GUT MICROBIOME AND BRAIN ACTIVITY

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REVIEW

BY

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This is to certify that **Ms. DEEP MAHESHWARI** of **Third year, B.Sc. Biotechnology** has satisfactorily completed the REVIEW REPORT for fulfillment of Bachelor Degree is Biotechnology as a part of curriculum at **Rajiv Gandhi Institute of IT and Biotechnology, B.V.D.U, Pune for the academic year 2015-2016.**

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GUT MICROBIOME AND BRAIN ACTIVITY

INDEX

INDEX

Gut microbiome and Brain Activity

1. Introduction

Conventionally, gut microbiome have not been considered of particular importance for the development and function of the CNS or in the patho-physiology of chronic brain diseases, such as disorders of mood and affect, Parkinson's disease, or Alzheimer's disease. Exception to the traditional view is autism spectrum disorder (ASD), a brain disease which is being suspected to be related to altered gut microbiota (Mayer et al., 2014a). Although gut-brain interactions have been studied for decades, providing a wealth of information about the close interactions microbiome-free worldview of neuroscience has dramatically changed with the discovery and characterization of the human microbiome and, in particular, with the gut microbiome. (Mayer, 2011). Gut microbiome added component to the complex bidirectional signaling between mind, brain, gut, and its microbiome. The initial reports suggested profound role of gut microbiota in shaping brain neurochemistry and emotional behavior were considered skeptical. These findings have given way to conceptualize many psychiatric and neurological diseases. Although many of the new concepts primarily based on the curios experimental findings in rodents, initial studies in humans seem to support the notion that there is a relationship between the gut microbiota and brain structure and function. Even though the majority of published studies of gut microbiome to brain signaling are based on microbiome analyses from stool samples, future studies will almost certainly expand the scope of investigations to mucosal samples taken from different regions of the gastrointestinal tract.

Bidirectional communication network includes the central nervous system (CNS), both brain and spinal cord, the autonomic nervous system (ANS), the enteric nervous system (ENS) and the hypothalamic pituitary adrenal (HPA) axis (Fig. 1). The autonomic system, with the sympathetic and parasympathetic limbs, drives both afferent signals, arising from the lumen and transmitted though enteric, spinal and vagal pathways to CNS, and efferent signals from CNS to the intestinal wall. The HPA axis is considered

the core stress efferent axis that coordinates the adaptive responses of the organism to stressors of any kind.

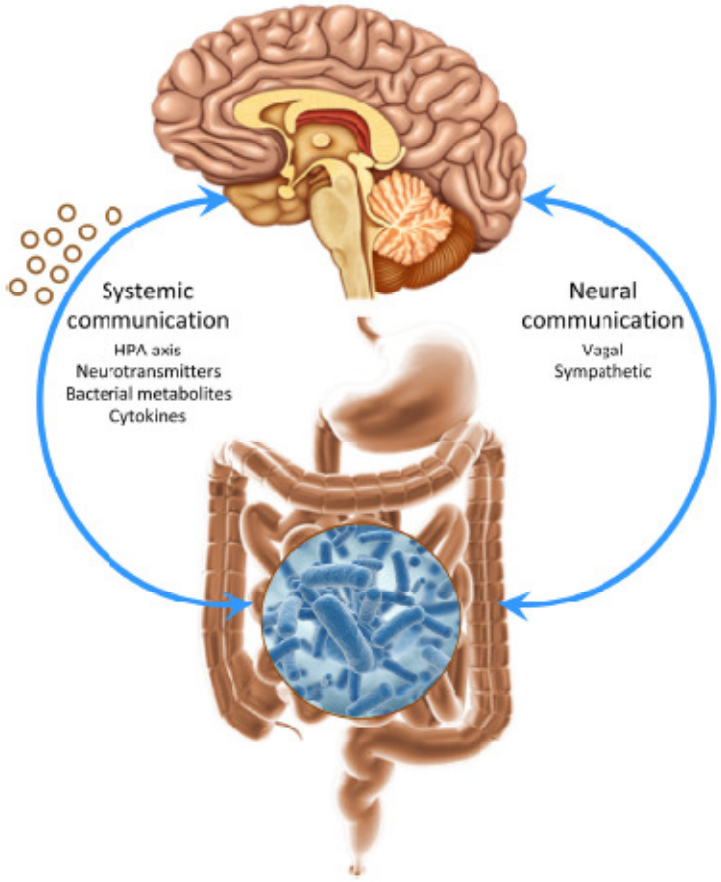

Fig. 1- Bidirectional communication channels between gut microbiome, gut & brain. Endocrine-,neurocrine- and inflammation-related signals are generated by gut microbiota and specialized cells of gut, which affects brain. While brain can alter microbial composition and function (Mayer et al, 2015).

Many reports have recently shown that the gut microbiota may be involved in controlling behaviors and brain functioning (Lyte M et al, 2009). Several experimental conditions have been used to study the role of the gut microbiota in preclinical models, including perturbation of the gut microbiome by ingestion of probiotics and antibiotics, fecal microbial transplant, and comparison of behaviors and biological readouts between germ-free animals (raised in a sterile environment from the time of birth) and those with a pathogen-free microbiome. Several researches revealed that brain behavior and activity could be controlled by synthesizing microbiota derived molecules such as neurotransmitters, neuropeptides etc. In a study Asano et al showed that gut microbiota is

capable of synthesizing acitive neuroendocrine hormones of class catecholamines. He also reported that the catecholamines, norepinephrine, and dopamine, were produced in significant working amounts in specific pathogen free mice(Asano et al, 2012). Another experimental study revealed production of GABA by intestinal microbes (Barret et al, 2012).

