

THE EFFECT OF INFECTION AND ANTIBIOTICS ON THE GUT BRAIN AXIS

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SUMMARY

Considerable experimental evidence largely from preclinical studies supports the concept of bidirectional interactions between the gut microbiota and the central nervous system. Multiple chemical, neural and immune signalling mechanisms have been identified which can mediate the transfer of information from complex microbial communities in the gut, to various cell types in the gut wall, including enterochromaffin cells and primary afferent nerves. In rodents, the perturbation of microbial homeostasis by introduction of pathogens can influence activity within brain circuits related to emotional behaviour, even before the development of gut inflammation. The suppression or perturbation of microbial communities by oral antibiotics can also influence such behaviours, and such behavioural changes can be associated with altered brain neurochemistry. Even though it is currently unknown if similar effects occur in adult humans on antibiotic therapy, it is intriguing to speculate that the regular antibiotic consumption early in life may affect brain development via the microbiota gut brain axis.

INTRODUCTION

The importance of interactions between the brain and the digestive system in health and disease has been recognized for many centuries (reviewed in *Mayer and Brunhuber, 2012*). A major scientific breakthrough in understanding these interactions occurred with the discovery of the enteric nervous system (ENS) in the middle of the 19th century (*Costa et al., 1987; Furness et al., 1998; Furness, 2006; Gershon et al., 1994*). The ENS has been referred to as the “second brain,” based on its size, complexity and similarity in neurotransmitters and signalling molecules with the brain (*Gershon, 1998*). The topic of top-down modulation of gas-

trointestinal (GI) function by stress and emotions (*Cannon, 1929*), as well as bottom signalling from visceral afferents to the brain in abdominal pain syndromes, and possible emotion regulation (*James, 1884; Mayer, 2011*) has received increased attention during the past two decades, largely due to a series of independent, yet converging scientific discoveries from various fields of research, including enteric neuroscience (reviewed in *Furness, 2006*), neuro-imaging (reviewed in *Mayer, 2009*), intestinal microbiology and host microbial interactions (reviewed in *Artis and Grencis, 2008; Round and Mazmanian, 2009*), and more recently

microbial gut-brain signalling (reviewed in *Collins and Bercik, 2009; Collins et al., 2012; Cryan and Dinan, 2012; Forsythe et al., 2010; Forsythe and Kunze, 2012; Rhee et al., 2009;*),

which discusses emerging pre-clinical and some clinical evidence supporting a role of infections and antibiotic treatment on bi-directional signalling between the gut and the brain.

THE GUT BRAIN AXIS IN HEALTH

There are an estimated 200-600 million neurons in the human ENS, equal to the number of neurons in the spinal cord (*Furness, 2006*). These neurons have been classified on the basis of their morphology, electrophysiological properties and chemical coding into distinct classes of functional specific neurons, including several classes of afferents (*Furness, 2006; Wood, 1987*). The size and complexity of the ENS is not surprising when considering some of the unique challenges posed by the interface of the organism with its luminal environment: It interfaces closely with our largest body surface (the intestinal surface area [100 m²] is approximately 100 times larger than the surface area of the skin), with the largest population of commensal microorganisms of all body surfaces (100 trillion, 40,000 species, 100 fold greater number of genes

compared to the human genome (*Kurokawa et al., 2007*), with the gut-associated immune system (containing two-thirds of the body's immune cells), and with thousands of entero-endocrine (EE) cells (containing more than 20 identified hormones). These unparalleled relationships between the GI tract and the brain, with multiple, bi-directional and often interacting interoceptive communication systems emphasize the importance of this system in the maintenance of homeostasis, and make the brain gut axis unique amongst all viscera. A rapidly growing body of evidence supports a crucial role of the gut microbial ecology in the normal functioning of the gut brain axis, with gastro-enteric infections and suppression of the normal microbial ecology by antibiotic treatment representing major threats to its homeostasis.

