to the HPA axis, works rapidly and is a part of the autonomic nervous system (ANS). The SAM system links the sympathetic nervous system (SNS) with the adrenal

medulla. Specifically, when under stress norepinephrine is released from sympathetic nerve endings which stimulates the adrenal medulla to release norepinephrine and epinephrine (Leigh, S et al 2023; Floriou-Servou et al, 2021). These hormones belong in the category of catecholamines which bind to adrenergic receptors present on the GI tract causing delayed gastric emptying, reduced motility and blood flow and inhibits GI secretions.

3.3.2 SMA axis under chronic stress conditions

Even though the SAM system is primarily involved in the acute stress response as it is mostly designed for short-term and immediate responses, chronic activation can still lead to problems regarding the gut brain axis especially due to the interconnection of the SMA and HPA axes. They are involved in a positive feedback loop, which means the activation of one axis stimulates the activation of the other affecting many organs, such as the GI tract (La Torre et al, 2023).
For example, during chronic stress prolonged activation of the SMA stimulates eosinophils to degranulate which lead to CRH release further stimulating mast cells to degranulate and release cytokines that increase intestinal permeability. This CRH production adds to the detrimental effects mentioned in the HPA axis section under chronic stress conditions. Furthermore, prolonged release of Catecholamines increases adherence of bacteria to the gut mucosa and contributes to dysbiosis as it is positively correlated with Bacteroidetes and negatively correlated with Firmicutes (Kasarello, K et al,2023; Paudel, D et al, 2022) .

3.4 ENS

3.4.1 ENS background information

The ENS is another important aspect of the gut-brain axis, alongside the HPA and SAM axis. As mentioned before, the ENS is made up of millions of neurons located in the GI tract and bidirectionally communicates with the central nervous system (CNS). It consists of two major parts, the submucosal plexus (SMP) and the myenteric plexus (MP) and both control different aspects. The SMP is involved in the control of absorptive and secretory functions, blood flow and neuroimmune interactions while the MP is involved in regulating motility during different digestive states (Li, S et al, 2016). Therefore, during acute stress through the release of the stress hormones (GCs and catecholamines) the ENS is activated and influences functions related to the gut such as motility, secretion and absorption of nutrients. These changes are associated with activation of the innate immune system and suppression of the adaptive immune system (Leigh, S et al, 2023).

The ENS also regulates the GI tract through the release of neurotransmitters by either excitatory or inhibitory motor neurons which innervate the smooth muscle cells of the GI tract. Excitatory neurons release acetylcholine (ACh) and substance P that stimulate the smooth muscle to contract and secrete, therefore increasing motility and digestion. Inhibitory neurons on the other hand release nitric oxide (NO) and

vasoactive intestinal peptide (VIP) which in contrast to ACh and Substance P promote relaxation of the smooth muscle and inhibit motility (González-Vergara et al 2023; Drokhlyansky, E et al, 2020).

3.4.2 ENS under chronic stress conditions

While the activation of the ENS is crucial during acute stress, prolonged exposure to stress can lead to maladaptive changes in its functioning. As mentioned, research has shown that chronic stress leads to elevated levels of GCs which act on neurons and enteric glial cells in the ENS, disrupting normal functioning. On one hand, this elevation alters the behavior of the glial cells who now instead of exhibiting their normal anti-inflammatory effects are now attracting white blood cells to the GI tract and therefore increase inflammation, contributing to the symptoms of conditions like IBS. On the other hand, this also influences the normal functioning of neurons that leads to altered gut motility and worsening of symptoms of GI disorders (University of Pennsylvania School of Medicine, 2023).

Another study using rats showed that enteric glial cells were activated during chronic water avoidance stress (WAS) and are involved in the disruption of their gut microbiota. Chronic WAS in rats is a model for investigating chronic psychological stress and minimal physical stress (Lee, J. Y et al, 2016). They also showed that important proteins involved in maintaining the integrity and correct function of the intestinal barrier were altered. Specifically, the expression of the protein occludin, claudin and PCNA in the colon of rats when undergoing chronic stress was reduced. Occludin and claudin1 are tight junction proteins, and their reduction can lead to increased intestinal permeability, subsequently triggering immune response and inflammation contributing to the development of GI disorders. PCNA is involved in DNA replication and repair so a decrease in expression suggests impaired repair mechanisms. This result was reversed with the use of a gliotoxin (L-AA), which is toxic to glial cells and therefore inhibits their normal function in the ENS. This aids in understanding how specifically the enteric glial cells contribute to stress-induced changes (Lu, T et al, 2024). Furthermore, L-AA treatment decreases the inhibitory neurotransmitter NO and increases the excitatory neurotransmitter ACh, which shows that chronic WAS influences neurotransmitter release eventually contributing to dysbiosis and altered barrier function. Enteric glial cells may serve as intermediaries between the HPA axis and immune cells in the gut, modulating these stress responses (Reed D, 2023).