This review explores the influence of gut microbiota in brain behavior and development, its use in controlling various psychiatric disorders like autism spectrum disorder and depression as well as highlighting the novel use of probiotics in human behavioral control.

2. Autism and depression

Autism spectrum disorder (ASD) is a range of developmental neuro-behavioral disorders characterized by impaired social interaction and communication. Autism is primary form of ASD. Various researches tried to establish link between gut microbiome and ASD, either as direct causality or as indirect consequences of atypical patterns of feeding and nutrition (Mulle et al., 2013). Disruption of gut microbiota might promote the over-colonization of neurotoxin-producing bacteria and thus contribute to autistic symptoms. However, use of antibiotics like vancomycin gives short-term benefits to regressive-onset autism children (Sandler et al., 2000). General gut microbiota alteration or specific gut commensal strains have been implicated in ASD. Bolte et al. postulated that Clostridium tetani could induce autism (Bolte, 1998). Indeed, two ensuing human gut microbiome studies illustrated a greater number of species under the Clostridium genus present in fecal samples of autistic children (Finegold et al., 2002; Parracho et al., 2005). An imbalance of Bacteroidetes and Firmicutes phyla also manifests in autistic children. Finegold et al. reported increased presence of Bacteroidetes in severe autistic group and predominant presence of Firmicutes in healthy controls (Finegold et al., 2010). Williams et al. revealed a reverse trend in comparing autism and GI disease comorbid (AUT-GI) children and GI disease alone controls (Williams et al., 2011). In addition, altered levels of other gut commensals, including those of Bifidobacterium, Lactobacillus, Sutterella, Prevotella and Ruminococcus genera and of the Alcaligenaceae family, were correlated with autism(Adams et al., 2011; Kang et al., 2013; Wang et al., 2013; Williams et al.,

2012). Nonetheless, there are studies refuting the microbiota alteration between autistic and healthy subjects (Gondalia et al., 2012). Further, gut microbiome mediated metabolism also impacts autism. Metabolites profile gathered from both urinary and fecal samples differed in autistic patients and healthy control, potentially consequent of microbiota changes (Ming et al., 2012; Wang et al., 2012; Yap et al., 2010)

Dysbiosis of the microbiota is implicated in the pathogenesis of several human disorders, including IBD, obesity and cardiovascular disease (Blumberg and Powrie, 2012). Commensal bacteria also affect a variety of complex behaviors, including social, emotional and anxiety-like behaviors, and contribute to brain development and function in mice (Collins et al., 2012; Cryan and Dinan, 2012) and humans (Tillisch et al., 2013). Long-range interactions between the gut microbiota and brain underlie the ability of microbe-based therapies to treat symptoms of multiple sclerosis and depression in mice (Bravo et al., 2011; Ochoa-Reparaz et al., 2010) and the reported efficacy of probiotics in treating emotional symptoms of chronic fatigue syndrome and psychological distress in humans (Messaoudi et al., 2011; Rao et al., 2009).

Depression is a major form of mood disorder that results from neuro-psychiatric disturbance or immunological deregulation (Dantzer et al., 2008). Probiotic treatment has shown efficacy in suppression of animal depression models. Species under Lactobacillus genus are particularly characterized as anti-depressant. Probiotic mixture comprising L. rhamnosus and L. helveticus strains ameliorated maternal separation-induced depression via normalizing corticosterone level (Gareau et al., 2007). Similarly, L. rhamnosus strain JB-1 reduced depression-related behavior through regulating corticosterone and GABA receptor in a vagal-dependent manner (Bravo et al., 2011). Species of Bifidobacterium are also potent anti-depressants. Bifidobacterium infantis alleviated depression as indicated by rat forced swim test (FST) and maternal separation models. Mechanisms involved include attenuation of pro-inflammatory cytokines, regulation of tryptophan metabolism and CNS neurotransmitters (Desbonnet et al., 2008; Desbonnet et al., 2010). Probiotics combining Lactobacilli and Bifidobacteria were tested in post-myocardial infarction depression models. L. helveticus and Bifidobacterium longum together ameliorated post-MI depression through reduction of pro-inflammatory cytokines and

restoration of barrier integrity at GI tract (Arseneault-Breard et al., 2012; Gilbert et al., 2013). In addition, gut microbial products, such as sodium butyrate (salt formed from butyrate acid, a type of SCFA) have been explored in animal depression model, without showing anti-depressant effects (Gundersen and Blendy, 2009). Further, a diet formulation containing high levels of polyunsaturated fatty acids (PUFAs) n-3 attenuated rat post-MI depression via similar mechanisms as did L. helveticus and B. longum(Gilbert et al., 2013).

2.1. Case Study-

In study done by Hsiao et al in 2013 showed how ASD may be linked to gut bacteria as a number of studies report that ASD individuals exhibit altered composition of the intestinal microbiota (Adams et al., 2011; Finegold et al., 2010; Finegold et al., 2012; Gondalia et al., 2012; Kang et al., 2013; Parracho et al., 2005; Williams et al., 2011; Williams et al., 2012). Altogether, evidence of GI complications and microbiota alterations in broadly defined ASD populations raises the intriguing question of whether such abnormalities can contribute to the clinical manifestations of ASD.

Based on emerging research on gut microbiome brain interaction, Hsiao et al used several mouse models of genetic and/or environmental risk factors are used to study ASD. Also use of maternal immune activation (MIA) model to study impact of ASD on GI abnormalities and microbe dysbiosis. To explore the role of GI complications to ASD symptoms, treatment with the gut bacterium Bacteroides fragilis was performed.

