

Emerging roles for microglia and microbiota in the development of social circuits

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ABSTRACT

Social withdrawal is a core component of the behavioral response to infection. This fact points to a deep evolutionary and biologic relationship between the immune system and the social brain. Indeed, a large body of literature supports such an intimate connection. In particular, immune activation during the perinatal period has been shown to have long-lasting consequences for social behavior, but the neuroimmune mechanisms by which this occurs are only partially understood. Microglia, the resident immune cells of the brain, influence the formation of neural circuits by phagocytosing synaptic and cellular elements, as well as by releasing chemokines and cytokines. Intriguingly, microbiota, especially those that reside within the gut, may also influence brain development via the release of metabolites that travel to the brain, by influencing vagal nerve signaling, or by modulating the host immune system. Here, I will review the work suggesting important roles for microglia and microbiota in social circuit formation during development. I will then highlight avenues for future work in this area, as well as technological advances that extend our capacity to ask mechanistic questions about the relationships between microglia, microbiota, and the social brain.

1. Introduction

The immune system has long been known to influence social behavior and the social brain (for review see [Smith and Bilbo, 2021](#)). Early studies of the behavior of sick animals identified ‘sickness behavior’ as an adaptive host response to infection that includes anorexia, lethargy, and social withdrawal ([Hart, 1988](#); [Dantzer and Kelley, 2007](#)). Social withdrawal following infection has been observed across the evolutionary continuum and while it may help to prevent disease transmission, it also comes at a cost in that it reduces parental care and limits mate selection ([Shakhar and Shakhar, 2015](#)). Animals, including humans, are also capable of recognizing, and therefore avoiding, other individuals who are sick ([Kavaliers and Choleris, 2018](#); [Arakawa et al., 2011](#); [MacRae et al., 2015](#); [Lasselin et al., 2017](#)). (see [Fig. 1](#))

The above examples illustrate how the immune system modulates social behavior during an acute immune challenge in adulthood. However, the immune system also plays a critical role in the developmental organization of social circuits. Several studies have shown that immune challenges during the perinatal period lead to long-lasting changes in social behavior in rodent models. For example, maternal immune activation during pregnancy, postnatal injection of the bacterial mimetic lipopolysaccharide (LPS), and other immune-activating exposures such

as maternal high-fat diet have all been shown to alter adult social behavior ([Hsiao et al., 2013](#); [Choi et al., 2016](#); [Carlezon et al., 2019](#); [Buffington et al., 2016](#); [Smith et al., 2020](#)). Excitingly, emerging work also suggests that neuroimmune interactions are critical to the development of neural circuits in the healthy brain, but only a handful of studies have explored this in the context of social circuits ([Stevens and Schafer, 2018](#)). Microglia, the resident immune cells of the brain, have stood out as important players in this work, as they are capable of both responding to immune challenges, and phagocytosing synapses and even whole cells during development ([Kopec et al., 2018](#); [VanRyzin et al., 2019](#)).

It is tempting to view all bacterial organisms as potentially pathogenic invaders. However, in recent years, our microbiota – the commensal bacteria that live alongside and within us – have gained recognition as important, often beneficial, intermediaries between ourselves and our environment. Indeed, microbes are required for the developmental education of our immune system, which, in turn, may impact brain development ([Zegarra-Ruiz et al., 2021](#); [Filiano et al., 2016](#)). This is likely to be the case in the realm of social behavior. For example, germ-free mice, who lack microbiota entirely, have abnormal social behavior ([Desbonnet et al., 2014](#); [Buffington et al., 2016](#); [Lu et al., 2018](#)). Furthermore, early studies suggest that the effects of immune challenges on social behavior may depend on the composition of the gut microbiome ([Kim et al., 2017](#)),

