The Role of the Microbiota-Gut-Brain Axis in Neurodevelopment and Mental Health in Childhood and Adolescence

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Abstract and Keywords

Evidence for the importance of the intestinal microbiota in the development and function of the central nervous system is growing apace. Most of the work in this area has been conducted in translational rodent models but clinical studies are increasing. Studies examining the role of intestinal bacteria in brain health and disease are of high interest but most focus on the extremes of age. There is a need for more work investigating the microbiota-gut-brain axis during the childhood and adolescent windows of critical brain development. Here we outline current thinking regarding general patterns of brain development and intestinal microbial change during early childhood and adolescence and emphasize that dynamic shifts in intestinal colonization occur concurrently with the journey to neurodevelopmental milestones. We review the body of evidence that supports the hypothesis that bugs can alter neural function, with specific focus on what is known regarding bacterial input on brain development in youth.

Keywords: adolescence, childhood, microbiome, microbiota-gut-brain axis, neurodevelopment, prebiotics, probiotics, psychobiotics

Introduction

There is a growing body of literature recognizing the importance of the intestinal microbiota in the development and function of the central nervous system (CNS). Animal studies ranging from those that use germ-free (GF) mice (animals that are bred and maintained in the total absence of bacteria), mice that have been treated with antibiotics during various stages in development, pre- and probiotic feeding studies, fecal microbial transplantation experiments, as well as work that has examined the impact of pathogenic infection have all indicated that the bacterial status of the intestinal lumen has far-reaching and long-term effects on brain neurochemistry, anatomy and behavior (Bercik et al., 2011b; Diaz Heijtz et al., 2011; Neufeld et al., 2011; Bravo et al., 2011; Collins et al., 2013; Hsaio et al., 2013; Ogbonnaya et al., 2015; Luczynski et al., 2016b; Bastiaanssen et al., 2019). In addition, preliminary evidence in human clinical trials indicates that feeding certain pre- and probiotics may have beneficial effects on brain function (Benton et al., 2007; Messaoudi et al., 2011a; Messaoudi et al., 2011b; Tillisch et al., 2013; Schmidt et al., 2015; Steenbergen et al., 2015; Huang et al., 2016; Cerdo et al., 2017; Reis et al., 2018). There is keen interest in microbiota studies particularly focused on early life, childhood and young adulthood, as this is the time of life when the brain is most plastic and vulnerable to peripheral influence and thus more likely to be sensitive to changes in the intestinal microbiome. Similarly, work focusing on the potential role of the intestinal microbiome in the pathogenesis of a variety of childhood neurodevelopmental illnesses is of high priority. In this chapter we will review the basic concepts of the microbiota-gut-brain axis as well as briefly touch on what is known with regards to potential mechanisms whereby this crosstalk occurs. We will outline current thinking regarding general patterns of intestinal microbial change during early childhood and adolescence and emphasize that dynamic shifts in intestinal colonization occur concurrently with the journey to neurodevelopmental milestones. We will review the body of evidence that supports the hypothesis that bugs can alter neural function, with specific focus on what is known regarding bacterial input on cognitive development in youth. Much of the neurodevelopmental work done to date is preclinical in nature, but there is some evidence of microbial alterations in children with neurodevelopmental disorders that will also be reviewed. Lastly, we will discuss the hypothesis that specifically targeting the intestinal microbiome will provide promising and novel therapeutic opportunities in the treatment of brain-related disease.

Microbiota-Gut-Brain Axis (a Primer) and Mechanisms for the Crosstalk

The gut-brain axis is a bidirectional highway of communication linking the peripheral functioning of the gastrointestinal tract with central brain processing, including emotional and cognitive centers. It has been studied both preclinically and clinically for several decades and its existence has been well established in large part due to work focused on the highly comorbid psychiatric and bowel disorders (Mayer, 2011; Al Omran and Aziz, 2014). During roughly the last decade, this axis has been reframed in order to highlight the intestinal microbiota as a key player, as an ever-growing body of research has brought to light the physiological importance of the trillions of bugs residing and working in the intestinal tract (Rhee et al., 2009; Bercik, 2011; Cryan and Dinan, 2012; Mayer et al., 2014; Quigley, 2018). Adult humans house roughly 0.2 kg of intestinal microbiota (Sender et al., 2016a), and the ratio of bacterial cells to human host cells is now thought to be ~ 1:1 (Sender et al., 2016b). Both bacteria and host benefit from a mutualistic relationship; the host gains as bacteria aid in the breakdown of otherwise indigestible food products, and in addition these bacteria provide a protective biofilm against pathogenic bugs. The bacteria benefit from their relationship with the host as they gain a protected environment, rich in life-giving resources (Backhed et al., 2005). Perhaps even more interesting than the existence of an essentially invisible but highly complex ecosystem flourishing within our guts is the more far-reaching consequences of host-microbe interactions; we now know that these bugs are essential for health and wellbeing, and that this extends to brain function and potentially human mental health. It is now clear that the microbiota influence the expression of host behavior and furthermore it is quite possible that the pathophysiology of psychiatric disease is at least in part dictated by our relationship with intestinal microbiota (Foster and McVey Neufeld, 2013).

While the body of literature surrounding the effects of microbes on the brain is steadily growing, there are far fewer studies examining mechanisms by which the bacteria may exert their influence. We do know that there are a number of systems involved in communication between the bugs, gut, and brain, and that these work in parallel. Neural (both autonomic and enteric), immune, metabolic, and endocrine pathways are all engaged in the constant crosstalk between microbiota and the brain (Cryan and Dinan, 2012; Foster and McVey Neufeld, 2013; Mayer et al., 2014; El Aidy et al., 2015). In addition, the microbiota themselves are known to produce a variety of chemical messengers including short-chain fatty acids, oligosaccharides, neurotransmitters, and other neuroactive agents (Puertollano et al., 2014; Wall et al., 2014; Donia and Fischbach, 2015; Levy et al., 2016). While it is currently unclear how these bacteria-derived neuroactive products might act on the brain, or indeed even if their production is being altered in physiologically relevant levels by bugs, this potential mechanism of action whereby intestinal bacteria can influence the host brain warrants careful examination (Forsythe et al., 2016).

Some of the difficulties in teasing apart mechanisms by which the microbiota may exert influence on the brain can be highlighted by an examination of work done in GF animals. First studies indicating a role for commensal bacteria on behavior and brain neurochemistry were initially conducted in GF mice, and these animals have been extremely useful in highlighting the importance of bacterial colonization to host health—particularly with respect to CNS function and behavior. GF mice are raised and maintained in aseptic environments and demonstrate significant alterations in behavior, with deficits observed in anxiety-like behavior (Diaz Heijtz et al., 2011; Neufeld et al., 2011; Clarke et al., 2013), social behavior (Desbonnet et al., 2014; Arentsen et al., 2015), cognition and memory (Gareau et al., 2011), and fear learning behavior (Hoban et al., 2018). In addition, these animals show altered expression of brain mRNA for molecules key to learning and memory and neurotransmission (Sudo et al., 2004; Gareau et al., 2011; Diaz Heijtz et al., 2011; Neufeld et al., 2011; Clarke et al., 2013; Arentsen et al., 2015). GF animals also show impairments in hypothalamic-pituitary-adrenal axis activity (Sudo et al., 2004; Neufeld et al., 2011; Clarke et al., 2013), neurogenesis (Ogbannaya et al., 2015), blood-brain barrier function (Braniste et al., 2014), microglial activation (Erny et al., 2015), and myelination (Hoban et al., 2016b). Anatomically, the brains of GF mice are found to be significantly different from conventionally housed mice, in terms of both increased hippocampal and amygdalar volume, as well as dendritic morphology (Luczynski et al., 2016b). These findings can be, however, difficult to interpret when trying to tease apart mechanisms of action. GF mice are known to be, from an immunological and metabolic perspective, distinctly abnormal. Indeed historically, GF mice were primarily known for their use in immunological research. Defects are clearly observed in the lymphoid tissue development of spleen, thymus, and lymph nodes (Gensollen et al., 2016). The mucosal immune system is significantly undeveloped, and the toll-like receptors therein are decreased or absent in the GF gut (Abrams et al., 1963; Wostmann et al., 1970). In addition these mice have reduced immunoglobulin A (IgA) secretion as well as fewer and smaller lymphoid patches in the gut wall and immune responses are blunted as compared to normally housed “conventional” animals (Clarke et al., 2013). Given these obvious immune impairments, and that immune function clearly impacts that of the brain, it is difficult then to ascertain if CNS alterations in GF mice are a direct result of the complete absence of colonizing bacteria, or are instead due to their significant immune deficiencies (Luczynski et al., 2016a). Interestingly, colonizing GF mice with intestinal bacteria is sufficient to generate an almost entirely normal immune response, particularly if bacteria are introduced in the early postnatal period (Crabbe et al., 1970; Rakoff-Nahoum et al., 2008; Geuking et al., 2011), while it seems only some of the observed CNS abnormalities can be normalized outside of a critical window for early life colonization. Similarly, the metabolic deficits inherent to GF mice may be responsible for observed CNS dysfunction. GF animals need to eat more in order to maintain body weight (Wostmann et al., 1983) and are in fact resistant to diet-induced obesity (Backhed et al., 2004; Backhed et al., 2007). Given that microbiota are responsible for harvesting otherwise inaccessible nutrients, it is also unsurprising that they have reduced short chain fatty acid production (Hoverstad and Midtvedt, 1986) and require vitamins B and K supplementation in order to survive (Gustafsson, 1959; Sumi et al., 1977). Again, it is difficult to ascertain if the metabolic deficiencies observed in GF animals are in fact responsible for some of the alterations observed in CNS functioning. The enteric nervous system (ENS) too is significantly different in GF mice as compared to their conventionally reared counterparts. The ENS is considered immature in GF mice, and it can be normalized after colonization with intestinal bacteria (McVey Neufeld et al., 2013; Collins et al., 2014; De Vadder et al., 2018). Neurons in the ENS control gut motility and other gastrointestinal physiological functions, but also directly communicate with the vagus nerve. Electrophysiological recordings made from the afferent nerve connecting gut to brain have also been demonstrated as abnormal in GF mice compared to controls (McVey Neufeld et al., 2015). Previous work in mice has demonstrated that an intact vagal nerve is necessary in order to transmit some aspects of microbiota-gut-brain communication, but it seems as though this varies depending on the specific bacterial species in question, as both vagal dependence (Lyte et al., 2006; Bercik et al., 2011b; Bravo et al., 2011) and independence (Bercik et al., 2010; Bercik et al., 2011a) have been demonstrated in vagotomy experiments. It is clear that the neural, immune, endocrine, and metabolic systems are all responsive to changes in the intestinal bacterial status, but also that all are able to reciprocally exert influence on the gut microbiota themselves (Hooper et al., 2012; Nicholson et al., 2012; Neuman et al., 2015; Zaneveld et al., 2017). However, due to the overlapping nature of these systems, and that they are all able to independently influence the function of the CNS, this also makes the study of the microbiota-gut-brain axis and the mechanisms of communication particularly challenging. There has thus been a well-reasoned call for an increase in multidisciplinary approaches to the study of the microbiota-gut-brain axis in order to further our understanding of how these systems work in concert to impact human health (Johnstone and Cohen Kadosh, 2019). However for the time being we are certain that cross-communication does occur, but are still in the relative infancy of understanding exactly how this happens.