Chapter 4: Implications of altered Gut-Brain Axis on Immune system

4.1 Introduction

Dysregulation of the gut-brain axis has broader implications on the immune system which will be explored in this chapter. Specifically, how alterations of the components of the GBA axis, such as dysbiosis, altered HPA and SAM axis function, as well as ENS impact broader immune responses.

4.2 Dysbiosis and implications on Immune system

4.2.1 Dysbiosis and GALT function

Various studies reveal that exposure to chronic stress leads to a myriad of problems, especially regarding the immune system. These include alterations in increase in the immune activation such evidence by elevated proinflammatory cytokines, increased monocytes and neutrophils as well as microglia, which upregulate further markers of the immune system. In the context of the gut-brain axis, chronic exposure to psychological stress as aforementioned alters the microbiome, leading to a state of dysbiosis (Warren, A et al, 2024).

One way dysbiosis affects the immune system is by altering GALT function. Galt is found in the epithelium of the GI tract and is a system of lymphoid tissues composed of a variety of immune cells of the innate and adaptive immune system which communicate directly with the gut microbiota. This means it is critical for initiating immune response against pathogens entering the gut, as well as maintaining homeostasis. It includes various structures, such as the Peyer's patches which contain Microfold cells (M cells), isolated lymphoid follicles, the appendix and mesenteric lymph nodes which collectively play a role in surveillance, defenses, and immune activation. (Celi, P et al, 2017; Donaldson, D. S et al, 2015; Westfall, S et al, 2021). The lymphoid tissues specifically interact closely with gut microbiota through toll-like receptors and nod-like receptors which are able to recognize any present disruptions in the microbiome or damaged tissues. In the state of dysbiosis, altered GALT function leads to hindered surveillance and failure to react to exposure to infectious agents, vaccines, or immunotherapy. (Dupont, H et al, 2020).

Another study found that alteration in the microbiome leads to M cell hyperplasia, a component of GALT allowing a higher number of microorganisms to infiltrate the gut lining activating blood monocytes that migrate to lymphoid tissue and initiate an immune response (Arrazuria, R et al, 2018).

4.2.2 Implications on the overall immune system

During chronic psychological stress and as mentioned in Chapter 3 the amount of beneficial gut flora gets reduced while there is an increase of harmful microbes.
The beneficial bacteria play an important role in maintaining immune homeostasis, with one of the most crucial ones being L*actobacilli* (Westfall, S et al, 2021; Zhao, M et al, 2023). These bacteria are known to play a role in producing anti-inflammatory cytokines and regulatory T cells which are involved in avoiding excessive immune responses and minimizing damage. This is therefore impaired due to their decrease, contributing to a state of inflammation.

The microbiome also plays an important role in maintaining the integrity of the intestinal biological barrier due to the short-chain fatty acids (SCFAs) they produce. Therefore, in a state of dysbiosis, specifically due to the increase of studies that have found that any intestinal epithelial damage worsens, goblet cells increase, protective antibody secretion is reduced and there is a reduction in tight junction protein levels. This all leads to activation of inflammatory pathways and increases intestinal permeability, allowing pathogens to penetrate easier and trigger systemic inflammation as the products they produce activate the intestinal immune system to release proinflammatory cytokines (Zhao, M et al, 2023).

4.3 Signaling pathway dysfunction implications on Immune system function

4.3.1 HPA dysfunction

Immune cells have both GC and noradrenergic receptors making them sensitive to SAM and HPA signals. GCs usually play a role in reducing inflammatory responses by promoting apoptosis in monocytes, macrophages and T lymphocytes. It is also involved in suppressing NF-kB which promotes the expression of various proinflammatory genes (Liu, T., Zhang et al, 2017). This usual anti-inflammatory property is diminished during chronic stress conditions which causes GC resistance. This means that the immune cells are less sensitive to this effect due to the prolonged exposure to high levels of GCs.