2.1.1.Experiments & Results-

A) MIA offspring exhibit dysbiosis of gut microbiota:

To show that MIA induces microbiota alterations, gene sequencing of 16sRNA in fecal bacterial population was done and samples were isolated from adult MIA or control offspring. UniFrac analysis (measures the degree of phylogenetic similarity between microbial communities), revealed a strong effect of MIA on the gut microbiota of adult offspring.

It was found that MIA leads to dysbiosis of the gut microbiota, driven primarily by alterations in specific Operational Taxonomic Unit (OTUs) of the bacterial classes Clostridia and Bacteroidia. Changes in OTUs classified as Lachnospiraceae and Ruminococcaceae of the order Clostridiales parallel select reports of increased Clostridium species in the feces of subjects with ASD (Finegold et al., 2012). Altogether, modeling MIA in mice induces not only behavioral and neuropathological features of ASD, but also microbiome changes as described in subsets of ASD individuals.

B) B. fragilis Treatment Restores Specific Microbiota Changes in MIA Offspring:

The finding that B. fragilis rectifies GI defects in MIA offspring prompted us to examine its effects on the intestinal microbiota. However, OTUs that discriminate adult MIA offspring from controls reveals that B. fragilis treatment significantly alters OTUs levels. Phylogenetic reconstruction of the OTUs that are altered by MIA and restored by B. fragilis treatment reveals that the Bacteroidia OTUs cluster together into a monophyletic group and the Lachnospiraceae OTUs cluster into 2monophyletic groups (Hsiao et al). This result suggests that, although treatment of MIA offspring with B. fragilis may not lead to persistent colonization, this probiotic corrects the relative abundance of specific groups of related microbes of the Lachnospiraceae family as well as unclassified Bacteriodales.

C) B. fragilis Treatment Corrects ASD-Related Behavioral Abnormalities:

The behavioral assays employed were used to measure the cardinal diagnostic symptoms of ASD, in addition to ASD-associated anxiety and deficient sensorimotor gating, and confirm that the MIA model reflects behavioral features of autism. Oral treatment with B. fragilis rectifies many of ASD-related behaviors. B. fragilis-treated MIA offspring do not exhibit anxiety-like behavior. B. fragilis improves sensorimotor gating in MIA. B. fragilis-treated mice also exhibit decreased levels of stereotyped marble burying and restored communicative behavior. Although B. fragilis-treated MIA offspring exhibit improved communicative, repetitive, anxiety-like and sensorimotor behavior, they retain deficits in sociability and social preference. These data suggest that there may be differences in the circuitry or circuit plasticity governing social behavior as compared to

the other behaviors, and that B. fragilis treatment may modulate specific circuits during amelioration of ASD-related behavioral defects.

2.1.2.Discussion

Authors found that B. fragilis treatment ameliorates abnormal communicative, sensorimotor and anxiety-like behaviors in MIA offspring, supporting evolving evidence for a gut-brain link in ASD. Results obtained from experimental study provides a novel mechanism by which a human commensal bacterium can improve ASD-related GI deficits and behavioral abnormalities in mice, possibly explaining the rapid increase in ASD prevalence by identifying the microbiome as a critical environmental contributor to disease. Thus transformative concept that autism was proposed by Hsiao et al is that, it is a disease involving the gut that impacts the immune, metabolic and nervous systems, and that microbiome-mediated therapies may be a safe and effective treatment for ASD.

3. Gut microbe derived products Modulating Brain Activity

3.1. Neuropeptides-

Neuropeptides are important mediators both within the nervous system and between neurons and other cell types. Neuropeptides such as substance P, calcitonin gene-related peptide and neuropeptide Y (NPY), vasoactive intestinal polypeptide, somatostatin and corticotropin-releasing factor are likely to play a role in the bidirectional gut-brain communication (Peter Holzer and Aitak Farzi, 2014). In this capacity they may influence the activity of the gastrointestinal microbiota and its interaction with the gutbrain axis. Current efforts in presenting the implication of neuropeptides in the microbiota-gutbrain axis address 4 information carriers from the gut to the brain i.e. (1)vagal and spinal afferent neurons; (2)immune mediators eg.cytokines; (3)gut hormones; (4)gut microbiota-derived signalling molecules. Apart from operating as neurotransmitters, many biologically active peptides also function as gut hormones. Given that neuropeptides and gut hormones target the same cell membrane receptors (typically G protein coupled receptors), the two messenger roles often converge in the same or similar biological implications. These are NPY and peptide YY (PYY), two members of the PP-

fold peptide family(Peter Holzer and Aitak Farzi, 2014). While PYY is almost exclusively expressed by enteroendocrine cells, NPY is found at all levels of the gut-brain and brain-gut axis. The function of PYY-releasing enteroendocrine cells is directly influenced by short chain fatty acids generated by the intestinal microbiota from indigestible fibre, while NPY may control the impact of the gut microbiota on inflammatory processes, pain, brain function and behavior(Peter Holzer and Aitak Farzi, 2014).

Experimental data shows significant part of the metabolites circulating in mammalian blood is derived from the intestinal microbial community (Wickoff et al, 2009;,Antunes et al, 2011; Nickolson et al, 2012). Importantly, the presence or absence of the gut microbiota also influences the profile of metabolites (including peptides) present in the brain (Matsumoto et al, 2013). LPS translocated from the gut through a leaky mucosal barrier carries a microbial message to distant organs including the brain. The behavioural responses to systemic exposure of excess LPS are well characterized in animals and humans and comprise acute sickness [18,19] and delayed depression-like behaviour [20-24]. LPS originating from the gut microbiota may give rise to alterations in brain function via 3 different pathways. Following translocation across the intestinal mucosa it may, on the one hand, stimulate the intestinal immune system to produce cytokines which (i) can signal directly to the brain or (ii) sensitize/stimulate vagal and spinal afferent neurons [18,19,25,26]. On the other hand, (iii) the circulation may carry LPS itself to the central nervous system where it may modify brain function.