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Fig. 1. Dr. Caroline J. Smith. Dr. Smith is a postdoctoral fellow in the lab of Dr. Staci Bilbo at Duke University. She began her academic career as an undergraduate student at the University of Massachusetts Amherst in the lab of Dr. Nancy Forger studying the epigenetic mechanisms underlying sex differences in the brain. She completed her PhD in the lab of Dr. Alexa Veenema at Boston College. Her graduate research aimed to elucidate sex differences in the roles of neuropeptides and endogenous opioids in the regulation of adolescent social behavior and was supported by a fellowship from the National Science Foundation. She is currently a postdoctoral fellow in the lab of Dr. Staci Bilbo at Duke University where her work focuses on understanding how neuroimmune interactions during development influence the organization of social circuits in the brain and how this process is disrupted by a variety of perinatal immune challenges (such as environmental toxicants, stress, opioids, and bacterial mimetics). This work is supported by a Ruth L. Kirschstein National Research Service Award from the National Institute of Environmental Health Sciences. In the future, she hopes to combine systems level circuit-based approaches and molecular/sequencing technologies to investigate the ways in which microglia and the gut-brain axis sculpt the social brain in both males and females.

and that intervening at the level of the microbiome may ameliorate social impairments in neurodevelopmental disorders such as autism spectrum disorders (ASD; Kang et al., 2017, 2019; 2020).

Here, I will first review the evidence suggesting a causal role for microglia in the developmental sculpting of social circuits in the brain. Next, I will discuss the findings suggesting that the gut microbiome may causally contribute to the organization of social circuits. Finally, I will discuss the need for further studies in this arena, as well as the potential ways in which microglia and microbiota may interact in this context and new technological advances that extend our ability to ask targeted and precise questions about the function of microglia and the microbiome.

2. What role do microglia play in shaping social neural circuits?

Microglia are the tissue-resident macrophages of the central nervous system (CNS) and migrate into the brain prior to the closure of the blood brain barrier during embryonic development. In addition to responding to direct insults such as infections and traumatic brain injuries, microglia participate in healthy brain development via the release of trophic factors (such as BDNF) and neuromodulators, and by engaging in activity-dependent synaptic pruning. This has been particularly well characterized within the retinogeniculate pathway where microglia have been shown to influence synaptic formation and elimination via both complement-dependent and independent mechanisms (Schafer et al., 2012; Sipe et al., 2016; Cheadle et al., 2020; Hammond et al., 2018).

Several studies now suggest that microglia play a role in the development of the neural circuits underlying social behavior as well. For instance, genetic manipulations that affect microglial function, such as deletion of P2Y₁₂ (a purinergic receptor expressed on homeostatic microglia in the CNS) alter social behavior. Indeed, chasing behavior and olfactory investigation towards other conspecifics are decreased in both male and female P2Y₁₂ knockout mice (Lowery et al., 2021). Similarly, mice lacking CX3Cr1 have fewer microglia during the perinatal period, reduced synaptic engulfment, and social behavior impairments (Zhan et al., 2014). Microglia-specific overexpression of the translation initiation factor eIF4E, which elevates protein synthesis, leads to reduced synapse engulfment and social behavior deficits in male mice (Xu et al., 2020). In rats, transient neonatal microglial depletion with liposomal clodronate induced changes in both adolescent social play behavior and passive adult social behaviors (Nelson and Lenz, 2017). These findings suggest that microglial phagocytosis is critical to the organization of the social brain.

Intriguingly, we recently found that in rats, microglial density, complement component 3 (C3) expression, and dopamine D1 receptor (D1R) density are all elevated within the nucleus accumbens (NAc; a critical node in the social reward network) during adolescence (Kopec et al., 2018). Importantly, social play behavior is also highest during the adolescent period in rats – declining sharply into adulthood as animals transition to mature behaviors such as sexual behavior and aggression. D1R activation within the NAc has previously been shown to facilitate social reward (Manduca et al., 2016). We found that microglial complement-mediated phagocytosis is required for the developmental decline in D1R density that occurs between adolescence and adulthood, which, in turn, causes the decline in social play behavior (Kopec et al., 2018). Importantly, these effects were present in males, but not females, demonstrating sex-specificity of this mechanism. This is in line with work demonstrating that microglia phagocytose more newborn astrocytes in the amygdala in males than in females during the neonatal period (VanRyzin et al., 2019). This increased phagocytosis in males is driven by testosterone and endocannabinoids and results in higher levels of social play behavior in males than in females (VanRyzin et al., 2019). These studies highlight the importance of sex differences in the microglial sculpting of social circuits. Indeed, it appears that microglial phagocytosis of newborn cells and synaptic elements is potentially a male-specific phenomenon in the social brain. In keeping with this idea, we recently showed that a neonatal LPS challenge leads to changes in sociability and social memory in females but not males (Smith et al., 2020). However, microglia-specific genetic knock-down of myeloid differentiation response protein 88 (MyD88; the removal of which prevents LPS from increasing proinflammatory cytokines such as TNF α and IL-1 β) did not prevent these LPS-induced changes (Smith et al., 2020). Thus, it is possible that non-microglial mechanisms of circuit organization are more important in the female brain. Understanding how social circuits are sculpted in the female brain represents an important avenue for future investigation.

