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Research report

Gut bacteria interaction with vagal afferents

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ABSTRACT

Contemporary techniques including the use of germ-free models and next generation sequencing have deepened our understanding of the gut microbiota dynamics and its influence on host physiology. There is accumulating evidence that the gut microbiota can communicate to the CNS and is involved in the development of metabolic and behavioral disorders.

Vagal afferent terminals are positioned beneath the gut epithelium where they can receive, directly or indirectly, signals produced by the gut microbiota, to affect host behavior, including feeding behavior.

Supplementation with *L. Rhamnosus* in mice notably causes a decrease in anxiety and these effects are abolished by vagotomy. Additionally, chronic treatment with bacterial byproduct lipopolysaccharide (LPS) blunts vagally-mediated post-ingestive feedback and is associated with increased food intake. Inflammation in the nodose ganglion (NG), the location of vagal afferent neurons' cell bodies, may be a key triggering factor of microbiota-driven vagal alteration. Interestingly, several models show that vagal damage leads to an increase in immune cell (microglia) activation in the NG and remodeling of the vagal pathway. Similarly, diet-driven microbiota dysbiosis is associated with NG microglia activation and decreased vagal outputs to the CNS. Crucially, preventing dysbiosis and microglia activation in high-fat diet fed rodents normalizes vagal innervation and energy intake, highlighting the importance of microbiota/vagal communication in controlling feeding behavior.

As of today, new consideration of potential roles for glial influence on vagal communication and new methods of vagal afferent ablation open opportunities to increase our understanding of how the gut microbiota influence its host's health and behavior.

1. Introduction

Early consideration of the gut microbiota looked principally at its role as an internal ecosystem that may have, via competition for resources, an ability to protect our guts from pathogenic invasions (Greenberg, 1969; Mushin and Dubos, 1965). Viewing the gut microbiota as having a larger impact on host health was not far behind (Whitt and Savage, 1980; Williams, 1973) and a role for the gut microbiota in the development of immunity was proposed early on (Klaasen et al., 1993). The use of germ-free animals (Backhed et al., 2004; Falk et al., 1998) combined with improved methods for microbial species identification (Gill et al., 2006) greatly advanced microbiota research and unraveled the crucial influence of microbiota composition on host physiology (Ley et al., 2006; Sen et al., 2017; Turnbaugh et al., 2006; van Tongeren et al., 2005) as well as behavior (Goehler et al., 2008; O'Mahony et al., 2009).

The prominent role of the microbiota in innate and adaptive immunity has been well established (Yamamoto et al., 2012). Pattern recognition receptors (PRRs), including Toll-Like receptors (TLRs) can recognize bacterial products, such as lipopolysaccharide (LPS) or peptidoglycans to induce the appropriate immune response, for example the release of pro-inflammatory cytokines (Aderem and Ulevitch, 2000). Chronic over-activation of PRRs has been implicated in inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis (Cario and Podolsky, 2000). A recent *meta*-analysis found fecal microbiota transfer to be an effective treatment in 45% of IBD cases overall and in as many as 60% of Crohn's disease patients (Colman and Rubin, 2014) Limited

Abbreviations: CCK, cholecystokinin; CNS, central nervous system; EEC, enteroendocrine cell; FFAR, free fatty acid receptor; GABA, gamma-aminobutyric acid; GI, gastrointestinal; GLP, glucagon-like peptide; IB4, isolectin B4; IBD, inflammatory bowel disease; LPS, lipopolysaccharide; NG, nodose ganglion; NGS, next generation sequencing; NTS, nucleus of the solitary tract; ob/ob, homozygous for Lep^{ob} mutation; PRR, pattern recognition receptor; SCFA, short chain fatty acid; SOCS3, suppressor of cytokine signaling-3; TLR, toll-like receptor; TRPV1, capsaicin receptor/vanilloid receptor-1; VAN, vagal afferent neurons.

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evidence suggests that donor microbiota characteristics influence the ability of fecal transfer treatments to induce remission in IBD. Increased donor microbiota species richness has been associated with increased likelihood of remission (Vermeire et al., 2016). However, the influence of gut bacteria is not limited to PRR signaling or to the gastrointestinal (GI) tract. Unfavorable microbiota composition and/or dysregulation of the intestinal epithelial barrier have been implicated in the etiology of several diseases including metabolic and behavioral disorders.

2. The bug-gut-brain axis

In mice, infection with *Campylobacter jejuni* increases anxiety-like behavior (Goehler et al., 2005, 2008) while supplementation with *Lactobacillus rhamnosus* reduces stress and anxiety behavior (Bravo et al., 2011). Changes in central gamma-aminobutyric acid (GABA) signaling are implicated in the etiology of anxiety disorders and *L. rhamnosus* supplementation is associated with alterations in $GABA_A$ and $GABA_B$ receptors in several cortical regions, including the amygdala and hippocampus. Such evidence of communication between commensal bacteria and the brain have supported the emergence of the "microbiota-gut-brain axis" concept (Bercik, 2011; Bienenstock and Collins, 2010). Interestingly, *C. jejuni* infection leads to vagal activation (Goehler et al., 2005) while *L. rhamonus*' positive effects on anxiety are abolished by vagotomy (Fig. 1) (Bravo et al., 2011), identifying the vagus nerve as a potential relay pathway for microbiota-to-brain signals. Vagal afferent neurons (VAN) express $GABA_A$ receptor (Ashworth-Preece et al., 1997) and strains of *Lactobacillus* and *Bifidobacterium*, common members of the gut microbial community, can produce GABA (Barrett et al., 2012) but *L. rhamnosus* was not found to be a GABA producer suggesting that bacteria can influence vagal signaling via both direct and indirect pathways.