Microbiota Development

Our understanding of what makes up the constituent bacteria in the average healthy human gut over the lifespan is under constant revision, in part due to the continual refinement of techniques used to identify and quantitatively analyze resident organisms (Mallick et al., 2017). While it has been challenging to link specific microbial types or combinations to human health outcomes, at least in broad strokes it has been possible through several large, population-scale studies to identify a working definition of what constitutes a “healthy microbiome” (Lloyd-Price et al., 2016).

The controversy and changing opinion with regards to what constitutes a healthy bacterial load begins with the fetus. While this topic is discussed in detail in the chapter of this textbook entitled “Development of the Microbiome-Gut-Brain Axis and its Effect on Behavior,” we will briefly say that for more than a century, the developing human was considered to be completely void of bacteria on any surface, including the placenta and womb itself. This is currently contentious, as it has recently been proposed that bacteria are in fact present during the gestational period and that the human placenta, meconium, and amniotic fluid contain their own constituent microbiome (Jimenez et al., 2005; Jimenez et al., 2008; Aagard et al., 2014; Collado et al., 2016; Mishra et al., 2021). However, some studies have failed to find evidence for cultivable bacteria (Perez-Munoz et al., 2017; Kennedy et al., 2021) or bacterial nucleic acids (de Goffau et al., 2019; Kuperman et al., 2020; Kennedy et al., 2021) in fetal meconium or associated tissues. Irrespective of the controversy, it is widely accepted that the majority of colonization of the gut occurs primarily during birth and in the immediate subsequent postnatal days and weeks (Dominguez-Bello et al., 2010; Funkhouser and Bordenstein, 2013; Dominguez-Bello et al., 2019).

The first 3–4 years of life are characterized by a rapid increase in bacterial diversity (Koenig et al., 2011; Yatsunenko et al., 2012; Stewart et al., 2018; Niu et al., 2020) driven in large part by environmental exposure and increasingly complex food intake (Koenig et al., 2011; Stewart et al., 2018) which then stabilizes in early childhood to a relatively constant state (Stewart et al., 2018 Yatsunenko et al., 2012. The childhood microbiome has, as a result, often been considered comparable to that of an adult; nonetheless there appear to be subtle continuing changes that occur with further maturation of the gut communities even into adolescence (Ihekweazu et al., 2018). For example, one study compared the fecal microbiota of adolescent children to those of healthy adults and found that, while the number of species detected was not significantly different between groups, the relative abundances of specific bacterial genera were different (Agans et al., 2011). Higher numbers of *Bifidobacterium* were observed in the younger cohort, with adults presenting with more *Bacteroides* (Agans et al., 2011; Hollister et al., 2015). Subsequent research found that children harbored bacteria linked to growth and development, while adult bacterial communities were associated with inflammation and obesity (Hollister et al., 2015). There is also some evidence that gut microbiota composition may be influenced by hormonal changes at puberty. Yuan and colleagues (Yuan et al., 2020) found that, while no differences in diversity measures were detectable between children pre- or post-puberty, there were distinct changes in some genera which in several cases correlated with testosterone levels. Interestingly, adolescence may also be a period during which microbiome composition is repaired following perturbations earlier in life. While microbiome development is known to be substantially influenced in early life by both delivery mode and feeding type (formula vs breastfed) (Stewart et al., 2018), a recent study found that these effects were largely undetectable by adolescence (Cioffi et al., 2020).

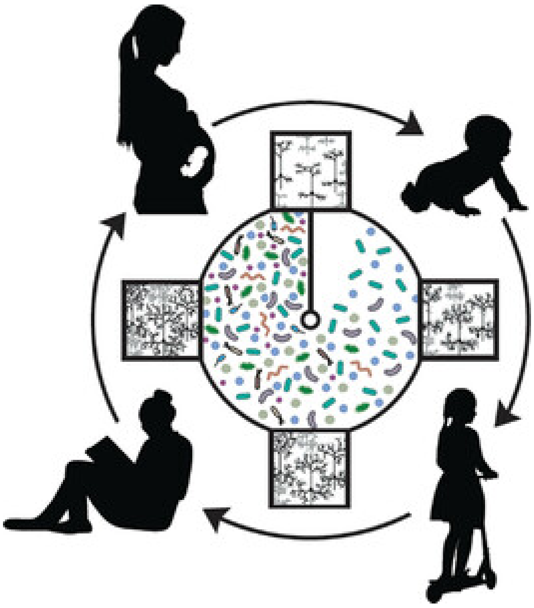

Figure 29.1 Microbiota and neural development from infancy to adulthood. The intestinal microbiota and the brain develop in parallel from infancy, through childhood and adolescence into adulthood. Reciprocal interactions occurring between the brain and gut highlight the importance of a normal trajectory of development for the intestinal bacteria, as perturbations may lead to adverse mental health outcomes and brain function later in life.

Altogether, research is beginning to demonstrate that childhood and adolescence are important periods of microbiome development, but more studies are needed to examine the normal development and change of intestinal bacteria over the lifespan. Most of the studies that have been carried out to date have dealt with the extremes of age, and we particularly need more information on the gradual change in bacterial communities that occurs during later periods of childhood and through adulthood. Importantly, we do know that intestinal bacteria are susceptible to environmental influence (McVey Neufeld et al., 2016a; McVey Neufeld et al., 2016b), and that this differs from person to person over the lifespan. Once we have established what can be considered normal and healthy gut colonization patterns, studies examining how life experience and environmental challenge may contribute to changes in the intestinal microbiota and thus to human health need follow. What is abundantly clear now is that, from the perinatal period throughout childhood and adolescence, the gut is continuously colonizing with bacterial species, and that this is happening in parallel with ongoing nervous system development (see Figure 29.1). Given that we know the intestinal microbiota have a lasting and significant impact on brain development and function (Foster and McVey Neufeld, 2013), it is crucial that we gain an understanding of how these two systems developmentally mature together.