In caregivers of brain cancer patients which correlates to being in a state of chronic psychological stress, there is decreased GC-related gene expression and increased NF-kB signaling in monocytes. Another study showed that chronically stressed adults exhibit higher cytokine production and NF-kB activation in mononuclear cells which worsens inflammation. Rodent studies have also shown that chronic psychosocial stress reduces the sensitivity of monocytes and splenic macrophages to GCs and therefore increased pro-inflammatory cytokine release (Ménard, C et al, 2016). This pro-inflammatory state in turn can also negatively impact the gut microbiota ultimately, to dysbiosis, and the inflammation resulting from dysbiosis also leads to HPA-axis activation. Furthermore, the increase of pro-inflammatory molecules increases intestinal permeability which allows Gram-negative bacteria to

enter the bloodstream resulting in chronic inflammation in the central nervous system (Ménard C et al, 2016; Bellavance, M., & Rivest, S, 2014).

4.3.2 SAM dysfunction

The SAM axis dysregulation also has important implications on the immune system. The released catecholamines produced in response to the HPA axis activation during chronic stress conditions are involved in a myriad of immune functions such as cell proliferation and cytokine production. They affect the immune system via adrenergic receptors on immune cells, specifically the b2 adrenergic receptors on macrophages and T lymphocytes. The activation of these receptors is connected to a cascade that involves G-proteins and adenylate cyclase, leading to increased itcAMP levels that stimulate transcription factors like CREB. This leads to increased expression of cytokines such as interleukin-6 (IL-6) that in turn activate the immune system such as by promoting T-cell proliferation. While during normal conditions catecholamines just lead to a temporal boost of the immune system, during chronic stress conditions this leads to a state of prolonged inflammation (Padgett, D. A., & Glaser, R, 2013).

4.3.3 ENS dysfunction

Chronic stress also affects the immune system by ENS dysregulation through Glucocorticoid receptor (GR) signaling. Through the use of a prolonged psychological stress mouse model, it was observed that deletion of GRs in cells other than ENS did not shield the mice from stress- induced colitis as well as a reduction in the accumulation of immune cells such as monocytes (Schneider, K. M et al, 2023). The study shows that a specific group of enteric cells called enteric glia associated with psychological stress (eGAPS) are triggered during chronic stress, which are associated in pathways related to inflammation and cell-death. These are characterized by heightened levels of stress-responsive factors. Furthermore, the release of the signaling molecule CSF1 by stressed enteric glia leads to the buildup and activation of monocytes that produce TNF. All these effects lead to a state of inflammation (Figure 2)

This study utilizing a chronic stress model and a synthetic GC, revealed the presence of neuronal changes. Specifically it shows that chronic stress, through the elevation of GCs induces transcriptional immaturity in enteric neurons, as there was a decrease of mature enteric neurons and an increase in immature neurons (Figure 2). This leads to a reduction of acetylcholine and nitric oxide. This in turn affects normal immune function as these neurotransmitters are involved in the suppression of inflammation through maintenance of the intestinal barrier and the reduction of pro-inflammatory cytokines (Fujii, T et al, 2017; Bogdan, C, 2001).