3.2. Neurohormones-

Microorganisms actively produce; wide range of neuroendocrine hormones has been reported for decades (12, 24). Range of hormones that are found in microorganisms is extremely diverse extending from somatostatin to acetylcholine to progesterone. Specially, microorganisms which inhabit the gut are capable of producing neuroendocrine(**Table 1**) hormones which have related host receptors which can easily be found both inside and outside intentine to which these neurohormones, can effect neurophysiological changes in the host. For example, certain Lactobacillus and Bifidobacterium strains isolated from the human gastrointestinal tract can produce in

vitro over 20 000 µg ml-1 of GABA in the presence of a suitable substrate. Members of the genera Candida, Streptococcus, Escherichia and Enterococcus synthesize 5-hydroxytryptamine (5-HT), members of the genera Escherichia, Bacillus and Saccharomyces generate dopamine and/or noradrenaline, members of the genus Lactobacillus produce acetylcholine, and members of the genera Lactobacillus and Bifidobacterium manufacture gamma-aminobutyric acid (GABA) [7,14,36-39]. The release of microbiota-derived dopamine into the lumen of the intestine has been suggested to play a proabsorptive role in the colon [38]. Signalling via opioid and cannabinoid receptors may also be modified by the gut microbiota, a conclusion based on the ability of certain probiotics to alter the expression of opioid and cannabinoid receptors in the gut [7]. *E. coli* is a prominent bacterial species that generates bioactive nitric oxide in both the small and large intestine using different oxygen dependent mechanisms (Seth et al., 2012) Asano et al. reported that the catecholamines, norepinephrine and dopamine, were produced in significant amounts in specific pathogen free mice. Although, in germ-free animals substantially lower amounts were detected in luminal contents. Critically, whereas the majority of catecholamines in pathogen-free animals were structurally determined to be free and biologically active, those found in germ-free animals were present in a biologically inactive, conjugated form. Inoculation of germ-free animals with the flora from specific pathogen free mice resulted in the production of free, biologically active, catecholamines within the gut lumen. Thus it was clear that in-vivo conditions gut microbiota is capable of producing neuroendocrine hormones that are commonly only associated with host production.

S.No.	Gut-Microorganisms Producing Neurotransmitters	Neurotransmitter Produced	Function
1	*Lactobacillus & Bifidobacterium*	GABA	Reduce neuronal excitability
2	*Candida, Streptococcus, Escherichia and Enterococcus*	Serotonin	Mood regulation
3	*E. coli, Bacillus and Saccharomyces*	Norepinephrine	increases alertness, promotes vigilance, memory retrieval &focuses attention
4	*Bacillus and Serratia*	Dopamine	Mood, behavior, memory, vasodilator
5	*L. reuteri*	Histamine	Regulates attention, sleep, feeding

Table 1- Gut microbes producing different neurotransmitters and their roles

3.3. Neuroactive factors released by the gut microbiota

Gut microbiota not only releases ligands for pattern recognition receptors, but also release factors that target specific neuronal systems involved in the gut-brain axis. Microbiota in the intestine is able to produce metabolites with benzodiazepine-like structures and effects, originating from the gut microbiota have been proposed to contribute to the encephalopathy associated with hepatic failure [40-42]. Under these conditions, benzodiazepine-like molecules are likely to reach the brain at increased concentrations that will enhance neurotransmission via GABAA receptors and thus contribute to the disease process [40]. The pyrrolobenzodiazepines (e.g., anthramycin) synthesized by a number of gut microbes display not only benzodiazepine-like but also antibiotic and antineoplastic activities and may thus influence the biology of the microbiota and host alike in many respects.