Brain Development During Childhood and Adolescence

This section focusses on neurodevelopment following the immediate postnatal period, leading up to and including adolescence. Again, for a full discussion of both pre- and early postnatal neurodevelopment, we direct the reader to the chapter in this textbook entitled “Development of the Microbiome-Gut-Brain Axis and its Effect on Behavior.” The infant brain is approximately four times smaller than it will be in adulthood and given that neurogenesis by this time is largely complete (van Dyck and Morrow, 2017), the growth in brain size over childhood and adolescence must therefore be primarily due to other factors. During the early years of childhood, the rapid increases seen in gray matter are likely due to increased synaptic density, dendritic elaboration, and glia production (van Dyck and Morrow, 2017), while increases in white matter reflect ongoing myelination of axons (Craik and Bialystok, 2006). Research demonstrates continued myelination and increased white matter volume in most brain regions through to early adulthood (Semple et al., 2013), but it is important to emphasize that otherwise much of the continued brain development during childhood and adolescence is region specific (Brenhouse and Andersen, 2011). For example, in regions of the brain involved in sensory processing, early synaptogenesis is quickly followed by a period of increased synaptic pruning with synaptic density reaching adult levels just prior to or around adolescence (Cragg, 1975; Huttenlocher, 1979; Huttenlocher et al., 1982; Bourgeois and Rakic 1993; Huttenlocher and Dabholkar, 1997). In contrast, synaptic development follows a very different time course in the prefrontal cortex. Here, there is a proliferation of synapses during both childhood and adolescence, followed by a period of senescence, then a subsequent elimination of synaptic connections post-puberty (Huttenlocher, 1979; Bourgeois et al., 1993 Woo et al., 1997).

While it was previously thought that the brain was largely fully developed by the time puberty was reached, we now know that this is not the case. Adolescence is a period of profound neuroanatomical change and maturation (Spear, 2000; Blakemore and Choudhury, 2006; Sturman and Moghaddam, 2011) and for a detailed discussion of these changes please see an excellent summary by Brenhouse and Andersen, 2011. Two major processes continue during the adolescent period: synaptic pruning and myelination. Myelination accelerates in adolescence, thus increasing the efficiency of interneuronal communication and resulting in increased speed of information flow between the various brain regions

(Benes et al., 1989; Spear, 2013). This is in contrast to synaptic density, which decreases after peaking in childhood. This synaptic refinement is especially prevalent in adolescence, and is thought to increase brain efficiency (Spear, 2013), and results in an overall decrease in synaptic connections moving into adulthood (Huttenlocher, 1979; Woo et al., 1997). Synaptic pruning can be significant, and can result in the loss of up to 50% of synaptic connections in some regions, while other regions can see little decline (Spear, 2013). Pruning is an experience-dependent process and results in a more nuanced wiring, whereby frequently used connections become stronger and less used connections are eliminated (Holtmaat and Svoboda, 2009). While it is still currently unclear exactly how these processes occur, resident microglia in the brain are necessary for both the strengthening and elimination of synapses (Hua and Smith, 2004; Schafer et al., 2012). These processes are especially notable in the prefrontal cortex and other areas associated with cognition, where structural and functional changes in adolescence correlate with improvements in inhibitory control and other adult-typical behaviors (Burnett et al., 2011; Spear, 2013).

Both brain imaging and postmortem studies have provided additional evidence of the ongoing maturation of the frontal cortex during adolescence, and adds to the evidence of delayed maturation in frontal regions thought to reflect immaturities in certain cognitive processes like attention regulation and cognitive control (Huttenlocher, 1979; Huttenlocher et al., 1982; Spear, 2013). Frontal and parietal lobes both show increases in grey matter volume over childhood, which peak in adolescence, and then slowly begin to decrease (Giedd et al., 1999; Sowell et al., 2001). At the same time, white matter shows a fairly constant increase, likely due to the observed increases in myelination (Barnea-Goraly et al., 2005). While further work is necessary examining the exact nature of change in the adolescent brain and the mechanisms by which these occur, what is clear is that the teenage brain is no longer viewed as existing in a static period of development. Increasingly we are recognizing that other physiological systems like the intestinal microbiota play an integral role in how these developmental changes manifest.

Childhood and Adolescent Microbiota-Gut-Brain Axis

The ongoing changes occurring in the brain during childhood and adolescence highlight both its plasticity but also potential vulnerability to environmental input. It is vital to stress that the critical window of brain development occurring during childhood and adolescence is occurring simultaneous to ongoing changes in the intestinal microbiome. The composition and diversity of the gut microbiota undergoes a shift in structure and balance during adolescence–the trajectory of which can also be altered by the environment (Borre et al., 2014; Cowan et al., 2021). In addition, a crucial factor that cannot be ignored is the developing youth’s exposure to a wide variety of environmental stimuli all of which are known to influence both the gut and brain. A host of environmental stimuli such as alcohol, prescription and nonprescription drug intake, changes to nutritional status and sleep schedule, as well as the occurrence of social stressors are all frequently part of a youth life experience and are all also known to impact the intestinal microbiota and the brain alike (McVey Neufeld et al., 2016a; McVey Neufeld et al., 2016b). It is also important to be cognizant that adolescence is often the first time when psychiatric disorders first become apparent. Given that gut microbiota can influence the development of the brain, changes occurring at the intestinal microbial level may contribute to the manifestation of psychiatric illnesses associated with puberty. It is thus imperative that we investigate the potential harmful effects of such environmental exposures to the ongoing development of the microbiota-gut-brain axis and how these may affect the incidence and manifestation of neurodevelopmental and psychiatric disorders.

Findings from Animal Studies

Much has been gleaned regarding the potential role for intestinal microbiota in neurodevelopment from experiments using GF mice. As stated previously these animals, which have been born and raised in the complete absence of bacteria, show abnormal brain development and functioning (Sudo et al., 2004; Diaz Heijtz et al., 2011; Neufeld et al., 2011; Clarke et al., 2013; Braniste et al., 2014; Desbonnet et al., 2014; Erny et al., 2015; Stilling et al., 2015; Gacias et al., 2016; Chen et al., 2017; Hoban et al., 2018; Thion et al., 2018). Of particular interest is work that has demonstrated both altered social behaviors and cognitive functioning. GF mice typically demonstrate sociability deficits and social memory impairments (Desbonnet et al., 2014; Arentsen et al., 2015; Buffington et al., 2016; Stilling et al., 2018; Sgritta et al., 2019), and these works have strengthened the hypothesis that microbiota-gut-brain axis dysfunction may play an important role in the etiology and development of autism spectrum disorder. Learning and memory is also impaired in GF animals (Gareau et al., 2011; Hoban et al., 2018; Luk et al., 2018; Chu et al., 2019) again emphasizing the importance of examining the role for intestinal microbial disruption in human cognitive disorders.

Given the limitations of GF studies (discussed previously) and their apparent lack of clinical relevance, work examining the impact of induced microbial disruption during early life and up to adolescence has gained importance. While this chapter focusses on studies examining the effects of microbial disruption occurring during periods relevant to childhood and adolescence, we direct the reader to the chapter in this text entitled “Development of the Microbiome-Gut-Brain Axis and its Effect on Behavior” for a discussion of the effects of microbial disruption occurring during the perinatal period. Data gathered from antibiotic feeding studies in adult rodents is increasingly showing resulting alterations to brain and behavior (Frohlich et al., 2016; Hoban et al., 2016a; Wang et al., 2015) indicating central effects following intestinal bacterial disruption. However antibiotic studies conducted in rodents around the period of weaning from their mothers up until what is considered an “early adulthood” developmental timepoint have not been plentiful to date. One such study examined the effects of feeding penicillin V to young mice for one week prior to weaning and then examined behavior and mRNA expression in brain tissue in adulthood. Antibiotic treated male mice showed altered social behavior with concomitant changes to hippocampal gene expression of the arginine vasopressin receptor 1A and 1B as well as changes to immune cell activation. Interestingly, feeding probiotic simultaneous with the antibiotic resulted in normalization of behaviors, brain gene expression and immune cell functioning (Kayyal et al., 2020). A separate antibiotic feeding study demonstrated that antibiotics administered during the adolescent period in mice resulted in significant changes to adult behavior, brain neurochemistry, and intestinal microbial content when compared to control animals (Desbonnet et al., 2015). At weaning, or 3 weeks of age in mice, animals were given an antibiotic treatment cocktail via the drinking water for 4 weeks, and then behavior was measured later in adulthood. These treated mice showed decreased anxiety-like behavior, altered cognitive function in the novel object recognition test, and also deficits in social behaviors. In addition, antibiotic treated mice showed significantly increased serum tryptophan and reduced kynurenine levels. They also had reduced gene expression of hippocampal brain-derived neurotrophic factor (BDNF), and less vasopressin messenger ribonucleic acids (mRNA) in the hypothalamus (Desbonnet et al., 2015). This study was the first to show that antibiotic treatment during adolescence in mice can result in significant changes to brain function and gene expression in adulthood and demonstrates that more work in this area is certainly required.

Another rodent study examined the effects of treating adolescent rats with propionic acid, a short chain fatty acid that is produced by intestinal bacteria (MacFabe et al., 2011). Propionic acid is a normal product of metabolism, but both it and its derivatives have been implicated in disease, particularly autism spectrum disorder. Male rats were treated with propionic acid intracerebroventriculary prior to behavioral testing, and the authors found that these animals showed impairments in social behavior and cognitive tests. When the brains were analyzed via immunohistochemistry they observed a significant upregulation of activated microglia. It should be noted that while the propionic acid was delivered via intracerebroventricular injection in these experiments, this acid does get manufactured in the gut by commensal bacteria and does readily pass the blood-brain barrier in a nonpathological state. The authors argue that their behavioral and immunohistochemical results provide evidence that propionic acid could be linked to autism spectrum disorder.