THE EFFECT OF INTESTINAL INFECTION ON GUT BRAIN INTERACTIONS

From a clinical perspective, profound behavioural changes, as well as changes in mood, affect, cognitive function (attention, concentration) and motivation have long been known as characteristic features of most acute gastro-enteric infections in humans. Similarly, the clinical presentation of

patients with diarrhoea and cramp like abdominal pain are hallmarks of these enteric infections. However, the neurobiological mechanisms underlying the effects of gastro-enteric infections on the human gut brain axis are largely unknown.

GASTRO-ENTERIC INFECTIONS IN RODENTS WITH VARIOUS PATHOGENS

The cellular and molecular mechanisms underlying the symptoms of acute abdominal pain in enteric infections have been identified in the form of peripheral and central sensitization of visceral afferent pathways. Similarly, many of the molecular immune and neurobiological mechanisms that mediate the behavioural changes associated with acute enteric infections, including the so-called sickness syndrome (Watkins and Maier, 1999) have been identified (Bercik et al., 2012). More recently a series of intriguing studies from a small number of laboratories have identified behavioural, cognitive and neurobiological effects resulting from perturbations of the normal gut microbiota by different pathogens, and have described similarities and differences in the way the signals from the gut are transmitted to the brain.

Campylobacter jejuni

A pivotal study by Lyte and colleagues demonstrated that mice display altered, anxiety-like behaviour during the early phase of acute infection with *C. jejuni*, a common food-borne pathogen (Lyte et al., 1998). This abnormal behaviour consisted of decreased exploratory and increased non-exploratory behaviour during elevated plus-maze task on the second day after oral infection with the intestinal pathogen, compared to saline-treated animals. Interleukin-6 (IL-6) levels and peripheral blood leukocyte populations did not differ significantly between infected and control animals indicating the absence of a fully engaged immune response at the time of the behavioural tests. Based on these findings, the authors suggested that the behavioural changes were not a consequence of cytokine-induced sickness behaviour

but were rather mediated by neuronal pathways transmitting information about the shift in gut microbiota to the brain. A subsequent study showed that infection with *C. jejuni* activated sensory visceral structures in the brainstem, on both the first and second day post-oral inoculation, in the nucleus tractus solitarius (NTS) and lateral parabrachial nucleus (LPBN) (Gaykema et al., 2004). On the second day, an increase in c-FOS mRNA expression, an indicator of neuronal activity, was observed in the hypothalamic paraventricular nucleus, a brain region involved in the stress response. Since serum levels of IL-6, IL-1 β , and tumour necrosis factor- α (TNF- α) were unchanged over both days, the data are most consistent with vagal transmission of pathogen-related signals to the CNS. Mediation by the vagal afferent pathways was confirmed by the combined result of bilateral increase of c-FOS expression in the NTS, 4-12 hours post oral inoculation with *C. jejuni*, and unchanged levels of circulating pro-inflammatory cytokines (Goehler et al., 2005). Other studies focusing on early (7-8 hours) responses to oral inoculation have demonstrated that infection with *C. jejuni* increased anxiety-like behaviour during the hole-board task, accompanied by activation of brain circuits related to emotional behaviour. Increased c-FOS mRNA expression was observed in central autonomic regions, including the paraventricular, basolateral nuclei of the amygdala and parts of bed nucleus striae terminalis. This data is consistent with previous studies as these brain regions are purportedly relaying the viscerosensory stimuli from vagal afferent pathways to higher order regions mediating behavioural stress responses (Goehler

et al., 2008). Together with the earlier identification of vagal afferent pathways in mediating cytokine triggered sickness behaviour (Watkins and Maier, 1999) these studies illustrate an important role of vagal afferents in detecting an acute pathogen induced change in the gut and transmitting this signal to central fear circuits, even though the peripheral encoding mechanisms of such signals (e.g. inflammatory, pathogen related) may differ.