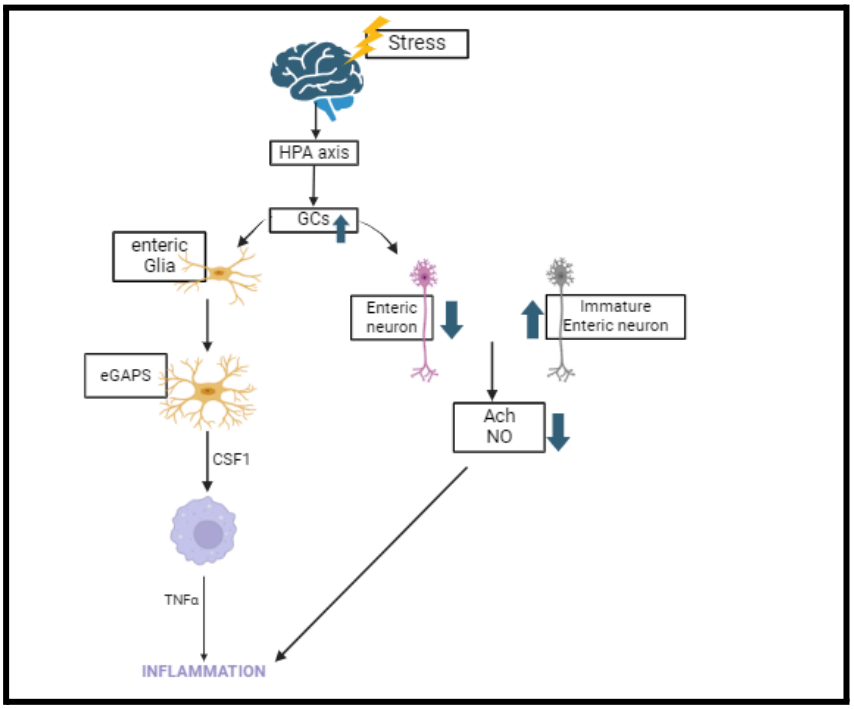

Figure 2: CPS induced ENS dysregulation via GR signaling. Prolonged stress either triggers eGAPS to release CSF1, therefore activating monocytes to produce TNF or leads to a reduction of mature enteric neurons, increase of immature neurons and therefore less Ach and Nitric oxide (NO) release. These all contribute to systemic inflammation (Yirka, B, 2023).

Chapter 5: Stress-Induced Dysregulation and Gastrointestinal Health

5.1 Introduction

Through the aforementioned information we can focus on how the disruptions specifically affect Gut health and their involvement in the development of Gastrointestinal disorders. Specifically, the two most common GI disorders will be analyzed: Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis and irritable bowel syndrome (IBS).

5.2. Inflammatory bowel disease

5.2.1 Crohn’s disease

Crohn's disease (CN), a form of IBD, is a chronic autoimmune disease that leads to inflammation of the gut. The exact cause of Crohn's is unknown, but it is believed that genetics, hereditary and environmental factors play a big role. Chronic stress is one environmental factor that studies have shown to lead to the exacerbation of Crohn's. (Pelc, C, 2021) It does so by disrupting the gut´s mucosal immunity and causing a state of dysbiosis. More specifically, a study showed that stress hormones killed off cells that are responsible for producing interleukin 22 (IL-22), which is a cytokine involved in maintaining mucosal defenses such as antimicrobial peptides and barrier integrity. This leads to gut barriers that are weak and with reduced antimicrobial responses which creates a favorable environment for pathogenic opportunistic bacteria such as the adherent- invasive Escherichia coli. (E. coli). As these harmful microbes grow it subsequently triggers further inflammation and dysbiosis, increasing the likelihood of CD flare-ups and relapse (Mangos, C, 2022; Pelc, C, 2021).

5.2.2 Ulcerative colitis

Another form of IBD is Ulcerative Colitis (UC) which is also a chronic autoimmune disease of unknown cause leading to inflammation in the gut. It is limited to the colon in comparison to CN which can affect the areas from mouth to anus (Feuerstein, J. D et al, 201). One study has shown that the resulting dysbiosis increases susceptibility to dextran sulfate sodium (DSS)- induced colitis. The hyperinflammatory response that is usually observed in colitis was not the main factor in stress sensitization as the removal of the IL-6 inflammatory cytokine did not reduce disease severity in mice but rather through achieving a state of eubiosis by antibiotic administration. Furthermore, as already discussed chronic stress worsens mucosal inflammation in

colitis by reduced protection from immune cells into the colonic lamina leading to the destruction of crypt architecture and faster colitis progression. Systemic immunity is also impaired, as a decreased mesenteric lymph node function and thymus size can be observed (Gao, X et al, 2019).

Another study found that long-term psychological stress increases the risk of exacerbation in patients with UC, using General Perceived stress questionnaires. This study emphasized on long term stress over months or years and showed that prolonged stress rather than acute is mostly linked to UC flare ups likely due to hyperactivation of the immune system (Levenstein, S et al, 2000).

5.3 Irritable bowel syndrome

Irritable bowel syndrome is a functional disorder meaning there is a disruption of the normal GI tract function rather than an autoimmune disorder involving the immune system, as seen in IBD. The pathophysiology is also not well understood but it most likely involves interactions between the immune, hormonal and nervous system (Qin, H et al, 2014). Nevertheless, recently the disturbance of the gut-brain axis is increasingly recognized as a potential model for IBS pathophysiology. Evidence from clinical studies show that patients with IBS usually have a 40-60% of having an additional psychiatric disorder, especially anxiety. One review showed that patients that progressed from non-IBS patients to IBS patients reported a significant increase in stressor score. Alterations of the HPA axis have also been noted in patients with IBS and higher morning cortisol levels which indicates chronic stress, the results have not been consistent though (Bhatia, V., & Tandon, R. K, 2005).