Findings from Human Studies

While interest is high, there have still been relatively few studies examining the relationship between the intestinal microbiome and behavior in children and adolescents. Again, while not covered in this chapter, there are a few studies examining the relationship between the intestinal bacteria and temperament prior to the first year of life but less at later timepoints in development. One association study measured the constituent microbiota and cognitive performance at later stages of development and showed that the composition of the microbiota at one year of age successfully predicted cognitive performance at age 2. In this study, lower bacterial diversity measures were predictive of better cognitive performance (Carlson et al., 2018). There have also been studies examining the potential ramifications of antibiotic use on children’s cognitive performance. In the first of these by Slykerman and colleagues, the authors reported that antibiotic use prior to one year of life was associated with lower IQ and reading ability as well as behavioral disturbances when assessed at early school age (Slykerman et al., 2017b). In a follow up study these authors went on to examine the relationship between the timing of antibiotic use prior to 2 years of age and neurocognitive outcomes at age 11 through use of a regression model (Slykerman et al., 2019). The authors reported that children who received antibiotics for the first time prior to 6 months of age had significantly lower cognitive abilities at age 11, including difficulties with verbal comprehension and increased problems with executive functioning, impulsivity, hyperactivity, and attention-deficit hyperactivity disorder (ADHD) (Slykerman et al., 2019). It is important to note however that these authors did not control for the reasons behind the antibiotic use in early childhood and thus a cautious interpretation of the results must follow. A more recent study examined premature babies that received perinatal antibiotics either via the mother prior to birth or during their stay at a neonatal hospital unit. In this case the authors did exclude those infants found to have been suffering from infection, and found an association between antibiotic use and attention issues at 4–5 years of age (Firestein et al., 2019). These children also demonstrated EEG patters consistent with those observed in children suffering from ADHD (Firestein et al., 2019).

There have been a number of pediatric studies examining the resident microbiota of individuals suffering from ADHD, and many report that these patients have an altered microbial profile as compared to age-matched, healthy control subjects (Aarts et al., 2017; Jiang et al., 2018; Wan et al., 2020). While these studies and others report alterations to the microbial signature of patients with ADHD as compared to controls, findings between studies have been inconsistent, and thus it has not been possible to find a specific microbial signature. For this reason, a larger scale study examining the gut microbiota of treatment-naïve adults suffering from ADHD was conducted, in which authors examined 100 ADHD subjects and 100 sex-matched controls. These authors found differing relative abundances of several microbial taxa in subjects with ADHD, and at the family level lower abundances of Gracilibacteraceae, and higher levels of Selenomonadaceae, and Veillonellaceae (Richarte et al., 2021).

There is currently keen interest in potential associations between altered gut microbiota and autism spectrum disorder (Vuong and Hsiao 2017). Autism is a neurodevelopmental disorder characterized by impairments in social communication and repetitive, stereotyped behaviors. Causes of autism are unclear, but there is high comorbidity with gastrointestinal dysfunction such as diarrhea/constipation and abdominal pain. Altered intestinal microbiota has also been reported in these patients (Parracho et al., 2005; Strati et al., 2017). There have been several studies reporting an improvement in autism symptoms in patients following a course of antibiotics (Sandler et al., 2000; Ramirez et al., 2013; Urbano et al., 2014), which has been similarly modeled in animal studies (Wellmann et al., 2014; Kumar and Sharma, 2016). In addition there have been a few probiotic studies designed to assess any improvement in behavioral symptoms following a course of treatment. One such study reported an improvement in bowel function and possibly some behavior alterations in a 12-week oral treatment study of 3–16-year-olds (Parracho et al., 2010). Another study reported that feeding a probiotic supplement up until 6 months of age resulted in a reduced risk for autism disorder at 13 years of age (Partty et al., 2015).

Again, future well-controlled studies are needed in this area in order to further our understanding of the potential role of the microbiota-gut-brain axis in autism.

Future Directions

While research into the microbiota-gut-brain axis has received an enormous amount of attention in the last decade, there is still a paucity of information regarding both mechanism of action of the bugs on brain neurodevelopment, and on human data and clinical trials. This is particularly true for the childhood and adolescent developmental periods. In spite of the lack of information regarding mechanism, interest and study in microbiota-gut-brain communication is expanding rapidly, and it is unsurprising that attention has now been directed toward developing therapeutics targeting this axis in the treatment of brain-related disease. Much work is currently aimed at understanding how pre- and probiotics may potentially treat these often-refractory illnesses. The therapeutic potential of bacteria in the treatment of psychiatric illnesses has indeed led to the development of the term *psychobiotic,* referring to a live organism that can be ingested to produce particular health benefits (Dinan et al., 2013). To date, some studies have been conducted examining the potential for mind altering effects of pre- and probiotics, and most have been conducted in healthy adults with mood and anxiety symptoms as outcome measures (Benton et al., 2007; Messaoudi et al., 2011a; Messaoudi et al., 2011b; Mohammadi et al., 2015; Steenbergen et al., 2015; Takada et al., 2016; Pinto-Sanchez et al., 2017; Slykerman et al., 2017a; Johnstone et al., 2021), although a few of these studies have been conducted in the depressed patient population (Akkasheh et al., 2016; Kazemi et al., 2018). Far fewer studies have focused on youth and their potential response to pre- or probiotic treatment (for a systematic review of these studies see Cohen Kadosh et al., 2021). This promising line of research needs to be expanded in childhood and adolescent clinical trials such as those reviewed in order to determine the potential benefits of pre- and probiotic treatment during neurodevelopment.

Concluding Remarks

The developing brain is unsurpassed in its potential for growth and plasticity, and yet at the same time incredibly vulnerable to outside environmental influence, including exposure to the changing status of intestinal microbes. From the prenatal period up to and including adolescence, the brain is maturing and changing, simultaneous with changes mirrored in the intestinal bacteria. This can be viewed as an exciting opportunity, as the potential for brain plasticity can be harnessed by therapeutics targeted to the peripheral gut microbiome. We would be wise to turn our attention to furthering our understanding of both the normal healthy progression of intestinal microbiota in the youth, as well as to the impact of abnormal microbial development on the brain. That the microbiome could act as a potential therapeutic target in the improvement of mental resiliency and in the treatment of brain disorders is a challenging but exciting possibility for the future of mental health.

References

Aagard, K., Ma, J., Antony, K. M., Ganu, R., Petrosino, J., and Versalovic, J. (2014). The placenta harbors a unique microbiome. *Science Translational Medicine*, 6: 237ra65.

Aarts, E., Ederveen, T. H. A., Naaijen, J., Zwiers, M. P., Boekhorst, J., et al (2017). Gut microbiome in ADHD and its relation to neural reward anticipation. *PLOS One*, 12: e0183509.

Abrams, G. D., Bauer, H., and Sprinz, H. (1963). Influence of the normal flora on mucosal morphology and cellular renewal in the ileum. A comparison of germ-free and conventional mice. *Laboratory Investigations*, 12: 355–364.

Agans, R., Rigsbee, L., Kenche, H., Michail, S., Khamis, H. J., and Paliy, O. (2011). Distal gut microbiota of adolescent children is different from that of adults. *FEMS Microbiology Ecology*, 77: 404–412.

Akkasheh, G., Kashani-Poor, Z., Tajabadi-Ebrahimi, M., Jafari, P., Akbari, H., et al. (2016). Clinical and metabolic response to probiotic administration in patients with major depressive disorder: A randomized, double-blind, placebo-controlled trial. *Nutrition*, 32: 315–320.

Al Omran, Y. and Aziz, Q. (2014). The brain-gut axis in health and disease. *Advances in Experimental Medicine and Biology*, 817: 135–153.

Arentsen, T., Raith, H., Qian, Y., Forssberg, H., and Heijtz, R. D. (2015). Host microbiota modulates development of social preference in mice. *Microbiota in Health and Disease*, 26: 29719.

Backhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., Semenkovich, C. F., and Gordon J. I. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the National Academy of Sciences* U S A, 101: 15718–15723.

Backhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A., and Gordon, J. I. (2005). Host-bacterial mutualism in the human intestine. *Science*, 307: 1915–1920.

Backhed, F., Manchester, J. K., Semenkovich, C. F., and Gordon, J. I. (2007). Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proceedings of the National Academy of Sciences* U S A, 104: 979–984.

Barnea-Goraly, N., Menon, V., Eckert, M., Tamm, L., Bammer, R., Karchemskiy, A., Dant, C. C., and Reiss, A. L. (2005). White matter development during childhood and adolescence: A cross-sectional diffusion tensor imaging study. *Cerebral Cortex*, 15(1848): 54.