The vagus nerve is a bidirectional communication line between visceral organs and the central nervous system (CNS). Vagal efferent neurons send motor information from the brain to peripheral organs but are outnumbered 9:1 by vagal afferents that convey sensory information from the periphery to the CNS (Foley and DuBois, 1937). VAN play a crucial role in regulating host behavior, including feeding behavior (de Lartigue et al., 2012). VAN, located in the nodose ganglion, notably relay information on quantity and quality of ingested nutrients to the nucleus of solitary tract (NTS) to regulate meal termination (Sutton et al., 2004). Vagal afferent terminals are located in the gastrointestinal lamina propria where they can directly or indirectly interact with the microbiota or sense immune or endocrine signals originating from the GI tract (Patterson et al., 2002). In animal models, chronic consumption of energy dense diet leads to microbiota dysbiosis (Turnbaugh et al., 2006) and disrupts vagal afferent signaling resulting in overeating (de La Serre et al., 2010, 2015; Sen et al., 2017; Vaughn et al., 2017).

3. The gut microbiota influences regulation of food intake

Commensal bacteria have been shown to produce neurotransmitters such as serotonin (Hsu et al., 1986), GABA (Barrett et al., 2012), dopamine, and noradrenaline (Oleskin et al., 2016) and microbiota-originating neural signals could potentially directly affect vagal signaling. However, there is accumulating evidence pointing towards indirect interactions between the gut microbiota and the vagal afferent pathway.

Firstly, microbiota composition appears to influence enteroendocrine cell (EEC) signaling. EEC release satiety peptides such as cholecystokinin (CCK) (Gutzwiller et al., 2000) and glucagon-like peptide (GLP)-1 (Novak et al., 1987) in response to feeding. Gut-originating peptides bind to their specific receptors on vagal afferents to reduce food intake. Supplementing a high-fat diet with oligofructose, a known prebiotic, induces an increase in GLP-1 expression in mice (Cani et al., 2005). More recent research suggests that oligofructose can be fermented into short chain fatty acids (SCFA) by members of the gut microbiota, especially *Bifidobacteria* (Meyer and Stasse-Wolthuis, 2009). SCFA can act as endogenous ligands for free fatty acid receptor (FFAR)2 and FFAR3, two G-protein coupled receptors that are involved in controlling the secretion of GLP-1 (Tolhurst et al., 2012). In another study, oligofructose supplementation in ob/ob mice led to significant increases in *Bifidobacteria* and *Lactobaccillus*. These increases were associated with increased GLP-2 expression (Cani et al., 2009). GLP-2 acts as a trophic factor in the gut promoting epithelial growth and strengthening the intestinal barrier (Cani et al., 2009). This is critical since high-fat diet fed rats' propensity towards obesity is associated with increased GI permeability while resistance to weight gain is correlated with an intact intestinal barrier (de La Serre et al., 2010). A leaky gut would allow for bacterial products and /or metabolites to cross the gut epithelia and come in close contact with vagal terminals providing another means for the microbiota to communicate with the host. In human (Creely et al., 2007) and rodent models (Cani et al., 2007) obesity is a state of metabolic endotoxemia, a chronic elevation in circulating bacterial LPS. VAN express PRRs, including the LPS receptors, TLR-4 (Hosoi

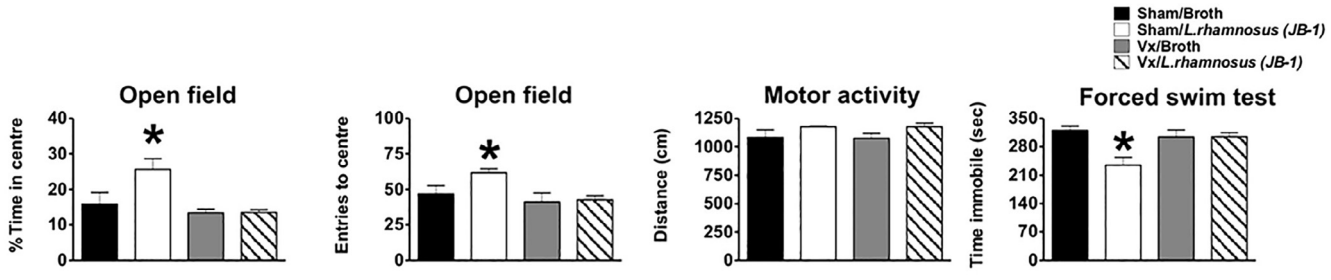

Fig. 1. Adapted from Bravo et al. (2011). Supplementation with Lactobacillus rhamnosus decreases anxiety via a vagally dependent pathway. Male BALB/c mice were vagotomized (Vx) or sham operated. Animals from each surgery group were then gavaged with either sterile broth or broth containing active *L. rhamnosus* for 28 days prior to behavioral testing. Mice with intact vagus and *L. rhamnosus* treatment displayed less anxiety in the open field and forced swim tests than vagotomized and/or untreated mice. (Sham)/*L. rhamnosus* (JB-1) treated mice (n = 10) (white bars) spent more time in the central area of an open field arena in comparison with sham/broth animals (n = 10) (black bars) as measured by the number of entries into the central area of the open field. Sham/*L. rhamnosus* (JB-1) mice (n = 10) entered the central area significantly more often than sham/broth treated animals. Vx animals did not display significant differences in behavior regardless of broth/*L. rhamnosus* group suggesting the anxiety-alleviating effects are mediated by the vagus nerve. Furthermore, these behavioral differences were not found to be related to an effect on locomotion as animals in all groups travelled similar distances. Sham/L. rhamnosus (JB-1) (n = 10) mice spent had significantly less immobile time than sham/broth animals (n = 10) in the forced swim test and this effect was also abolished by vagotomy. $^{*}p < .05$.

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