2.1. How does the gut microbiome influence the social brain?

Changes in the gut-brain axis have been suggested to contribute to the pathophysiology of many neurological disorders including Alzheimer's disease, Parkinson's disease, anxiety, depression, and ASD (for review see Cryan et al., 2020). Altered social functioning is a characteristic of many of these disorders, most notably ASD, which is primarily characterized by deficits in social communication and social interaction (Baio et al., 2018). Several studies now show that the composition of the gut microbiome is shifted in individuals with ASD, and that fecal microbiota transfer (FMT) therapy may have therapeutic potential for alleviating both gastrointestinal and behavioral symptoms in ASD (Kang et al., 2017, 2019; 2020). Of note, FMT comes with some important risks, such as the potential transmission of multi-drug resistant bacterial species (U.S. Food and Drug Administration, 2019). This highlights the importance of

identifying specific bacterial taxa that might prove efficacious in the amelioration of ASD symptoms. Together, these findings have spurred a surge of animal studies in recent years investigating the links between the gut microbiome and the social brain.

Germ-free mice, which lack microbiota entirely, display social deficits at baseline, as compared to conventionally housed mice (Desbonnet et al., 2014; Buffington et al., 2016; Lu et al., 2018). Importantly, colonization of germ-free mice with conventional microbiota at weaning restores appropriate social behavior in male mice (Desbonnet et al., 2014). Excitingly, this has also recently been extended to social hierarchies. Specifically, dominant and submissive male mice have significantly different gut microbiome compositions, with less diversity in submissive males as compared to dominant males. Fecal microbiota transplantation from dominant males into germ-free mice increased sociability, while microbiota transplanted from submissive males lead to reduced social behavior (Agranyoni et al., 2021). Finally, germ-free mice colonized with microbiota from human patients with ASD display reduced sociability as compared to those colonized with microbiota from typically developing children (Sharon et al., 2019).

Gut microbiota have also been shown to causally contribute to social behavior deficits in mouse models of ASD. In a seminal study, Hsiao et al. (2013) found that maternal immune activation (MIA) in utero leads to social behavior impairments (social interaction and social communication), changes in the composition of the gut microbiome, and greater intestinal permeability in male offspring. In particular, *Bacteroides fragilis* was less abundant in the gut microbiome of offspring exposed to MIA as compared to control (Hsiao et al., 2013). Supplementation with *B. fragilis* restored both social communication deficits and intestinal permeability, although social interaction itself was not rescued (Hsiao et al., 2013). In line with this finding, more recent work suggests that the effects of MIA on offspring social behavior also depend on the presence of segmented filamentous bacteria (SFB) within the gut microbiome (Kim et al., 2017).