Bastiaanssen, T. F. S., Cowan, C. S. M., Claesson, M. J., Dinan, T. G., and Cryan, J. F. (2019). Making sense of …the microbiome in psychiatry. *International Journal of Neuropsychopharmacology*, 22: 37–52.

Benes, F. M. (1989). Myelination of cortical-hippocampal relays during late adolescence. *Schizophrenia Bulletin*, 15: 585–593.

Benton, D., Williams, C., and Brown, A. (2007). Impact of consuming a milk drink containing a probiotic on mood and cognition. *European Journal of Clinical Nutrition*, 61: 355–361.

Bercik, P. (2011). The microbiota-gut-brain axis: Learning from intestinal bacteria? *Gut*, 60: 288–289.

Bercik, P., Verdu, E. F., Foster, J. A., Macri, J., Potter, M., Huang, X., Malinowski, P., Jackson, W., Blennerhassett, P., Neufeld, K. A., Khan, W. I., Corthesy-Theulaz, I., Cherbut, C., Bergonzelli, G. E., and Collins, S. M. (2010). Chronic gastrointestinal inflammation induces anxiety-like behavior and alters central nervous system biochemistry in mice. *Gastroenterology*, 139: 2102–2112.

Bercik, P., Denou, E., Collins, J., Jackson, W., Lu, J., Jury, J., Deng, Y., Blennerhassett, P., Macri, J., McCoy, K. D., Verdu, E. F., and Collins, S. M. (2011a). The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology*, 141: 599–609.

Bercik, P., Park, A. J., Sinclair, D., Khoshdel, A., Lu, J., Huang, X., Deng, Y., Blennerhassett, P. A., Fahnestock, M., Moine, D., Berger, B., Huizinga, J. D., Kunze, W., McLean, P. G., Bergonzelli, G. E., Collins, S. M., and Verdu, E. F. (2011b). The anxiolytic effect of Bifidobacterium longum NCC3001 involves vagal pathways for gut-brain communication. *Neurogastroenterology & Motility*, 23: 1132–1139.

Blakemore, S. J. and Choudhury, S. (2006). Brain development during puberty: State of the science. *Developments in Science*, 9: 11–14.

Borre, Y. E., O’Keefe, G. W., Clarke, G., Stanton, C., Dinan, T. G., et al. (2014). Microbiota and neurodevelopmental windows: Implications for brain disorders. *Trends in Molecular Medicine*, 20: 509–518.

Bourgeois, J. P. and Rakic, P. (1993). Changes of synaptic density in the primary visual cortex of the macaque monkey from fetal to adult stage. *Journal of Neuroscience*, 13: 2801–2820.

Braniste, V., Al-Asmakh, M., Kowal, C., Anuar, F., Abbaspour, A., Toth, M., Korecka, A., Bakocevic, N., Ng, L. G., Gulyas, B., Halldin, C., Hultenby, K., Nilsson, H., Hebert, H., Volpe, B. T., Diamond, B., and Pettersson, S. (2014). The gut microbiota influences blood-brain barrier permeability in mice. *Science Translational Medicine*, 6: 263ra158.

Bravo, J. A., Forsythe, P., Chew, M. V., Escaravage, E., Savignac, H. M., Dinan, T. G., Bienenstock, J., and Cryan, J. F. (2011). Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proceedings of the National Academy of Sciences*, U S A, 108: 16050–16055.

Brenhouse, H. C. and Andersen, S. L. (2011). Developmental trajectories during adolescence in males and females: A cross-species understanding of underlying brain changes. *Neuroscience and Biobehavioral Reviews*, 35: 1687–1703.

Buffington, S. A., Di Prisco, G. V., Auchtung, T. A., Ajami, N. J., Petrosino, J. F., and Costa-Mattioli, M. (2016). Microbial reconstitution reverses maternal diet-induced social and synaptic deficits in offspring. *Cell*, 165: 1762–1775.

Burnett, S., Sebastian, C., Cohen Kadosh, K., and Blakemore, S. J. (2011). The social brain in adolescence: Evidence from functional magnetic resonance imaging and behavioural studies. *Neuroscience and Biobehavioral Reviews*, 35: 1654–1664.

Carlson, A. L., Xia, K., Azcarate-Peril, M. A., Goldman, B. D., Ahn, M., et al. (2018). Infant gut microbiome associated with cognitive development. *Biological Psychiatry*, 83: 148–159.

Cerdo, T., Ruiz, A., Suarez, A., and Campoy, C. (2017). Probiotic, prebiotic, and brain development. *Nutrients*, 9: 1247.

Chen, J. J., Zeng, B. H., Li, W. W., Zhou, C. J., Fan, S. H., et al. (2017). Effects of gut microbiota on the microRNA and mRNA expression in the hippocampus of mice. *Behavioural Brain Research*, 322: 34–41.

Chu, C., Murdock, M. H., Jing, D., Won, T. H., Chung, H., et al. (2019). The microbiota regulate neuronal function and fear extinction learning. *Nature*, 574: 543–548.

Cioffi, C. C., Tavalire, H. F., Neiderhiser, J. M., Bohannan, B., and Leve, L. D. (2020). History of breastfeeding but not mode of delivery shapes the gut microbiome in childhood. *PLOS One*, 15: e0235223.

Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R. D., Shanahan, F., Dinan, T. G., and Cryan, J. F. (2013). The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Molecular Psychiatry*, 18: 666–673.

Cohen Kadosh, K., Basso, M., Knytl, P., Johnstone, N., Lau, J. Y. F., et al. (2021). Psychobiotic interventions for anxiety in young people: A systematic review and meta-analysis, with youth consultation. *Translational Psychiatry*, 11: 352.

Collado, M. C., Rautava, S., Aakko, J., Isolauri, E., and Salminen, S. (2016). Human gut colonization may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. *Scientific Reports*, 6: 23129.

Collins, S. M., Kassam, Z., and Bercik, P. (2013). The adoptive transfer of behavioral phenotype via the intestinal microbiota: Experimental evidence and clinical implications. *Current Opinions in Microbiology*, 16: 240–245.

Collins, J., Borojevic, R., Verdu, E. F., Huizinga, J. D., and Ratcliffe, E. M. (2014). Intestinal microbiota influence the early postnatal development of the enteric nervous system. *Neurogastroenterology and Motility*, 26: 98–107.

Cowan, C. S. M., Dinan, T. G., and Cryan, J. F. (2021). Annual Research Review: Critical windows—the microbiota-gut-brain axis in neurocognitive development. *Journal of Child Psychology*, 6: 353–371.

Crabbe, P. A., Nash, D. R., Bazin, H., Eyssen, H., and Heremans, J. F. (1970). Immunohistochemical observations on lymphoid tissues from conventional and germ-free mice. *Laboratory Investigations*, 22: 448–457.

Cragg, B. G. (1975). The development of synapses in the visual system of the cat. *Journal of Comparative Neurology*, 160: 147–166.

Craik, F. and Bialystock, E. (2006). Cognition through the lifespan: Mechanisms of change. *Trends in Cognitive Science*, 10: 131–138.

Cryan, J. F. and Dinan, T. G. (2012). Mind-altering microorganisms: The impact of the gut microbiota on brain and behaviour. *Nature Reviews Neuroscience*, 13: 701–712.

de Goffau, M. C., Lager, S., Sovio, U., Gaccioli, F., Cook E., Peacock, S. J., Parkhill, J., Charnock-Jones, D. S., and Smith, G. C. S. (2019). Human placenta has no microbiome but can contain potential pathogens. *Nature*, 572: 329–334.

Desbonnet, L., Clarke G., Shanahan, F., Dinan, T. G., and Cryan, J. F. (2014). Microbiota is essential for social development in the mouse. *Molecular Psychiatry*, 19: 146–148.

Desbonnet, L., Clarke, G., Traplin, A., O’Sullivan, O., Crispie, F., Moloney, R. D., Cotter, P. D., Dinan, T. G., and Cryan, J. F. (2015). Gut microbiota depletion from early adolescence in mice: Implications for brain and behaviour. *Brain, Behavior, and Immunity*, 48: 165–173.

De Vadder, F., Grasset, E., Manneras Holm, L., Karsenty, G., Macpherson, A. J., Olofsson, L. E., and Backhed, F. (2018). Gut microbiota regulates maturation of the adult enteric nervous system via enteric serotonin networks. *Proceedings of the National Academy of Sciences* U S A, 115: 6458–6463.

Diaz Heijtz, R., Wang, S., Anuar F., Qian, Y., Bjorkholm, B., Samuelsson, A., Hibberd, M. L., Forssberg, H., and Pettersson, S. (2011). Normal gut microbiota modulates brain development and behavior. *Proceedings of the National Academy of Sciences* U S A, 108: 3047–3052.

Dinan, T. G., Stanton, C., and Cryan, J. F. (2013). Psychobiotics: A novel class of psychotropic. *Biological Psychiatry*, 74: 720–726.

Dominguez-Bello, M. G., Costello, E. K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., and Knight, R. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proceedings of the National Academy of Sciences* U S A, 107: 11971–11975.

