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Nourishing the gut microbiota: The potential of prebiotics in microbiota-gut-brain axis research

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Abstract:	Dietary fiber and prebiotics consistently modulate microbiota composition and function and hence may constitute a powerful tool in microbiota-gut-brain axis research. However, this is largely ignored in Hooks et al.'s analysis, which highlights the limitations of probiotics in establishing microbiome-mediated effects on neurobehavioural functioning and neglects discussing the potential of prebiotics in warranting the microbiota's role in such effects.

1. Name of Target Article

Microbiota-gut-brain research: A critical analysis
Authors: Katarzyna B. Hooks, Jan Pieter Konsman, and Maureen A. O'Malley

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3. Title of invited commentary

Nourishing the gut microbiota: The potential of prebiotics in microbiota-gut-brain axis research

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3. Abstract

Dietary fiber and prebiotics consistently modulate microbiota composition and function and hence may constitute a powerful tool in microbiota-gut-brain axis research. However, this is largely ignored in Hooks et al.'s analysis, which highlights the limitations of probiotics in establishing microbiome-mediated effects on neurobehavioural functioning and neglects discussing the potential of prebiotics in warranting the microbiota's role in such effects.

4. Main text

Central to Hooks et al.'s analysis is their critique of unwarranted causal claims about the impact of the gut microbiota on psychobiological functioning following probiotic interventions, given the inconsistent evidence on probiotic's capacity to modulate gut microbiota composition and function (Hooks et al., n.d.). Although we agree, evidence on the effects of dietary fiber (DF) and prebiotics on microbiota composition and function and subsequent psychobiological changes are not discussed. We argue that such findings illustrate the potential of prebiotic interventions in supporting causal claims about the impact of gut microbiota on psychobiological functioning. Nonetheless, before meriting such claims, direct investigation into the mechanisms that mediate DF/prebiotic effects on brain function are needed.

Critical to warranting causal claims in microbiota-gut-brain (MGB) axis research is the availability of tools to steer microbial ecosystem into a desired composition/function that subsequently improves brain function. DF/prebiotics may constitute such a tool. DF is defined as carbohydrate polymers with 3 or more monomeric units, which are not hydrolysed by the endogenous small intestinal human enzymes, are naturally occurring or isolated from foods, and demonstrate a physiological health benefit (Jones, 2014). Fermentable fibers provide metabolic substrates for most gut bacteria, influence their diversity and richness, and increase levels of fermentation products such as short chain fatty acids (SCFA) (den Besten et al., 2013). Some fibers can be classified as "prebiotic" if they are "selectively utilized by host microorganisms conferring a health benefit" (Gibson et al., 2017).

A systematic review and meta-analysis of 64 intervention studies in healthy adults found that DF resulted in consistently higher abundance of *Bifidobacterium* spp. and *Lactobacillus* spp. (So et al., 2018). However, the application of next-generation sequencing techniques that allow microbiota-wide assessment of relative abundance shifts suggests that modification of microbiota composition by means of DF/prebiotics is not limited to these specific taxa (Davis et al., 2011; Everard et al., 2011; Everard et al., 2014; Holscher et al., 2015; Martínez et al., 2010; Vandeputte et al., 2017; Walker et al., 2010). Notably, these studies indicate that changes in gut microbiota composition are reversible, maintained only with continued consumption of DF/prebiotics, and exhibit inter-individual variation probably dependent on baseline microbiota

profile, including presence of keystone species or variation in enzymatic capacity of certain strains (Falony et al., 2016; Ze et al., 2013).

While DF/prebiotics consistently modify gut microbiota composition, only a few studies explored their effect on neurobehavioural functioning. Human studies, while notably scarce, showed positive effects on hypothalamic-pituitary-adrenal axis activity, emotional attention, and anxiety and depression symptomology (Azpiroz et al., 2017; Farhangi et al., 2017; Schmidt et al., 2015). Animal studies revealed effects on stress response (Forsatkar et al., 2017), anxiety- and depressive-like behavior (Mika et al., 2017; Mika et al., 2018; Savignac et al., 2016), stress-induced sleep alterations (Thompson et al., 2016), cognition (Gronier et al., 2018), and related neurobiological mechanisms such as GABA and serotonin receptor gene expression (Burokas et al., 2017) and BDNF and N-Methyl-D-Aspartate receptor subunit levels (Savignac et al., 2013; Williams et al., 2016). None of the human, and only some of these animal studies, concurrently quantified microbiota composition and found increased abundance in faecal *Bifidobacterium* and *Lactobacillus* spp. using selective bacterial culture or quantitative polymerase chain reaction (Azpiroz et al., 2017; Gronier et al., 2018; Kao et al., 2018; Mika et al., 2017; Mika et al., 2018; Savignac et al., 2013; Savignac et al., 2016; Thompson et al., 2016). One study (Burokas et al., 2017) used 16S rRNA sequencing and showed changes in β-diversity and shifts at different taxonomic levels. A limited subset of these studies, exclusively in rats, correlated prebiotic-induced changes in microbiota composition and relative abundance with changes in brain function. Prebiotic-induced increases in faecal *Lactobaccillus* spp. positively correlated with altered cfos and serotonin receptor gene expression in multiple brain regions (Mika et al., 2018), and predicted stress-protective alternations in mRNA expression in serotonergic dorsal raphe nucleus neurons during inescapable stress (Mika et al., 2017). Furthermore, lower levels of Deferribacteres following a prebiotic diet correlated with longer non-rapid eye movement episode durations (Thompson et al., 2016). Following ingestion of fructo- or galacto-oligosaccarides, number of faecal *Bifidobacteria* correlated positively with frontal cortex NR1 protein (Savignac et al., 2013).

Bacterial fermentation of DF leads to the production of SCFA (den Besten et al., 2013). SCFA – predominantly acetate, propionate, and butyrate – constitute the major anions in the colon and serve as energy source for colonocytes. Furthermore, they inhibit histone deacetylation and activate G-protein coupled receptors, thereby acting as signaling molecules linking diet, gut microbiota, and host (Tan et al., 2014) and interacting with gut-brain signaling pathways (Dalile et al., in press). Few studies explored whether the effects of DF/prebiotics on brain function are mediated by SCFA. Fructo- and galacto-oligosaccaride-induced increases in cecal SCFA correlated with effects on depressive and anxious behavior and stress responses as well as changes in gene expression in mice (Burokas et al., 2017). Prebiotic Bimuno galacto-oligosaccharides increased plasma acetate levels (Gronier et al., 2018; Kao et al., 2018), cortical GluN2B subunits (involved in glutamate neurotransmission), and Acetyl Co-A Carboxylase mRNA, all of which also increased following direct administration of acetate (Gronier et al., 2018), suggesting that acetate may play a mechanistic role in the observed effects. Other studies that explored the effects of SCFA administration on brain function have been reviewed elsewhere (Dalile et al., in press).

Although current evidence does not convincingly support a causal role of gut microbiota in modulating neurobehavioral functioning, we believe that prebiotics have a higher potential than probiotics to warrant such causal claims. However, before maintaining such claims, high-quality, adequately powered (human) prebiotic intervention studies, measuring both changes in microbiota composition or function (Bindels et al., 2015) and psychobiological functioning using state-of-the-art methodology are needed. Such interventions should utilize mediation analysis to estimate the contribution of microbiota composition/function in the observed psychobiological effects. Claims that causally implicate the role of gut microbiota in MGB axis should be based on studies that isolate products of microbial activity and directly demonstrate their causal effects on the brain.

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