Citrobacter rodentium

Oral inoculation of mice with another pathogen, *C. rodentium*, has been found to be associated with cognitive dysfunction in non-spatial and working memory, 10 days (time of maximal inflammation) and 30 days post-inoculation (Gareau et al., 2011). However, these inflammation associated behavioural effects were only seen in the context of an acute water avoidance stress. On the other hand, germ-free mice, 10 days post oral pathogen inoculation had an exaggerated stress response regardless of the presence or absence of the acute stressor, presumably due to an alteration in responsiveness of the hypothalamic-pituitary-adrenal axis (Gareau et al., 2011). When a Lactobacillus-containing probiotic regimen was given to non-germ-free mice before and during infection, serum corticosterone responses to stress were attenuated, c-FOS expression and BDNF levels in the CA1 region of the hippocampus stabilized, and cognitive dysfunction was prevented. Based on these findings, the authors made several speculations:

1. Information about specific enteric bacterial infection reaches the brain through either enterochromaffin cell derived serotonin or corticotropin-releasing factor, priming the HPA-axis to stress.
2. At the time of the stress, abnormal

behaviour is observed due to HPA axis induced alterations in hippocampal memory.

3. The primed stress response is heightened in germ free mice due to an absence of a gut microbiome.

Cognitive dysfunction and anxiety-like behaviour 7-8 hours after oral inoculation with *C. rodentium* was most likely mediated by vagal afferent pathways since vagal sensory ganglia from infected mice had higher c-FOS expression (Lyte et al., 2006). The data from various pathogenic infections suggests that vagal afferent transmission from gut to brain plays a crucial role in mediating the behavioural and cognitive changes, as well as the observed activation of brain circuits observed during gut infection.

Helicobacter pylori

Studies in chronic *H. pylori* infection of mice have also shown correlation with abnormal feeding behaviour consisting of frequent bouts of feeding with less food consumed per feeding than control animals. Increased levels of plasma ghrelin and postprandial CCK, higher TNF- α in the hypothalamic median eminence (ME), and lower pro-opiomelanocortin (POMC) expression in the arcuate nucleus were associated with the observed delay in gastric emptying and the development of visceral hypersensitivity in infected mice. The ME of the circumventricular organ contains a leaky area of the blood-brain barrier of the CNS, thus allowing metabolites and molecules from the systemic circulation to enter the brain directly rather than being mediated by neuronal activation (Bercik et al., 2009). Another study from the same group of mice chronically infected with *H. pylori* found delayed gastric emptying and visceral sensitivity associated with an up-regulation of substance P and calcitonin gene-related

peptide expression in the gut and spinal cord (Bercik et al., 2002). Bacterial eradication normalized gut dysfunction symptoms, but did not alter abnormal feeding behaviour and the increase in TNF- α in the brain and gastric CD3(+) T-cell counts remained elevated, suggesting different pathways monitor post-infective gut dysfunction. Interestingly these behavioural feeding changes and the biochemical changes both lasted for 2 months after the bacteria were eradicated, implying that the effects of chronic infection in GI tract can be long lasting (Bercik et al., 2009). When the infected animals were given probiotics, abnormal feeding behaviour was normalized and CD3(+) T-cell counts decreased, further suggesting that there are various mechanisms at play during recovery from infection that are not well understood and need further investigation (Verdu et al., 2008).

Trichuris muris

Chronic intestinal infestation of mice with *T. muris*, a close relative of the human parasite *Trichuris trichiura*, was accompanied by increased anxiety-like behaviour, as well as decreased expression of brain-derived neurotrophic factor (BDNF) in the hippocampus, a multifunctional brain region involved in memory formation and in the inhibition of the central stress response. Intestinal inflammation, observed by the increased plasma levels of pro-inflammatory cytokines TNF- α and interferon- γ , was also accompanied by an increase in the plasma kynurenine:tryptophan ratio, reflecting an alteration of tryptophan metabolism in the mice. The *T. muris* infection induced anxiety-like behaviour was not affected by a vagotomy performed prior to the infection, arguing against an important role of afferent vagal pathways in mediating the observed behavioural effects during this