Dominguez-Bello, M. G., Godoy-Vitorino, F., Knight, R., and Blaser, M. J. (2019). Role of the microbiome in human development. *Gut*, 68: 1108–1114.

Donia, M. S. and Fischbach, M. A. (2015). HUMAN MICROBIOTA. Small molecules from the human microbiota. *Science*, 349: 1254766.

El Aidy, S., Dinan, T. G., and Cryan, J. F. (2015). Gut microbiota: The conductor in the orchestra of immune-neuroendocrine communication. *Clinical Theory*, 37: 954–967.

Erny, D., Hrabe de Angelis, A. L., Jaitin, D., Wieghofer, P., Staszewski, O., David, E., Keren-Shaul, H., Mahlakoiv, T., Jakobshagen, K., Buch, T., Schwierzeck, V., Utermohlen, O., Chun, E., Garrett, W. S., McCoy, K. D., Diefenbach, A., Staeheli, P., Stecher, B., Amit, I., and Prinz, M. (2015). Host microbiota constantly control maturation and function of microglia in the CNS. *Nature Neuroscience*, 18: 965–977.

Firestein, M. R., Myers, M. M., Austin, J., Stark, R. I., Barone, J. L., et al. (2019). Perinatal antibiotics alter preterm infant EEG and neurobehaviour in the Family Nurture Intervention trial. *Developmental Psychobiology*, 61: 661–669.

Forsythe, P., Kunze, W., and Bienenstock, J. (2016). Moody microbes or fecal phrenology: What do we know about the microbiota-gut-brain axis? *BMC Medical*, 14: 58.

Foster, J. A. and McVey Neufeld, K. A. (2013). Gut-brain axis: How the microbiome influences anxiety and depression. *Trends in Neuroscience*, 36: 305–312.

Frohlich, E. E., Farzi, A., Mayerhofer, R., Reichmann, F., Jacan, A., et al. (2016). Cognitive impairment by antibiotic-induced gut dysbiosis: Analysis of gut microbiota-brain communication. *Brain, Behavior, and Immunity*, 56: 140–155.

Funkhouser, L. J. and Bordenstein, S. R, (2013). Mom knows best: The universality of maternal microbial transmission. *PLOS Biol*, 11: e1001631.

Gacias, M., Gaspari, S., Santos, P.-M. G., Tamburini, S., Andrade, M. et al. (2016). Microbiota-driven transcriptional changes in the prefrontal cortex override genetic differences in social behavior. *eLife*, 5: e13442.

Gareau, M. G., Wine, E., Rodrigues, D. M., Cho, J. H., Whary, M. T., Philpott, D. J., MacQueen, G., and Sherman, P. M. (2011). Bacterial infection causes stress-induced memory dysfunction in mice. *Gut*, 60: 307–317.

Gensollen, T., Iyer, S. S., Kasper, D. L., and Blumberg, R. S. (2016). How colonization by microbiota in early life shapes the immune system. *Science*, 352: 539–544.

Geuking, M. B., Cahenzli, J., Lawson, M. A., Ng, D. C., Slack, E., Hapfelmeier, S., McCoy, K. D., and Macpherson, A. J. (2011). Intestinal bacterial colonization induces mutualistic regulatory T cell responses. *Immunity*, 34: 794–806.

Giedd, J. N., Blumenthal, J., Jeffries, N. O., Castellanos, F. X., Liu, H., Zijdenbos, A., Paus, T., Evans, A. C., and Rapoport, J. L. (1999). Brain development during childhood and adolescence: A longitudinal MRI study. *Nature Neuroscience*, 2: 861–863.

Gustafsson, B. E. (1959). Vitamin K deficiency in germfree rats. *Annual Report of the New York Academy of Science*, 78: 166–174.

Hoban, A. E., Moloney, R. D., Golubeva, A. V., McVey Neufeld, K. A., O’Sullivan, O., et al. (2016a). Behavioural and neurochemical consequences of chronic gut microbiota depletion during adulthood in the rat. *Neuroscience*, 339: 463–477.

Hoban, A. E., Stilling, R. M., Ryan, F. J., Shanahan, F., Dinan, T. G, Claesson, M. J., Clarke G., and Cryan J. F. (2016b). Regulation of prefrontal cortex myelination by the microbiota. *Translational Psychiatry*, 6: e774.

Hoban, A. E., Stilling, R. M., Moloney, G., Shanahan, F., Dinan, T. G., Clarke, G., and Cryan J. F. (2018). The microbiome regulates amygdala-dependent fear recall. *Molecular Psychiatry*, 23: 1134–1144.

Hollister, E. B., Riehle, K., Luna, R. A., Weidler, E. M., Rubio-Gonzales, M., Mistretta, T.-A., Raza, S., Doddapaneni, H. V., Metcalf, G. A., Muzny, D. M., Gibbs, R. A., Petrosino, J. F., Shulman, R. J., and Versalovic, J. (2015). Structure and function of the healthy pre-adolescent pediatric gut microbiome. *Microbiome*, 3: 36.

Holtmaat, A. and Svoboda, K. (2009). Experience-dependent structural synaptic plasticity in the mammalian brain. *Nature Reviews Neuroscience*, 10: 647–658.

Hooper, L. V., Littman, D. R., and Macpherson, A. J. (2012). Interactions between the microbiota and the immune system. *Science*, 336: 1268–1273.

Hoverstadt, T. and Midtvedt, T. (1986). Short-chain fatty acids in germfree mice and rats. *Journal of Nutrition*, 116: 1772–1776.

Hsiao, E. Y., McBride, S. W., Hsien, S., Sharon, G., Hyde, E. R., et al. (2013). Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell*, 155: 1451–1463.

Hua, J. Y. and Smith, S. J. (2004). Neural activity and the dynamics of central nervous system development. *Nature Neuroscience*, 7: 327–332.

Huang, R., Wang, K., and Hu, J. (2016). Effect of probiotics on depression: A systematic review and meta-analysis of randomized controlled trials. *Nutrients*, 8: 483.

Huttenlocher, P. R. (1979). Synaptic density in human frontal cortex—developmental changes and effects of aging. *Brain Research*, 163: 195–205.

Huttenlocher, P. R. and Dabholkar, A. S. (1997). Regional differences in synaptogenesis in human cerebral cortex. *Journal of Comparative Neurology*, 387: 167–178.

Huttenlocher, P. R., de Courten, C., Garey L. J., and Van der Loos, H. (1982). Synaptogenesis in human visual cortex-evidence for synaptic elimination during normal development. *Neuroscience Letters*, 33: 247–252.

Ihekweazu, F. D. and Versalovic J. (2018). Development of the pediatric gut microbiome: Impact on health and disease. *American Journal of Medical Science*, 356: 413–423.

Jiang, H.-Y., Zhou, Y.-Y., Zhou, G.-L., Li, Y.-C., Yuan, J., et al. (2018). Gut microbiota profiles in treatment-naïve children with attention deficit hyperactivity disorder. *Behavioural Brain Research*, 347: 408–413.

Jimenez, E., Fernandez, L., Marin, M. L., Martin, R., Odriozola, J. M., Nueno-Palop, C., Narbad, A., Olivares, M., Xaus, J., and Rodriguez, J. M. (2005). Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. *Current Microbiology*, 51: 270–274.

Jimenez, E., Marin, M. L., Martin, R., Odriozola, J. M., Olivares, M., Xaus, J., Fernandez, L., and Rodriguez, J. M, (2008). Is meconium from healthy newborns actually sterile? *Research Microbiology*, 159: 187–193.

Johnstone, N. and Cohen Kadosh, K. (2019). Why a developmental cognitive neuroscience approach may be key for future-proofing microbiota-gut-brain research. *Behavior Brain Science*, 42: e73.

Johnstone, N., Milesi, C., Burn, O., van den Bogert, B., Nauta, A., et al. (2021). Anxiolytic effect of a galacto-oligosaccharides prebiotic in healthy females (18–25 years). with corresponding changes in gut bacterial composition. *Scientific Reports*, 11: 8302.

Kayyal, M., Javkar, T., Mian, M. F., Binyamin, D., Koren, O., et al. (2020). Sex dependent effect of post-natal penicillin on brain, behavior and immune regulation are prevented by concurrent probiotic treatment. *Science Reports*, 10: 10318.

Kazemi, A., Noorbala, A. A., Azm, K., Eskandari, M. H., and Djafarian, K. (2018). Effect of probiotic and prebiotic vs placebo on psychological outcomes in patients with major depressive disorder: A randomized clinical trial. *Clinical Nutrition*, 38: 522–528.

Kennedy, K. M., Gerlach, M. J., Adam, T., Heimesaat, M. M., Rossi, L., Surette, M. G., Sloboda, D. M., and Braun, T. (2021). Fetal meconium does not have a detectable microbiota before birth. *Nature Microbiology*, Doi:10.1038/s41564-021-00904-0 [Online ahead of print].