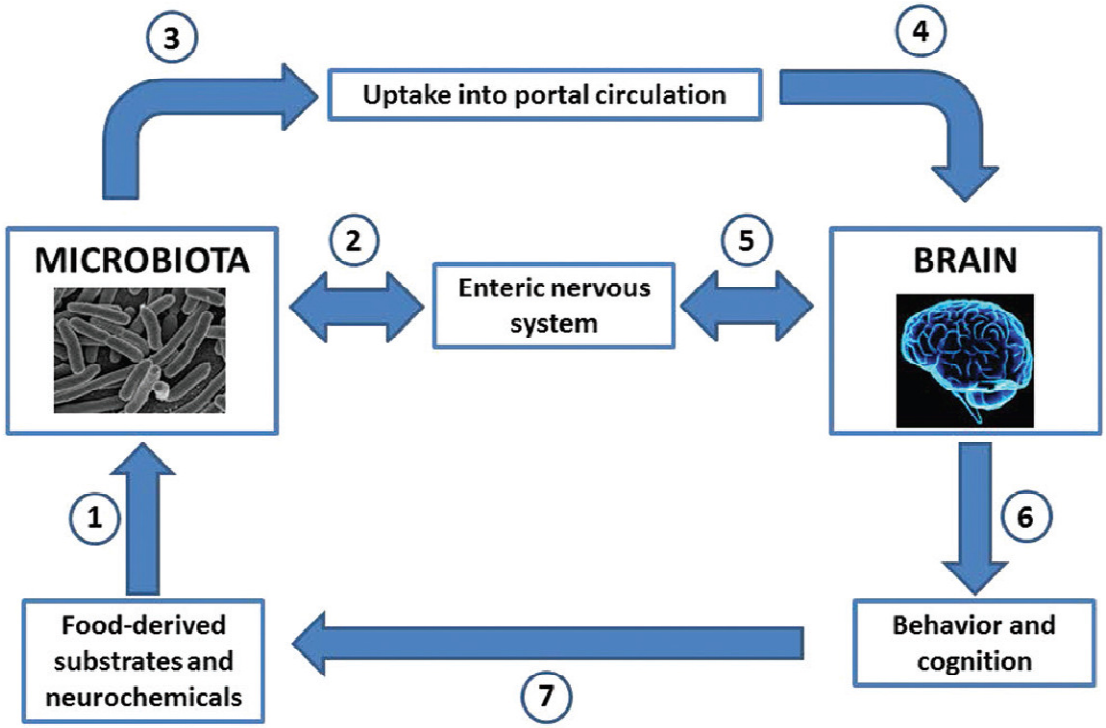

Fig.- Microbial endocrinology-based pathways by which neuroactive compounds produced by both the host and the microbiota can serve as a mechanism by which the brain and behavior can be modulated within the microbiota-gut-brain axis

4. Model organism used to study gut microbe- brain relationship

It is now a decade since Sudo et al. (2004) discovered that germ-free mice have an exaggerated hypothalamic-pituitary adrenal axis response to restraint stress, an effect that was reversed by monocolonization with a particulart Bifidobacterium species. GF mice was susceptible to reversal when animals were colonized early in life (Clarkeet al., 2013). Interestingly, recent studies in germ-free animals in the stress-sensitive F344 rat strain showed similar exaggerated neuroendocrine responses but also revealed an increase in anxiety-like behavior (Crumeyrolle-Arias et al., 2014). Moreover, it has recently been shown that short-term colonization of germ-free mice in adulthood reduced anxiety-like behaviors(Nishinoetal.,2013).Together, it is clear that studies in germ-free animals clearly show a relationship between gut microbiota and stress and anxiety-related behaviors, the nature of this relationship being influenced by various factors. number of

studies are also investigating gene expression changes in different brain regions in germ-free mice.

Most commonly, decreases in the hippocampal expression of BDNF, a protein involved in neuronal plasticity and cognition, have been observed in germ-free mice relative to conventionally raised or conventionalized (i.e., initially germ-free mice colonized with normal mouse gut microbiota) controls. Similar changes in BDNF expression have also been reported following antibiotic administration(Berciketal.,2011b). Alterations in neurotransmitter signaling, including neurotransmitters and associated metabolites and neurotransmitter receptors, have also been described in specific brain regions of germ-free mice.(Diaz Heijtz et al, 2011) took a genome-wide transcriptomic approach showing that genes associated with the citrate cycle (synaptic long-term potentiation steroid hormone metabolism, and cyclic adenosine5-phosphate-mediatedsignaling)were upregulated in germ-free mice.

Microbiota modifies the levels of metabolites that are relevant to the synthesis of transmitters in the nervous system. For example, the concentrations of tryptophan (the precursor of 5-HT), tyrosine (the precursor of dopamine and noradrenaline) and glutamine in the total brain of germ-free mice are lower than in mice that have been re-colonized by the gut microbiota (Matsumoto et al, 2013). Colonization of the germ-free animals restores peripheral tryptophan levels to control values but fails to reverse the changes in hippocampal 5-HT levels [43]. The concentrations of tryptophan, 5-HT and tyrosine in the blood plasma are likewise increased in germ-free animals [11,43], the elevation of tryptophan being likely due to the absence of bacterial tryptophanase [11]. Another explanation could be that the gut microbiota re-directs the metabolism pathways of tryptophan which lead either to the production of 5-HT or kynurenine [7]

In these studies, the cerebellum and hippocampus have robust changes in gene expression, but the hypothalamus, the brain region involved in the stress response, showed almost no differential gene expression. Some behavioral and biochemical parameters (including anxiety, sociability, hypothalamic-pituitary-adrenalaxis,andtryptophan metabolism) could be reversed in germ-free mice by

recolonization with a conventional microbiota or probiotic treatment, but others were unaffected by restoration of a normal microbiota. (Stilling et al., 2014a).

Germ- free studies are powerful in that they test definitively whether the microbiota is involved in a specific aspect of brain function. Germ-free mice also enable the study of the impact of a particular bacterial or dietary intervention on the microbiota-gut-brain axis in isolation. Studies in germ-free mice can also be expanded to enable research on the "humanization" of the gut microbiota (i.e., transplanting fecal microbiota from specific human conditions or fromanimal models of disease).In this regard, intriguing studies have shown that the transplantation of microbiota from a high anxiety mouse strain to a germ-free low-anxiety recipient in adulthood was sufficient to increase anxiety in the recipient, and the converse was also true(Collinsetal.,2013).

5. Interaction of the gut microbiota with brain function and behaviour: emerging neurochemical mediators:

Several studies shows that the absence or disturbance of the gut microbiota has a significant impact on brain function and behaviour. There are molecular factors which can play an important role in this interrelationship. Use of germ-free mice exhibit a number of neuro-chemical and functional alterations relative to conventionally colonized animals. For instance, the expression of the NMDA receptor subunit 2A (NR2A) in the cortex and hippocampus [57] and of the NR2B unit in the central amygdala [58] is decreased in germ-free mice, as is the expression of the 5-HT receptor 1A (5HT1A) in the dentate granule layer of the hippocampus [58]. In contrast, inconsistent changes in the levels of brain-derived neurotrophic factor (BDNF), a key neurotrophin involved in neuronal growth and survival, have been reported: two studies hold that BDNF in the hippocampus, amygdala and cortex of germ-free mice are decreased [57,59], while another study purports that the level of BDNF in the hippocampus of germ-free mice is increased [58].

impact of the gut microbiota on brain function has been confirmed by the impact of antibiotic-induced dysbiosis on the gut-brain axis and by the effects of selective probiotics on behaviour and brain chemistry. Disturbance of the gastrointestinal

microbiota with a combination of nonabsorbable antibiotics (neomycin, bacitracin, and pimaricin) increases exploratory behaviour and enhances BDNF expression in the hippocampus [61]. Similar observations have been made with another combination of nonabsorbable antibiotics (neomycin, cefoperazone and ampicillin) which has an anxiety like effect and impairs learning/memory in the object recognition test [62].