In another line of investigation, Buffington et al. (2016) demonstrated that maternal high-fat diet (mHFD) during pregnancy induced autism-relevant social behavior deficits and changes in the gut microbiome in offspring. Critically, these deficits could be reversed by co-housing with naïve cage mates at weaning – a procedure that restores the composition of the gut microbiome. The authors went on to show that the bacterial species *Lactobacillus reuteri* is decreased within the gut microbiome of mHFD-exposed offspring and that reconstitution with *L. reuteri* alone was sufficient to restore oxytocin-mediated neuroplasticity with the ventral tegmental area (VTA), as well as social behavior (Buffington et al., 2016). Building on this study, Sgritta et al. (2019) found that treatment with *L. reuteri* also rescues social behavior deficits in three mouse models of ASD. This rescue also depended on oxytocin-mediated neuroplasticity in the VTA. Importantly, they further demonstrated that vagal nerve activation was required to translate these signals from the gut into neural changes (Sgritta et al., 2019). Finally, Buffington et al. (2021) found that in *Cntnap2*^{-/-} mice (which display decreased sociability and hyperactivity), *L. reuteri* treatment increased social behaviors, but did not reduce hyperactivity (Buffington et al., 2021), demonstrating behavior- and circuit-specificity. The bacterial species implicated in these studies are all commensal bacteria – typically beneficial members of a healthy, diverse microbiome. Indeed, the genera *Parabacteroides* and *Bacteroides* (of which *B. fragilis* is a member) are less abundant in the gut microbiomes of human individuals with ASD (Sharon et al., 2019). These findings provide crucial mechanistic evidence that microbiota are capable of organizing social circuits within the developing brain.

2.2. Future directions

Several outstanding questions remain in our understanding of how the gut microbiome and microglia regulate social behavior. Importantly, the field is only just beginning to uncover the precise molecular mechanisms by which microbiota communicate with the brain in the context

of social circuits. Studies show that metabolites from the gut microbiome such as short-chain fatty acids (SCFAs) and vagal nerve activation can act directly on neural systems such as the oxytocin and dopamine systems in the brain to change social behavior (Buffington et al., 2016; Sgritta et al., 2019; Sharon et al., 2019). Importantly, there is also striking evidence demonstrating that microglial function is shaped by the composition of the gut microbiome during the perinatal period (Erny et al., 2015; Castillo-Ruiz et al., 2018; Thion et al., 2018; Luck et al., 2020). Specifically, Erny et al. (2015) found that adult male and female germ-free mice have higher densities of microglia in brain regions including the cortex and cerebellum and that these microglia are hyper-ramified as compared to specific pathogen free (SPF) mice. The ramification state of microglia is often taken as an indicator of function with more amoeboid (less ramified) microglia being more pro-inflammatory, although there are limits to morphology as an indicator of function (Buttini et al., 1996). Moreover, germ-free microglia were less reactive to an LPS challenge (as assessed by RNA sequencing), and oral supplementation with SCFA's rescued these phenotypes (Erny et al., 2015). Interestingly, during early postnatal development, germ-free mice have fewer or more microglia depending on the brain region, as well as lower expression of proinflammatory cytokines, and these changes appear to have important functional consequences for processes such as programmed cell death and synapse remodeling (Castillo-Ruiz et al., 2018; Luck et al., 2020). In an investigation of sex differences in the impact of the gut microbiome on microglia, Thion et al. (2018) used RNA sequencing of isolated microglia from both conventionally housed mice and germ-free mice. They found that at embryonic day 18.5, germ-free male, but not female, mice had more microglia and highly differential microglial gene expression patterns as compared to controls. In contrast, adult microbiota depletion with antibiotics had a greater impact on microglial number and gene expression in females than in males (Thion et al., 2018). These findings suggest that there are both direct and microglia-mediated mechanisms by which gut microbes influence behavior. It is also likely that these mechanisms depend highly on factors such as sex, age, and experiential history.

Another important future direction will be extending this work to understand how microglia and microbiota sculpt canonical social circuits in the brain. Indeed, the social behavior neural network (SBNN) has been well described and extended to comprise a social decision-making neural network (Newman, 1999; O'Connell and Hofmann, 2012). Moreover, neuropeptide systems such as the oxytocin and vasopressin systems are potent regulators of social behavior within these networks and their receptors and synaptic connections change with sex, age, and developmental exposure to immune challenges and stress (Smith et al., 2019; Dumais and Veenema, 2016; Raam and Hong, 2021). However, how microglia and microbiota might contribute to these changes is only just beginning to be explored. For example, we have previously found that oxytocin receptor (OTR) and vasopressin V1a receptor (V1aR) densities differ between males and females in brain regions such as the NAC, lateral septum, and amygdala in rats (Smith et al., 2017, 2017). There are also substantial decreases in OTR and V1aR, as well as in mu-opioid receptors, in many of the same brain regions between adolescence and adulthood (Smith et al., 2017, 2017; Smith et al., 2019). Could microglial phagocytosis contribute to these developmental declines? Do shifts in the gut microbiome following early life insults influence these processes? Interestingly, germ-free male, but not female rats, have higher densities of OTR in the prefrontal cortex and septum as compared to conventionally housed rats (Effah et al., 2021). Rodent species such as prairie voles could also prove to be incredibly useful in these investigations given that they exhibit complex social behaviors (such as pair bond formation) and that the social circuits underlying these behaviors have been well characterized in voles (Loth and Donaldson, 2021; Walum and Young, 2018).