Koenig, J. E., Spor, A., Scalfone, N., Fricker, A. D., Stombaugh, J., Knight, R., Angenent, L. T., and Ley, R. E. (2011). Succession of microbial consortia in the developing infant gut microbiome. *Proceedings of the National Academy of Sciences* U S A, 108 (Suppl 1): 4578–4585.

Kumar, H. and Sharma, B. (2016). Minocycline ameliorates prenatal valproic acid induced autistic behaviour, biochemistry and blood brain barrier impairments in rats. *Brain Research*, 1630: 83–97.

Kuperman, A. A., Zimmerman, A., Hamadia, S., Ziv, O., Gurevich, V., Fichtman, B., Gavert, N., Straussman, R., Rechnitzer, H., Barzilay, M., Shvalb, S., Bornstein, J., Ben-Shachar, I., Yagel, S., Haviv, I., and Koren, O. (2020). Deep microbial analysis of multiple placentas shows no evidence for a placental microbiome. *British Journal of Obstetrics and Gynaecology*, 127: 159–169.

Levy, M., Thaiss, C. A., and Elinav, E. (2016). Metabolites: Messengers between the microbiota and the immune system. *Genes Developments*, 30: 1589–1597.

Lloyd-Price, J., Abu-Ali, G., and Huttenhower, C. (2016). The healthy human microbiome. *Genome Medicine*, 8: 51.

Luczynski, P., McVey Neufeld, K. A., Oriach, C. S., Clarke, G., Dinan, T. G., and Cryan, J. F. (2016a). Growing up in a bubble: Using germ-free animals to assess the influence of the gut microbiota on brain and behavior. *International Journal of Neuropsychopharmacology*, 19: pyw020.

Luczynski, P., Whelan, S. O., O’Sullivan, C., Clarke, G., Shanahan, F., Dinan, T. G., and Cryan, J. F (2016b). Adult microbiota-deficient mice have distinct dendritic morphological changes: Differential effects in the amygdala and hippocampus. *European Journal of Neuroscience*, 44: 2654–2666.

Luk, B., Veeraragavan, S., Engevik, M., Balderas, M., Major, A., et al. (2018). Postnatal colonization with human “infant-type” Bifidobacterium species alters behavior of adult gnotobiotic mice. *PLOS One*, 13: e0196510.

Lyte, M., Li, W., Opitz, N., Gaykema, R. P. A., and Goehler, L. E. (2006). Induction of anxiety-like behavior in mice during the initial stages of infection with the agent of murine colonic hyperplasia Citrobacter rodentium. *Physiological Behavior*, 89: 350–357.

MacFabe, D. F., Cain, N. E., Boon, F., Ossenkopp, K. P., and Cain, D. P. (2011). Effects of the enteric bacterial metabolic product propionic acid on object-directed behavior, social behavior, cognition, and neuroinflammation in adolescent rats: Relevance to autism spectrum disorder. *Behavior Brain Research*, 217: 47–54.

Mallick, H., Ma, S., Franzosa, E. A., Vatanen, T., Morgan, X. C., and Huttenhower, C. (2017). Experimental design and quantitative analysis of microbial community multiomics. *Genome Biology*, 18: 228.

Mayer, E. A. (2011). Gut feelings: The emerging biology of gut-brain communication. *Nature Reviews Neuroscience*, 12: 453–466.

Mayer, E. A., Knight, R., Mazmanian, S. K., Cryan, J. F., and Tillisch, K. (2014). Gut microbes and the brain: Paradigm shift in neuroscience. *Journal of Neuroscience*, 34: 15490–15496.

McVey Neufeld, K. A., Mao, Y. K., Bienenstock, J., Foster, J. A., and Kunze, W. A. (2013). The microbiome is essential for normal gut intrinsic primary afferent neuron excitability in the mouse. *Neurogastroenterology and Motility*, 25: e183–e188.

McVey Neufeld, K. A., Perez-Burgos, A., Mao, Y. K., Bienenstock, J., and Kunze, W. A. (2015). The gut microbiome restores intrinsic and extrinsic nerve function in germ-free mice accompanied by changes in calbindin. *Neurogastroenterology and Motility*, 27: 627–636.

McVey Neufeld, K. A., Luczynski, P., Dinan, T. G., and Cryan, J. F. (2016a). Reframing the teenage wasteland: Adolescent microbiota-gut-brain axis. *Canadian Journal of Psychiatry*, 61: 214–221.

McVey Neufeld, K. A., Luczynski, P., Seira Oriach, C., Dinan, T. G., and Cryan, J. F. (2016b). What's bugging your teen?—the microbiota and adolescent mental health. *Neuroscience and Biobehavioral Reviews*, 70: 300–312.

Messaoudi, M., Violle, N., Bisson, J. F., Desor, D., Javelot, H., and Rougeot, C. (2011a). Beneficial psychological effects of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in healthy human volunteers. *Gut Microbes*, 2: 256–261.

Messaoudi, M., Lalonde, R., Violle N., Javelot H, Desor D., Nejdi A., Bisson, J. F., Fougeot, C., Pichelin, M., Cazaubiel, M., and Cazaubiel, J. M. (2011b). Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. *British Journal of Nutrition*, 105: 755–764.

Mishra, A., Lai, G. C., Yao, L. J., Aung, T. T., Shental, N., et al. (2021). Microbial exposure during early human development primes fetal immune cells. *Cell*, 184: 3394–3409.

Mohammadi, A. A., Jazayeri, S., Khosravi-Darani, K., Solati, Z., Mohammadpour, N., Asemi, Z., Adab, Z., Djalali, M., Tehrani-Doost, M., Hosseini, M., and Eghtesadi, S. (2015). The effects of probiotics on mental health and hypothalamic-pituitary-adrenal axis: A randomized double-blind, placebo-controlled trial in petrochemical workers. *Nutritional Neuroscience*, 19: 387–395.

Neufeld, K. M., Kang, N., Bienenstock, J., and Foster, J. A. (2011). Reduced anxiety-like behaviour and central neurochemical change in germ-free mice. *Neurogastroenterology and Motility*, 23: 255–264.

Neuman, H., Debelius J. W., Knight R., and Koren O. (2015). Microbial endocrinology: The interplay between the microbiota and the endocrine system. *FEMS Microbial Review*, 39: 509–521.

Nicholson, J. K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W., and Pettersson, S. (2012). Host-gut microbiota metabolic interactions. *Science*, 336: 1262–1267.

Niu, J., Xu, L., Qian, Y., Sun, Z., Yu, D., Huang, J., Zhou, X., Wang, Y., Zhang, T., Ren, R., Li, Z., Yu, J., and Gao, X. (2020). Evolution of the gut microbiome in early childhood: A cross-sectional study of Chinese children. *Frontiers in Microbiology*, 11: 439.

Ogbannaya, E. S., Clarke G., Shanahan, F., Dinan, T. G., Cryan, J. F., and O'Leary, O. F. (2015). Adult hippocampal neurogenesis is regulated by the microbiome. *Biological Psychiatry*, 78: e7–9.

Parracho, H. M., Bingham, M. O., Gibson, G. R., and McCartney, A. L. (2005). Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. *Journal of Medical Microbiology*, 54(Pt 10): 987–991.

Parracho, H. M. R. T., Gibson, G. R., Knott, F., Bosscher, D., Kleerebezem, M., et al. (2010). A double-blind, placebo-controlled, crossover-designed probiotic feeding study in children diagnosed with autistic spectrum disorders. *International Journal of Probiotics and Prebiotics*, 5: 69–74.

Partty, A., Kalliomaki, M., Wacklin, P., Salminen, S., and Isolauri, E. (2015). A possible link between early probiotic intervention and the risk of neuropsychiatric disorders later in childhood: A randomized trial. *Pediatric Research*, 77: 823–828.

Perez-Munoz, M. E., Arrieta, M. C., Ramer-Tait, A. E., and Walter, J. (2017). A critical assessment of the "sterile womb" and "in utero colonization" hypotheses: Implications for research on the pioneer infant microbiome. *Microbiome*, 5: 48.

Pinto-Sanchez, M. I., Hall, G. B., Ghajar, K., Nardelli, A., Bolino, C., et al. (2017). Probiotic Bifidobacterium longum NCC3001 reduces depression scores and alters brain activity: A pilot study in patients with irritable bowel syndrome. *Gastroenterology*, 153: 448–459.

Puertollano, E., Kolida, S., and Yaqoob, P. (2014). Biological significance of short-chain fatty acid metabolism by the intestinal microbiome. *Current Opinion in Clinical Nutrition and Metabolic Care*, 17: 139–144.

Quigley, E. M. M. (2018). The gut-brain-axis and the microbiome: Clues to pathophysiology and opportunities for novel management strategies in irritable bowel syndrome (IBS). *Journal of Clinical Medicine*, 7: 6.

Rakoff-Nahoum, S. and Medzhitov, R. (2008). Innate immune recognition of the indigenous microbial flora. *Mucosal Immunology Supplement*, 1: S10–14.