There has also been evidence through animal models investigating the impact of chronic psychological stress on IBS. Results show that through the activation of mucosal mast cells and endocrine cells, leading to increased serotonin levels and pro-inflammatory cytokines there is altered intestinal motility and permeability, characteristics of IBS. The changes in normal immune cell numbers and intestinal barrier function further exacerbates through increase of inflammation. Studies also show that patients with IBS also usually have different GI microbiomes as well as small intestinal bacterial overgrowth (SIBO) (Ford, A. C et al, 2020) which results from chronic stress weakening the immune system and allowing the overgrowth of bad bacteria (Bures J et al, 2010).

Chapter 6: Interventions for Mitigating the Impact of Chronic Stress

6.1 Introduction

In this chapter potential interventions for minimizing the impact of Chronic stress of the disruption of the gut brain axis and its associated resulting effects will be analyzed through existing literature and studies.

6.2 Prebiotics and Probiotics

Prebiotics and probiotics have been proven effective in reducing the impact of chronic stress on the gut-brain axis. Prebiotics such as fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) support the function of the gut-brain axis by promoting growth of beneficial gut bacteria, reducing the usual resulting state of dysbiosis. This shift towards an eubiotic state enhances microbial diversity and promotes the production of short-chain fatty acids (SCFAs) leading to a reduction of inflammation, a key contributor to the exasperation of gastrointestinal disorder (Zhao, M et al, 2023).

One study also wanted to investigate the effect of prebiotics on the central nervous system, specifically under chronic stress conditions. Prebiotics were administered to mice subjected to chronic psychosocial stress. Behavioral assessments for anxiety as well as biochemical analysis for plasma corticosterone, L-tryptophan, and serotonin were performed. Furthermore microbiota composition and SCFA levels were investigated. The results were that mice receiving prebiotics showed significant reduction of corticosterone levels, usually elevated during stressful conditions indicating their ability to normalize the HPA axis dysregulation. The combination of two prebiotics was also effective in normalizing the state of dysbiosis by normalizing the Actinobacteria ratio, and preventing the reduction of beneficial bacteria (Burokas, A et al, 2017).

Probiotics on the other hand contain live organisms with the purpose of maintaining or enhancing the beneficial bacteria in the gut. Usually strains like *Lactobacillus* and *Bifidobacterium* are administered to help prevent dysbiosis and the resulting inflammation as well as directly modulating immune responses. They also improve gut permeability and are involved in the maintenance of intestinal barrier integrity. Also, through the lowering of plasma corticosterone they reverse the effects of the typical resulting HPA axis dysfunction. So, in summary, prebiotics are involved in the improvement of the gut microbiota composition and SCFA levels while probiotics enhance gut integrity and reduce inflammation making them both important intervention therapies to minimize the effects of chronic stress (Butel M-J. et al, 2014).

6.3 Antibiotics

Antibiotics have come to light as potential treatment for IBS as they are able to target SIBO and are generally known to significantly affect the gut microbiome. Studies have shown that their administration can have both a variety of benefits but also drawbacks. One of the potential antibiotics are Rifaximin and Neomycin that target the gut flora directly and are involved in the treatment of SIBO while also displaying minimal systemic side effects. The difference is that Neomycin displays resistance over time which can be a problem for long term use (Yuan, C et al, 2023).

In the context of IBD studies have shown the involvement of antibiotics in both Crohns and Ulcerative colitis as they impact the gut microbiome and influence inflammation. They showed that for Crohn's disease randomized control trials showed that antibiotics can reduce disease activity. This is supported by uncontrolled studies that show reduction of inflammatory markers after the administration of antibiotics. In the context of Ulcerative colitis results vary greatly. Studies show that antibiotics lead to increased remission but also depend on a lot of factors such as the administration route and duration. For example, oral antibiotics are more effective than intravenous as act locally on the gut microbiome (Basseri, R. J et al, 2011).

Antibiotics can also have negative effects as they kill resident microbiota which leaves space for the overgrowth of pathogen microbes furthering the state of dysbiosis which is usually the result of chronic psychological stress. There have also been studies that show the negative effects of treating IBS and IBD, with increasing risk of development and promoting flare-ups. Therefore, when considering antibiotics as a course of treatment the harmful effects and the potential resistance must be taken into account (Ledder O, 2019).