6. Use of Probiotics in modulating brain activity

Animal models, a range of probiotics have been investigated. Bifidobacterium and Lactobacillus are the main genera showing beneficial effects on anxiety-and depression-like behavior .However, even within bacterial genera, marked strain differences occur, and only a few strains have any positive effects (Dinan et al., 2013). Chronic treatment with *Bifidobacterium infantis* attenuated early-life stress-induced immune changes and depressive-like behaviors in adulthood (Desbonnet et al., 2010). Lactobacillus helveticus ROO52 has also been shown to reduce anxiety-like behavior and alleviate memory dysfunction (Ohland et al., 2013). Lactobacillus rhamnosus JB-1 reduced anxiety- and depression-related behaviors in the elevated plus maze and forced swim test, respectively (Bravo et al., 2011). Recent work by Matthews and Jenks (2013) demonstrated reduced anxiety and improved performance on a complex maze task after treatment with live Mycobacterium vaccae. Bifidobacterium longum normalizes anxiety-like behavior in a colitis model (Berciketal.,2011a). Furthermore, a B.longum, but not L.rhamnosus strain ,normalized infection-induced anxiety-like behavior (Bercik et al., 2010). A combination of L. rhamnosus and L. helveticus reversed stress-induced memory dysfunction in Citrobacter rodentium-infectedmice(Gareauetal.,2011).More recently, it has been shown that VSL#3 (a mixture of eight different probiotics) was able to reverse age- associated deficits in long-term potentiation, the electrophysiological correlate of memory formation (Distrutti et al., 2014). Probiotic treatment has also proved efficacious in alleviating visceral pain responses in animal models (Rousseaux et al., 2007; McKernan et al., 2010).

Chronic treatment of mice with the probiotic Lactobacillus rhamnosus has been found to cause region-dependent alterations of GABAAα2 and GABAB1b receptor mRNA in the brain, which are associated with a decrease in the stress-induced corticosterone response

and a reduction of anxiety- and depression-related behaviour [63]. Importantly, these neurochemical and behavioural effects of probiotic treatment are prevented by bilateral subdiaphragmatic vagotomy. This role of vagal afferent neurons in communicating between gut bacteria and brain was confirmed by another study in which vagotomy abolished the anxiolytic effect of the probiotic *Bifidobacterium longum* NCC3001 in mice with experimentally induced colitis [64]. Probiotic treatment with several species of Lactobacillus genus restored the barrier integrity (AitBelgnaoui et al., 2012; Zareie et al., 2006)

Lactobacillus reuteri attenuates sensory neuron excitability [67] and alleviates the pain-related response to gastric distension [68]. Lactobacillus acidophilus also reduces experimentally evoked visceral pain, an effect that is associated with enhanced expression of opioid and cannabinoid receptors in the intestinal mucosa [69]. Lactobacillus paracasei has been found to attenuate antibiotic induced visceral hypersensitivity in mice [65], while *Lactobacillus rhamnosus* GG has a beneficial effect in abdominal pain-related functional gastrointestinal disorders in childhood [70].

Study conducted by Kirsten et al(2013), showed chronic ingestion of FMPP(Fermented Milk Product with Probiotics) for 4 weeks on healthy women affected activity of brain regions that control central processing of emotion and sensation.

ASD is a complex disorder with poorly defined etiologies and no effective or targeted cure. Use of probiotic using *Bacillus fragilis* treatment corrects MIA-associated changes in specific serum metabolites that appear to have a gut origin, suggesting B. fragilis may prevent leakage of harmful molecules from the GI lumen.(Hsiao et al, 2013). B. fragilis treatment has significantly reduced abnormal communicative and anxiety-like behaviors in MIA offspring, supporting emerging evidence for a gut-brain link in ASD.

7. Factors linking microbiome and the CNS

As microbiome is the collective genomes of total microbiota, microbiome research is broad in its scope, which incorporates general microbiota composition or specific bacterium, microbiota-generated products, external alteration of microbiota, and barrier integrity status that affects host-microbiota contact. It is thus worthy summarizing the factors that mediate the influence of microbiome on CNS disorders.