Finally, a major challenge to drawing causal inferences about the specific roles of microglia has been the paucity of techniques available to manipulate their function. Pharmacological agents that block CD11b function, such as neutrophil inhibitory factor (NIF) or OX-42 clone CD11b blocking antibodies, have proven to be very useful for allowing

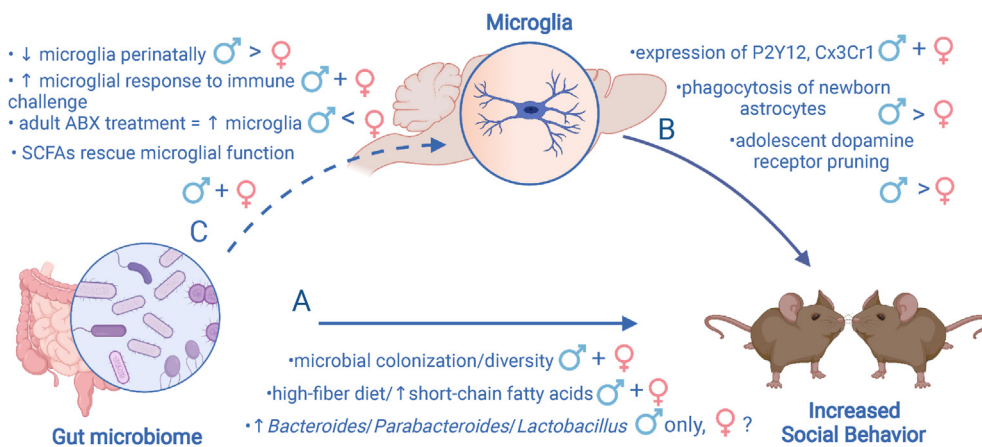


Fig. 2. Mechanisms by which microbiota and microglia influence social behavior. A) Work to date suggests that a healthy, diverse microbiome supports sociability and social hierarchy formation and that supplementation of the gut microbiome with species such as *B. fragilis* and *L. reuteri* can facilitate social behavior. B) The presence of microglia, as well as their developmental sculpting of the social circuits in the brain, supports social behavior. Microglial phagocytosis may be more important for social circuits/behavior in males than in females. C) Microbiota support the maturation and function of microglia. More work is needed to understand the complex interplay between the gut microbiome and microglial function in the context of social behavior. Please see main text for refs. ABX = antibiotic, SCFA = short chain fatty acid. Figure created with Biorender.com.

local manipulation of microglial phagocytosis (Kopec et al., 2018; Van-Ryzin et al., 2019). Another recent development that may prove advantageous is the use of chemogenetic strategies to manipulate microglial behavior. To date, only four studies have used chemogenetics to manipulate microglial function and have done so in the context of neuropathic pain and inflammation (Binning et al., 2020; Saika et al., 2020, 2021; Yi et al., 2021). Future studies should aim to expand our use and understanding of this approach in the context of social systems.

3. Conclusion

In closing, both microglia and microbiota are emerging as critical architects of the neural circuits that support social behavior (Fig. 2). Future studies should aim to extend these findings to the canonical social neural networks within the brain, to elucidate the ways in which microbiota and microglia interact during development, and to clarify the molecular mechanisms of each with greater spatial and temporal resolution. Furthermore, it is critical that we better understand sex differences in the developmental organization of the social brain. This is particularly important given that social impairments are a commonality across numerous neuropsychiatric disorders, many of which are sex-biased in their prevalence.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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