6.4 Diet

Another key factor that should be considered that can help alleviate some of the disruptions caused by chronic psychological stress is Diet. Poorer quality diets such as the western diet, associated with the consumption of processed and refined foods, are known to be involved in the exacerbation of gut-brain axis dysfunction. Studies on rodents on a high fat diet or westernized diet have shown decreased microbial diversity and reduction of the beneficial *Lactobacillus*. Male Wistar rats fed a high-fat diet were also found to have increased circulating cytokines and less clostridium and bacteroides abundance. Furthermore, they have also been proven to impair intestinal barrier integrity and the mucus layer of the gut, as well as increase inflammation (Zhao, M et al, 2023).

On the other hand, good quality plant based diets such as the Mediterranean Diet (MD) have shown to be beneficial for mitigating the impact of chronic stress. Studies have shown that following the MD has beneficial effects on IBD and inflammation as it promotes anti-inflammatory microbes and prevents the occurrence of dysbiosis.

This is possible through the reduction of pathogenic bacteria and increase of beneficial. Lowering oxidative stress and inflammatory biomarkers such as Nf-Bk also plays a crucial role (Ratajczak, A. E. et al, 2023; Zhao M et al, 2023).

6.5 Fecal Microbiota Transplantation (FMT)

FMT is a novel approach that helps minimize the effects by restoring microbial balance and diversity. It involves transferring fecal matter from healthy donors to patients with a compromised GI tract microbiome decreasing intestinal and systemic inflammation as well as restoring barrier disruption. It involves several steps such as selection of the optimal donor, fecal processing and routes of administration. Several studies show varying effectiveness of FMT in achieving remission in patients with IBD. For UC studies show successful remission after receiving FMT portraying rapid improvements in symptoms. On the other hand, these results were not always consistent in patients with CD. FMT is also generally well-tolerated with some adverse effects that rarely lead to any more serious complications. Further studies need to be performed in order to find optimal parameters for FMT such as a personally tailored therapy for each individual patient's microbiome or even investigate the potential of combining therapies such as with immunosuppressants (De Fátima Caldeira et al, 2020; Boicean, A et al, 2023).

Discussion

This literature review shows the result of current research on the impact chronic psychological stress (CPS) has on the gut-brain axis. Chronic stress disrupts the key components composing the GBA. These include the HPA axis, SAM axis and ENS axis, all of which contribute to a state of chronic inflammation and exacerbate GI disorders such as IBS and IBD. Dysregulation of the HPA axis leads to altered GC receptor sensitivity leading to systemic inflammation through dysfunction of the gut barrier. SAM axis dysfunction leads to an extended release of catecholamines negatively affecting gut motility and promoting a state of dysbiosis (Stephens, M. a. C., & Wand, G, 2012; Baritaki, S et al, 2019). ENS further disrupts the integrity of the gut barrier through alteration of neurotransmitter levels and enteric glial activation. All of these findings have significant implications, as they give insight into the interaction between CPS and gut health (Schneider, K. M. et al, 2023). This is of relevance, as with the increasing prevalence of chronic stress in modern day society, these findings are of great importance revealing how these conditions majority of people are subjected to affect GI health.

The disruption of the GBA also has important implications on the immune function as it contributes to a chronic inflammatory state that further exacerbates the gut microbiome dysregulation and systemic inflammation. This creates a never-ending cycle that needs targeted interventions in order to mitigate these effects of CPS and promote gut health. Therapies nowadays include prebiotics and probiotics that have proven effective and with little to no side effects, leading to reduced inflammation and restoration of gut microbiome by growth of beneficial bacteria and productions of anti-inflammatory SCFAs. Diet is an additional important factor to minimize the effect of CPS by creating an environment more suitable for beneficial bacteria. Antibiotics have also been studied as potential therapy interventions, such as treating SIBO but due to its large effect on the gut microbiome homeostasis it might lead to the opposite of the wanted effects and further outbalances the microbiome and might lead to microbial resistance. The promising new therapy known as FMT shows promising results in restoring microbial balance but there are still conflicting results yet.

Despite there being a myriad of solutions available there are still research gaps and opportunities for further research. For example, while acute stress is extensively studied, chronic stress needs to be further studied, especially in long-term conditions. Additionally, further research is needed to explore individual differences in GI responses to stress and to define a “typical " healthy microbiome. This will lead to the opportunity for more personalized treatments and aid in identifying specific imbalances that increase susceptibility to stress. The limitation of this study is that even though it provides insight into the effects of CPS on GBA, not all of the components of the GBA are analyzed nor the intricate mechanistic pathways involved.

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