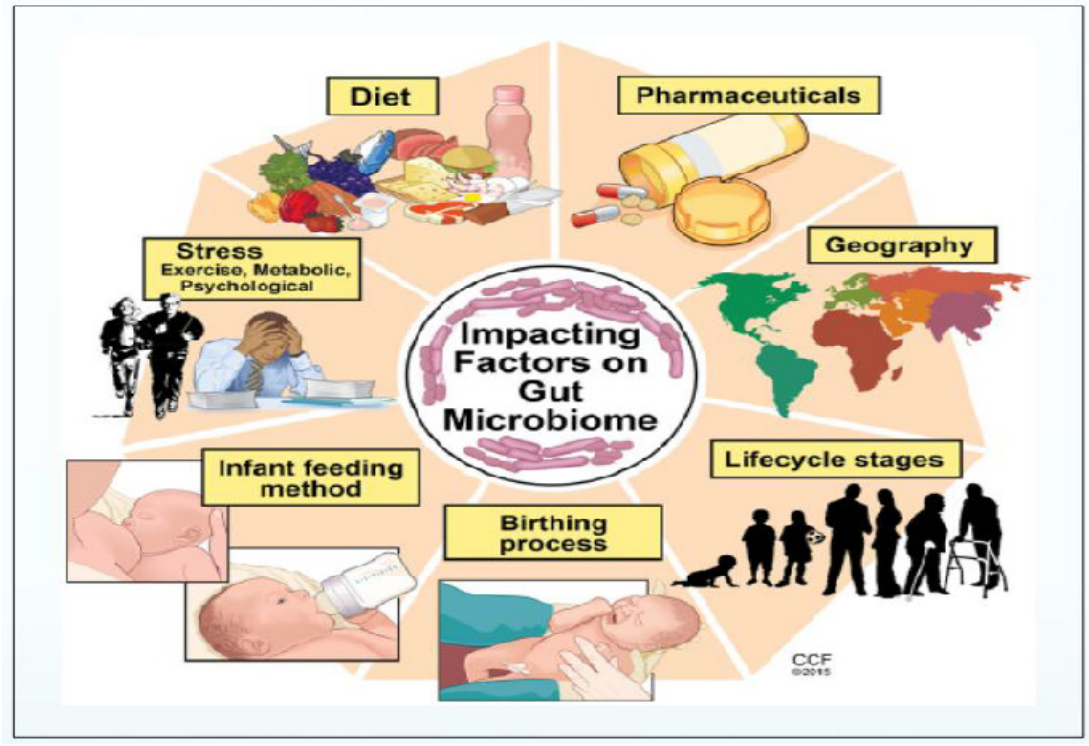

Fig.- Various factors affecting microbiome –CNS relationship(Cleveland Clinical Foundation)

7.1. Hygiene-

The hygiene hypothesis states that a lack of childhood exposure to infectious agents, parasites and commensals increases susceptibility to T helper 2 (Th2)-mediated allergic diseases. Th1(T helper 1) response targets intracellular microbes, mediated by cytokine IFNγ; while Th2 response targets helminthes and allergens, characterized by cytokines IL-4 and IL-13. Irregular immune development is therefore a potential mechanism that links hygiene and immune-mediated CNS disorders. GF mice displayed reduced EAE symptoms, concurrent with attenuated Th1, Th17 and B cell responses, which related to

the hygiene hypothesis yet contradicted findings in human MS(Berer et al., 2011; Lee et al., 2011). This discrepancy might be explained by intricate etiologies underlying human MS and intrinsic differences between murine GF condition and human hygienic state. In murine models, GF condition is also linked to neurobehavioral disorders. Total sterility results in reduction of BDNF levels and enhancement of HPA axis responses, correlated by elevated neurotransmitters in the plasma. GF animals displayed increased stress and impaired cognition (Gareau et al., 2011; Sudo et al., 2004). However, GF condition in other studies is identified as anxiolytic and can resolve anxiety, correlated by decreased neurotransmitter receptors levels (Kuss et al., 2011; Neufeld et al., 2011). Hence, hygiene exerts case-specific rather than universal influences on neurochemistry and neuro-behavioral manifestations.

7.2. Use of Antibiotics-

Antibiotics leads to selective alteration of gut microbiota. Mice pre-conditioned with oral antibiotics are less susceptible to autoimmune models such as EAE. In studies conducted by Ochoa-Reparaz et al., elimination of EAE was associated with reduced IFNγ and IL-17, increased IL-13 and IL-10, and systemic stimulation of Tregs and Bregs(Ochoa-Reparaz et al., 2009; Ochoa-Reparaz et al., 2010b). That antibiotics poise the Th1/Th2 equilibrium towards Th2 direction is consistent with hygiene hypothesis. An earlier study conducted by Yokote et al. also observed reduced pro-inflammatory cytokines, including IFNγ and IL-17, in antibiotic treatment of EAE. Different antibiotic agents were utilized in these EAE studies, which could result in different gut microbiome profiles and explain the variability of immune mechanisms. Current studies support a beneficial role of antibiotic treatment of neuro-behavioral disorders. Antibiotic treatment reduced stress response and increased exploratory behavior in mice and offered short-term benefit to regressive-onset autism children. Underlying mechanisms may involve the reduction of luminal LPS concentration (and thus potentially reduced chronic inflammation) and changes of CNS signals, such as hippocampal expression of BDNF(Ait-Belgnaoui et al., 2012; Bercik et al., 2011a; Sandler et al., 2000). In sum, antibiotics might reset the default immune and neuro-hormonal status shaped by commensal microbiome and therefore alter predisposition to CNS disorders.

7.3. Microbiota composition-

Microbiota composition can impact CNS disorders can be indicated by a variety of methodologies, including infection-induced microbiome perturbation, studies using SPF and gnotobiotic mice, mono-colonization of GF mice, and metagenomic. Further, compositional changes of microbiota can be indirectly reflected by profiling the metabolites and co-metabolites of microbiota and serum titers of antibodies against microbiota and diet components. Hildebrand et al. defined two murine enterotypes, ET1 and ET2 that bore striking similarity to Ruminococcus and Bacteroides enterotype in human, respectively. ET2 mice showed higher levels of fecal calprotectin, a biochemical marker for IBD (Hildebrand et al., 2013). For CNS disorders, a concrete link with enterotypes has yet to be established. While it is tempting to infer enterotypes from the scattered studies of certain disease type, opposing data often obstruct consensus. For instance, there are favorable and unfavorable results for the link between Bacteroides enterotype and autism(Finegold et al., 2010; Williams et al., 2011). Further, heed must be taken to clarify the cause and effect as CNS disorders could impact diet patterns or be concurrent with gut epithelial impairment, both scenarios affecting microbiota composition.