type of infection. In contrast, anxiety like behaviour was not observed when mice were treated with the anti-inflammatory agents etanercept (a TNF- α inhibitor) and the steroid budesonide after infection, even though hippocampal BDN expression was similar to the infected-only mice. These findings suggest that in this model, the observed anxiety-like behaviour is induced by inflammatory mediators originating in the gut, and exert their effects either via circulating cytokines and inflammation-related changes in altered tryptophan metabolites, rather than mediation by vagal afferents. On the other hand, the authors reported that administration of the probiotic *Bifidobacterium longum* was able to restore normal behaviour and hippocampal BDNF expression, while plasma cytokine and kynurenine levels remained unaffected. Even though the precise mechanisms and pathways underlying the infection induced behavioural changes in this model remain to be determined, the findings suggest that multiple signalling mechanisms can be involved in the transmission of gut pathogen-related information to the brain (e.g. vagal afferents, inflammatory mediators, amino acid metabolites), and that the engagement of these mechanisms can vary depending on the pathogen and the experimental model (Bercik et al., 2009). Bercik and co-workers have shown similar results in SCID mice, which had been orally administered chronic *T. muris*, which caused anxiety-like behaviour and also expressed higher levels of BDNF mRNA in the hippocampus using *in situ* hybridization. The effect of BDNF was avoided when *B. longum* was introduced, although anxiety-like behaviour remained; and the introduction of *L. rhamnosus* normalized anxiety-like behaviour but not BDNF mRNA expression. This further confirms the plethora

of data to date suggesting strongly that there are different signalling mechanisms for how information on the state of the gut's microbiota, specifically its contents work (Bercik et al., 2009).

In summary, considerable preclinical evidence has been reported to clearly support the ability of enteric pathogens to signal to the central nervous system and affect behaviour. This pathogen to gut to brain communication system is capable of detecting acute changes in the gut microbial ecology, and of selectively signalling the presence of a pathogen to brain circuits, which are involved in the stress

response, behaviour, memory, and learning. Furthermore, there is good evidence to support the existence of multiple pathways or mechanisms mediating the transmission of pathogen related information from the GI tract to the brain. One may speculate that, while signalling via circulating immune mediators plays the dominant role during fully developed mucosal inflammation, peripheral afferent pathways, including enteric and vagal afferents may play an important role at the earliest stages of infection, prior to the full engagement of the immune response (Bercik et al., 2012).

THE EFFECT OF ANTIBIOTICS ON GUT BRAIN INTERACTIONS

Despite the well known side effects of orally ingested antibiotics reported in human patients, there are no reports in the literature or anecdotal reports suggesting that suppression of normal microbiota by antibiotics (dysbiosis) are associated with significant changes in mood, affect, cognition or behaviour. This is surprising, given the widespread use of antibiotics both in paediatric and adult populations, the now well established communication between the gut microbiota and the central nervous system, and the recent results from rodent studies suggesting that antibiotic induced suppression of the normal flora is associated with significant behavioural and even neurochemical changes in the brain.

Antibiotic treatment of experimental mice has become one of the most commonly used methods to induce artificial intestinal dysbiosis. Perturbation of the microbiota in adult mice by oral administration of non-absorbable antibiotics neomycin and bacitracin and the antifungal agent nystatin has been shown to increase a viscerosomatic nociceptive reflex to