Ramirez, P. L., Barnhill, K., Gutierrez, A., Schutte, C., and Hewitson, L. (2013). Improvements in behavioral symptoms following antibiotic therapy in a 14-year-old male with autism. *Case Reports in Psychiatry*, 2013: 239034.

Reis, D. J., Ilardi, S. S., and Punt, S. E. W. (2018). The anxiolytic effect of probiotics: A systematic review and meta-analysis of the clinical and preclinical literature. *PLOS One*, 13: e0199041.

Rhee, S. H., Pothoulakis, C., and Mayer, E. A. (2009). Principles and clinical implications of the brain-gut-enteric microbiota axis. *National Reviews in Gastroenterology and Hepatology*, 6: 306–314.

Richarte, V., Sanchez-Mora, C., Corrales, M., Fadeuilhe, C., Vilar-Ribo, L., et al. (2021). Gut microbiota signature in treatment-naïve attention-deficit/hyperactivity disorder. *Translational Psychiatry*, 11: 382.

Sandler, R. H., Finegold, S. M., Bolte, E. R., Buchanan, C. P., Maxwell, A. P., et al. (2000). Short-term benefit from oral vancomycin treatment of regressive-onset autism. *Journal of Child Neurology*, 15: 429–435.

Schafer, D. P., Lehrman, E. K., Kautzman, A. G., Koyama, R., Mardinly. A. R., et al. (2012). Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron*, 74: 691–705.

Schmidt, K., Cowen, P. J., Harmer, C. J., Tzortzis, G., Errington, S., and Burnet, P. W. J. (2015). Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers. *Psychopharmacology* (Berl), 232: 1793–1801.

Semple, B. D., Blomgren, K., Gimlin, K., Ferriero, D. M., and Noble-Haeusslein, L. J. (2013). Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Progress in Neurobiology*, 106–107: 1–16.

Sender, R., Fuchs, S., and Milo, R. (2016a). Revised estimates for the number of human and bacterial cells in the body. *PLoS Biology*, 14: e1002533.

Sender, R., Fuchs, S., and Milo, R. (2016b). Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell*, 164: 337–340.

Sgritta, M., Dooling, S. W., Buffington, S. A., Momin, E. N., and Francis, M. B. (2019). Mechanisms underlying microbial-mediated changes in social behavior in mouse models of autism spectrum disorder. *Neuron*, 101: 246–259.

Slykerman, R. F., Hood, F., Wickens, K., Thompson, J. M. D., Barthow, C., et al. (2017a). Effect of Lactobacillus rhamnosus HN001 in pregnancy on postpartum symptoms of depression and anxiety: A randomized double-blind placebo-controlled trial. *EBioMedicine*, 24: 159–165.

Slykerman, R. F., Thompson, J, Waldie, K. E., Murphy, R., Wall, C., and Mitchell, E. A. (2017b). Antibiotics in the first year of life and subsequent neurocognitive outcomes. *Acta Pediatrica*, 106: 87–94.

Slykerman, R. F., Coomarasamy, C., Wickens, K., Thompson, J. M. D., Stanley, T. V., et al. (2019). Exposure to antibiotics in the first 24 months of life and neurocognitive outcomes at 11 years of age. *Psychopharmacology* (Berl), 236: 1573–1582.

Sowell, E. R., Thompson, P. M., Tessner, K. D., and Toga, A. W. (2001). Mapping continued brain growth and gray matter density reduction in dorsal frontal cortex: Inverse relationships during postadolescent brain maturation. *Journal of Neuroscience*, 21: 8819–8829.

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neuroscience Biobehavior Review*, 24: 417–463.

Spear, L. P. (2013). Adolescent neurodevelopment. *Journal of Adolescent Health*, 52(2 Suppl 2): S7–13.

Steenbergen, L., Sellaro, R., van Hemert, S., Bosch, J., and Colzato, L. S. (2015). A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood. *Brain Behavior and Immunity*, 48: 258–264.

Stewart, C. J., Ajami, N. J., O'Brien, J. L., Hutchinson, D. S., Smith, D. P., et al. (2018). Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nature*, 562: 583–588.

Stilling, R. M., Ryan F. J., Hoban, A. E., Shanahan, F., Clarke, G., et al. (2015). Microbes and neurodevelopment—Absence of microbiota during early life increases activity-related transcriptional pathways in the amygdala. *Brain Behavior and Immunity*, 50: 209–220.

Stilling, R. M., Moloney, G. M., Ryan, F. J., Hoban, A. E., Bastiaanssen, T., et al. (2018). Social interaction-induced activation of RNA splicing in the amygdala of microbiome-deficient mice. *ELife*, 7: ee33070.

Strati, F., Cavalieri, D., Albanese, D., De Felice, C., Donati, C., et al. (2017). New evidences on the altered gut microbiota in autism spectrum disorders. *Microbiome*, 5: 24.

Sturman, D. A. and Moghaddam, B. (2011). The neurobiology of adolescence: Changes in brain architecture, functional dynamics, and behavioral tendencies. *Neuroscience and Biobehavioral Reviews*, 35: 1704–1712.

Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X.-N., Kubo, C., and Koga, Y. (2004). Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *Journal of Physiology*, 558(Pt 1): 263–275.

Sumi, Y., Miyakawa, M., Kanzaki, M., and Kotake, Y. (1977). Vitamin B-6 deficiency in germfree rats. *Journal of Nutrition*, 107: 1707–1714.

Takada, M., Nishida, K., Kataoka-Kato, A., Gondo, Y., Ishikawa, H., et al. (2016). Probiotic Lactobacillus casei strain Shirota relieves stress-associated symptoms by modulating the gut-brain interaction in human and animal models. *Neurogastroenterology and Motility*, 28: 1027–1036.

Thion, M. S., Low, D., Silvin, A., Chen, J., Grisel, P., et al. (2018). Microbiome influences prenatal and adult microglia in a sex-specific manner. *Cell*, 172: 500–516.

Tillisch K., Labus J., Kilpatrick L., Jiang Z., Stains J., et al. (2013). Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology*, 144: 1394–1401.

Urbano, M., Okwara, L., Manser, P., Hartmann, K., Herndon, A., and Deutsch, S. I. (2014). A trial of D-cycloserine to treat stereotypes in older adolescents and young adults with autism spectrum disorder. *Journal of Neuropsychiatry and Clinical Neuroscience*, 27: 133–138.

van Dyck, L. I. and Morrow, E. M. (2017). Genetic control of postnatal human brain growth. *Current Opinion in Neurology*, 30: 114–124.

Vuong, H. E., and Hsiao, E. Y. (2017). Emerging roles for the gut microbiome in autism spectrum disorder. *Biological Psychiatry*, 81: 411–423.

Wall, R., Cryan, J. F., Ross, R. P., Fitzgerald, G. F., Dinan, T. G., and Stanton C. (2014). Bacterial neuroactive compounds produced by psychobiotics. *Advances in Experimental Medicine and Biology*, 817: 221–239.

Wan, L., Ge, W.-R., Zhang, S., Sun, Y.-L., Wang, B., et al. (2020). Case-control study of the effects of the gut microbiota composition on neurotransmitter metabolic pathways in children with attention deficit hyperactivity disorder. *Frontiers of Neuroscience*, 14: 127.

Wang, T., Hu, X., Liang, S., Li, W., Wu, X., et al. (2015). Lactobacillus fermentum NS9 restores the antibiotic induced physiological and psychological abnormalities in rats. *Beneficial Microbes*, 6: 707–717.

Wellmann, K. A., Varlinskaya, E. I., and Mooney, S. M. (2014). D-cycloserine ameliorates social alterations that results from prenatal exposure to valproic acid. *Brain Research Bulletin*, 108: 1–9.

Woo, T. U., Pucak, M. L., Kye, C. H., Matus, C. V., and Lewis, D. A. (1997). Peripubertal refinement of the intrinsic and associational circuitry in monkey prefrontal cortex. *Neuroscience*, 80: 1149–1158.

Wostmann, B. S., Pleasants, J. R., Bealmear, P., and Kincade, P. W. (1970). Serum proteins and lymphoid tissues in germ-free mice fed a chemically defined, water soluble, low molecular weight diet. *Immunology*, 19: 443–448.

Wostmann, B. S., Larkin, C., Moriarty, A., and Bruckner-Kardoss, E. (1983). Dietary intake, energy metabolism, and excretory losses of adult male germfree Wistar rats. *Laboratory Animal Science*, 33: 46–50.

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., et al. (2012). Human gut microbiome viewed across age and geography. *Nature*, 486: 222–227.

Yuan, X., Chen, R., Zhang, Y., Lin, X., and Yang, X. (2020). Gut microbiota: Effect of pubertal status. *BMC Microbiology*, 20: 334.

Zaneveld, J. R., McMinds, R., and Vega Thurber, R. (2017). Stress and stability: applying the Anna Karenina principle to animal microbiomes *Nature Microbiology*, 2: 17121.

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