7.4. Probiotics-

Ingestion of beneficial live bacteria, also known as probiotics, is a therapeutic way of using microbiota components for treatment. Probiotics can regulate immune subsets, especially in the case of CNS autoimmunity. Neural mechanisms that involve direct bacterial activation or inhibition of neurons may account for anti-nociceptive effects of probiotics. Probiotics can alleviate neuro-psychiatric disorders via hormonal and neuro-chemical mechanisms. For example, B. longum NCC3001 can normalize murine hippocampal BDNF expression and L. rhamnosus (JB-1) can exert differential regulation of GABA transcription in different CNS regions (Bercik et al., 2011b; Bravo et al., 2011).

7.5. Microbiota-derived products-

Microbiota-derived products are often effective components responsible for microbiota-gut CNS signaling. PSA is a unique zwitterion and referred to as a symbiosis factor for commensalism (Mao et al., 2013; OchoaReparaz et al., 2010c). Microbiota-derived metabolites and co-metabolites are critical intermediaries for microbiota-gut-CNS signaling. Commensals spawn a range of neuroactive substances. For example, Lactobacillus and Bifidobacterium species can produce the inhibitory neurotransmitter GABA(Barrett et al., 2012). SCFAs, a group of fatty acids with aliphatic tails of 2 to 6 carbons, are fermentation products of dietary fibers by microbiota. While SCFAs have been found to be important immune regulators, there is a scarcity of studies that target at their impacts on CNS disorders(MacFabe et al., 2011; Thomas et al., 2012).

7.6. Diet-

Diet patterns may modulate gut microbiome via alteration of nutrient availability. Recent developments have suggested that dietary intervention can impact gut microbial gene richness. Lower microbiome richness was identified as less healthy and associated with metabolic dysfunction and low-grade inflammation. Dietary formula with higher fiber contents can improve microbiome richness (Cotillard et al., 2013; Le Chatelier et al., 2013). Unhealthy diet patterns containing high levels of fat or salt could accelerate neuro-inflammation during EAE(Kleinewietfeld et al., 2013; Piccio et al., 2008). Western-style diet could negatively affect anxiety-like behavior and memory, depending on immune status (Ohland et al., 2013). Supplementation with high levels of PUPAs could alleviate depression (Gilbert et al., 2013). These experimental findings could indicate saturated fat as a risk factor for both neuro-immune and neuro-psychiatric disorders. Collectively, microbiome modulation is an integral mechanism underlying diet-based treatment.

7.7. Gut permeability-

Gut permeability has been directly and indirectly associated with the role of microbiome in CNS disorders. Humoral and cellular immune reaction to microbiota in the circulation, persistent low-grade inflammation and neuro-psychiatric co-morbidity with IBD may breach mucosal epithelial barrier(Banati et al., 2013; Bercik et al., 2011b; Lyte et al.,

2006; Maes et al., 2012; Severance et al., 2013; Varrin-Doyer et al., 2012). Probiotic treatment with several species of Lactobacillus genus restored the barrier integrity(AitBelgnaoui et al., 2012; Zareie et al., 2006). Dysbiosis and breakdown of mucosal barrier are interrelated phenomena. Microbiota and their ligands maintain the cell-cell junctions critical to barrier integrity(Hooper et al., 2001; Rakoff-Nahoum et al., 2004). Abnormal gut microbial composition is seen in IBD(Fava and Danese, 2011). In return, the cascade of inflammatory process during IBD may amplify intestinal dysbiosis.

Conclusion

As more research is carried out in field of gut microbiota and brain, greater the affect gut microbiota appears to have, on nearly all systems and levels in the human body. However, these clinical diseases further show the vital role played by gut microbiota in the human body, both through healthy, beneficial states and in altered and dysfunctional states. Therefore mentioned studies, which focus on the impact of gut microbiota on the host, are essential to our understanding the influence of these systems. In the absence or dysfunction of normal gut flora, a multitude of diseases may occur, shedding light on the important role maintained by the gut–brain axis.

Clinical studies have led researchers to speculate on possible adverse effects of gut microbes in ASD, schizophrenia, mood disorders, anxiety and depression like psychiatric disorders. Adverse effects have been attributed to change in gut microbiome composition (dysbiosis) and increased intestinal permeability. Several mechanisms may enable gut microbiota to influence function or dysfunction in the CNS: (1) Induction of host immune responses leading to cytokine activation; (2) synthesis of absorbable neuroactive metabolites, including neurotransmitters, etc.

Use of Prebiotics, probiotics, and fermented foods influences the impact of the gut microbiome on the CNS and have shown significant effects on brain function in a number of experimental trials and clinical studies. Use of probiotics have reduces anxiety like behavior and are also used for treating disorders like ASD.

Characterization of the gut microbiome has initiated a paradigm shift not only in medicine, but also in the basic and clinical domains of neuroscience. Not only is the concept of gut-microbiome-brain interactions in health and disease paradigm breaking, the emerging data-driven, analytical methodologies that are required to pursue the integration of massive amounts of data are equally revolutionary. It is difficult to predict the trajectory of exciting period of discovery: Will the gut microbiome add paradigm-transforming insights to our existing understanding of human brain function in health and disease, resulting in novel therapies, or will it represent an incremental step in understanding the inner workings of our brains? The next few years of research hold the

potential of uncovering intriguing connections between gut bacteria and neurological conditions that may possibly impact human health.

Experimental studies by Asano et al showed that microbes can actively respond to catecholamines coupled with the extensive and varied microbial possession of a wide-ranging spectrum of neuroendocrine hormones raises the obvious question whether the development of microbial neuroendocrine production and recognition systems means that within the gastrointestinal tract the microbial organ itself may possess its own nervous system?

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