colorectal distension consistent with the development of visceral hypersensitivity following antibiotic induced dysbiosis (Verdu et al., 2006). In the same study, the antibiotic induced visceral hypersensitivity was reversed by the oral administration of the probiotic *Lactobacillus paracasei*. This study demonstrated that antibiotics can severely disrupt the central processes responsible for visceral responses by altering the neurotransmitter content of the colon, while certain probiotics may be able to re-establish intestinal symbiosis and neurotransmitter content, thereby normalizing visceral function. In another study by the same group (Bercik et al., 2011a), oral administration of neomycin and bacitracin along with the antifungal agent pimelic acid to adult BALB/c mice, did not lead to quantitative changes in culturable bacteria but was associated with a transient change in microbial ecology: Antibiotic ingestion was associated with an increase in Actinobacteria and Lactobacillus species and decrease in Proteobacteria and Bacteroidetes. The antibiotics also induced changes in be-

haviour, with treated animals demonstrating evidence of increased exploratory behaviour in both the step-down and light/ dark preference tests, indicating reduced levels of anxiety. As was demonstrated in comparisons between germ-free and conventional animals, behavioural changes in antibiotic-treated animals were associated with reduced BDNF levels in the amygdala, and increased levels in the hippocampus (*Bercik et al., 2011a*). The effects of antibiotic treatment on the composition of the intestinal microbiota and on behaviour were transient with treated mice resembling controls after a 2-week washout period. In these studies, a causal relationship between microbiota changes and behavioural effects is supported by the demonstration that, in

contrast to oral antibiotic treatment, i.p. treatment did not influence behaviour. Furthermore, antibiotic treatment had no effect on the behaviour of germ-free animals (*Bercik et al., 2011a*). Whether the behavioural changes can be attributed to specific alterations in the microbiota, e.g., increased lactobacilli and acinto- bacteria or decreased c-proteobacteria and bacteroidetes, was not investigated. However, this is an intriguing idea especially given subsequent studies demonstrated anxiolytic effects of feeding certain lactobacilli and bifidobacterium strains (*Bercik et al., 2011b*), and as such it would be interesting to assess the effects of prebiotics, e.g. agents that promote the growth of bifidobacteria and lactobacilli, on behaviour.

SUMMARY AND CONCLUSIONS

Growing preclinical literature supports the concept of bidirectional microbiota gut brain communications providing the basis for interactions between three “super systems” within the body: The gut microbiome, the gut associated immune system and the central nervous system. However, despite the excitement about the recent findings demonstrating important influences of the gut microbiota on behaviour and brain signalling systems in rodent models, caution is in place when extrapolating the findings obtained from perturbations of the gut microbiota from rodent behaviours to complex human experiences and subjective symptoms.

In the case of gastro-enteric infections, characteristic human symptoms of nausea, fatigue, lack of motivation, anxiety, depression and abdominal pain may be the human homologues of rodent behaviours and molecular mechanisms reported in the publications discussed above. Functional brain imaging

studies in human subjects may provide further evidence to support the translational significance of these observations.

In the case of antibiotics, there is currently no convincing clinical or experimental evidence in human subjects to mimic the dramatic findings observed in rodent models. The reason for this translational gap is currently unknown, but may in part be related to the fact that antibiotics are generally not given to otherwise healthy subjects, but generally in the context of on-going infections. It is conceivable that targeted evaluations of cognitive function, affect and mood in subjects undergoing oral antibiotic therapy will reveal subtle effects in these domains. In view of the widespread use of non-absorbable antibiotics for the treatment of patients with irritable bowel syndrome and symptoms of bloating and abdominal distension, it would be important to know if such treatments have effects on

the CNS. An important aspect of suppression of normal gut microbiota by antibiotics and the resulting effects on the gut brain axis may be related to brain development. Germ-free conditions, an extreme form of dysbiosis, have been found to profoundly influence brain development in rodents. Given the widespread use of antibiotics in neonatal intensive care units, and in paediatrics for common diseases as si-

nusitis, bronchitis and respiratory tract infections, it is conceivable that the dysbiosis resulting from these interventions may affect brain development in children. Future studies evaluating possible correlations between antibiotic use, dysbiosis and cognitive and emotional functioning and underlying brain networks in children should address this important question.

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